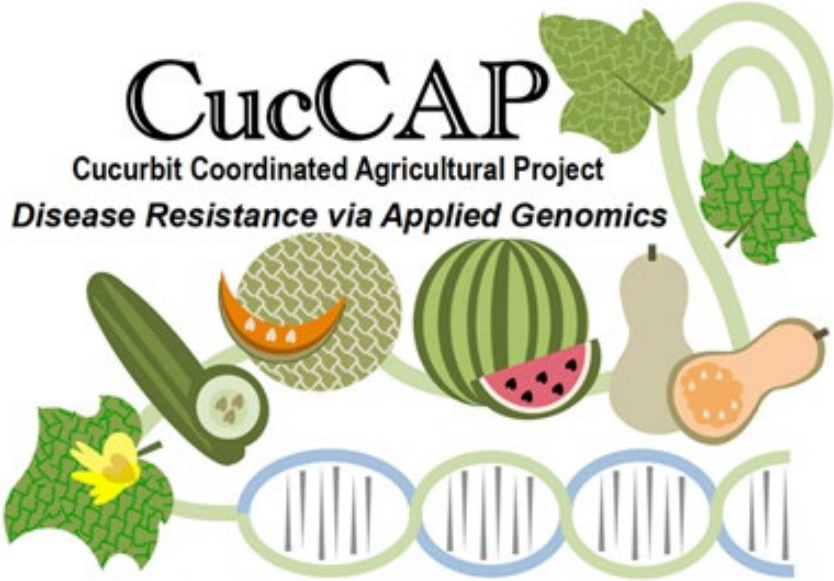
CucCAP2

Harnessing genomic resources for disease resistance and management in cucurbit crops – bringing the tools to the field



CucCAP2 Team Meeting

March April 21-22, 2024

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AGENDA

CucCAP2 team meeting – March 21-22, 2024

(Note: all times are U.S. Pacific Daylight Time)

THURSDAY, MARCH 21

8:00-8:20 Arrival, welcome, Introduction of participants, Introduction to meeting

Session I – Genomic Tools
Objective 1: Develop novel advanced bioinformatic, pan-genome, and genetic mapping tools for cucurbits.
- Develop high-resolution genotyping and advanced genome and pan-genome platforms for cucurbit species.
- Perform de novo genome assembly and pan-genome construction
- Develop breeder-friendly web-based databases for phenotypic, genotypic and QTL information.
- Perform seed multiplication and sequencing analysis of core collections of the four species, provide community resources for genome wide association studies (GWAS).
- [E/O] Provide access to cucurbit genomics tools and databases via the Cucurbit Genomics website (cucurbitgenomics.org) and genomics and bioinformatics workshops

8:20-9:00 Overview of progress: bioinformatics platforms, databases, genomic analyses
(Fei, Wu)
9:00-9:15 Discussion and feedback
Priorities, data access and distribution, phenotype data being collected
9:15-9:30 Status and use of core panels
(seed stocks; resequencing; seed handling and distribution; phenotyping)
(Grumet)
9:30-9:45 Discussion

9:45 – 10:00 Break

Session II – Breeding for disease resistance
Objective 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.
- Utilize genomic approaches to map resistance loci for key cucurbit diseases (QTL mapping, BSA, GWAS)
- Fine map and develop and verify molecular markers for efficient trait selection
- Introgress, pyramid, stack 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

10:00-11:30 **Watermelon**: Status for each disease
Fusarium race 1 and 2, gummy stem blight, Phytophthora, powdery mildew, CYSDV, GCMMV, PRSV-W, ZYMV
(Levi, Branham, Kousik, Ling, McGregor, Reddy, Wechter)
11:30-11:40 Report/priorities from watermelon industry
(Arney/Szczepanski)
11:40-11:55 Discussion

12:00-1:00 Lunch and Planning Session 1
Crop Team Meetings (Watermelon, Melon, Cucumber, Squash)

1:00-2:00 **Melon**: Status for each disease
powdery mildew, CMV, CYSDV, Fusarium
(McCreight, Branham, Kousik, Wechter, Wintermantel)
2:00-2:10 Feedback/priorities from industry

2:10-2:50 **Cucumber**: Status for each disease
downy mildew, Phytophthora, CGMMV
(Weng, Grumet, Keinath, Ling)
2:50-3:00 Feedback/priorities from industry

3:00-3:15 ***Break***

3:15-4:15 **Squash**: Status for each disease
C. moschata – powdery mildew, *Phytophthora*, *C. maxima – Phytophthora*,
C. pepo – powdery mildew, *Phytophthora*
(Mazourek, Hausbeck, Kousik, Meru, Ramirez, Smart)
4:15-4:25 Feedback/priorities from industry

Planning Session 2
4:30-5:30 Integrated Disease Management
Genomic Tools

6:30 ***CucCAP Networking Dinner***

FRIDAY, MARCH 22

8:00-8:15 Arrive

Session III – Integrated disease management and economic analysis
Objective 3. Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.
- Perform multi-location, multi-isolate trials and pathogen population analyses
- Determine economic impacts of disease and control tools and valuation of crop attributes
- [E/O] Provide readily accessible disease management information and recommendations via multiple means including the CucCAP website (cuccap.org)

8:15-9:45 Multi-location trials, pathogen population analyses, economic analyses, delivery of disease information
(Quesada, Hausbeck, Keinath, Kousik, Schulthies, Smart, Tregeagle, Lorscheider)

9:45-10:00 Discussion and feedback from industry

10:00-10:15 ***Break***

Planning Session 3
10:15-11:00 Looking to the future –
Logistics - timeline, no-cost extension, funds, CucCAP meetings (Grumet)
Future plans - CucCAP3? Industry priorities? Research priorities? Timeline?
(Branham, Mazourek)
11:00-11:30 Wrap up discussions, feedback from external reviewers

CucCAP Team

Project Director

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Stakeholder Advisory Board		
Organization	**Representative**	**Position**
Commodity Groups - Growers, Shippers, Processors, Marketing		
National Watermelon Promotion Board	Mark Arney	Executive Director
National Watermelon Association	George Szczepanski	Executive Director
California Melon Research Board	Steve Smith	Former Chair, California Melon Research Board
California Melon Research Board	Bart Fisher	Chair, California Melon Research Board President Fisher Ranch Corporation
Michigan Vegetable Council	Greg Bird	Executive Director
Pickle Packers International	John Cox	Executive Vice President
Swanson Pickles and Pickle Packers International	John Swanson	President Swanson Pickle Company; Research Board, Pickle Packers International
Seed Industry		
BASF	Mona Mazaheri	
Bayer Crop Science	Nischit Shetty	NAM Cucurbit Breeding Lead, Seminis Vegetable Seeds
East-West Seeds	Caleb Orchard Marilyn Hinlo	
Enza Zaden	Bart Kay Walter Verweij Tilly Elridge	Research Molecular Biology
Johnny's Selected Seeds	Lindsay Wyatt	Squash and pumpkin breeder
Limagrain Vegetable Seeds/HM Clause	Kishor Bhattarai Peter Kraan	Research Discovery Manager, HM Clause, Vegetable Seeds Division
Origene America	Eyal Vardi	Founder and CEO
Sakata Seeds	Nihat Guner Jeff Zischke	Senior watermelon breeder
Syngenta Seeds Inc.	Matt Kinkade Sanhu Ajay	Team Lead, watermelon breeding
Taki Seeds	Yasushi Tokairin, Yamamoto Gaku	
United Genetics	Raquel Salaki Xuemei Zhang	

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CucCAP2 Project Objectives

Objective 1. Develop advanced bioinformatic, pan-genome, and genetic mapping tools for cucurbits.

1.1. Develop genomic and bioinformatic platforms for cucurbit crops: high-resolution genotyping platforms; advanced genome and pan-genome platforms; de novo genome assembly and pan-genome construction; breeder-friendly web-based database for phenotypic, genotypic and QTL information.

1.2 Perform seed multiplication and sequencing analysis of core collections of the four species, define phylogenetic relationships and population structure, provide community resources for genome wide association studies (GWAS).

[E/O] Maintain and enhance the Cucurbit Genomics Database (http://cucurbitgenomics.org/), providing publicly available tools to analyze and integrate genotype, phenotype, and pan-genome data.

Objective 2. Utilize genomic approaches to identify, map, and develop markers for resistances to priority diseases identified by cucurbit industries.

2.1. Map resistances and identify QTL for key cucurbit diseases: identify QTL by standard mapping, bulked-segregant analysis, GWAS, synteny; fine map, refine QTL

2.2. Develop and verify markers for MAS.

[E/O] Share QTL information and markers with scientific community and seed industry through publications, scientific and extension presentations, and collaborative research.

Objective 3. Introduce and pyramid/stack resistances into advanced breeding lines.

3.1. Introgress resistance alleles into advanced breeding lines.

3.2. Pyramid/stack resistances: multi-locus marker-assisted selection (MAS); genomic selection; MAGIC population development in watermelon.

[E/O] Provide breeding lines and testing results through germplasm releases, publications, scientific presentations, field trials, and web-based and face-to-face extension venues

Objective 4. Perform multi-location, multi-isolate trials of resistances to improve integrated disease management, assess economic impacts, and provide state-of-the-art disease control recommendations.

4.1. Perform disease management information and recommendations.

4.2. Perform multi-location, multi-isolate trials and pathogen population analyses: evaluate cucurbit cultivars and breeding lines for disease resistance; evaluate integrated disease management in cucurbits combining host resistance and chemical control; analyze pathogen populations to inform breeding and disease management.

4.3. Economic impacts of disease and gains from control tools and valuation of crop attributes: determine economic impacts of disease and control tools; estimate industry valuation of improvement in crop attributes.

[E/O] Maintain and update the CucCAP website (https://cuccap.org) which provides diagnostic and disease control information, disease alerts, links to forecasting tools and project related news. Distribute trial and economic data through publications, extension venues, and the CucCAP website.

Project Structure – Team Organization

Table 4. CucCAP Teams			
Team	**PD, PIs and Co-PIs**		**Institution[a]**
	PD: Rebecca Grumet (RG)		MSU
Watermelon	***Amnon Levi – Team Leader***	(AL)	ARS-SC
	Sandra Branham	(SB)	CLU
	Shaker Kousik	(SK)	ARS-SC
	Kai-Shu Ling	(KSL)	ARS-SC
	Cecilia McGregor	(CM)	UGA
	Umesh Reddy	(UR)	WVSU
	Pat Wechter	(PW)	CLU
Melon	***Jim McCreight – Team Leader***	(JM)	ARS-CA
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	Pat Wechter	(PW)	ARS-SC
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Cucumber	***Yiqun Weng- Team Leader***	(YW)	ARS-WI
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	Anthony Keinath	(AK)	CLU
	Kai-Shu Ling	(KL)	ARS-SC
Squash	***Michael Mazourek – Team Leader***	(MM)	CU
	Mary Hausbeck	(MH)	MSU
	Shaker Kousik	(SK)	ARS-SC
	Geoffrey Meru	(GM)	UFL
	Angela Linares Ramírez	(ALR)	UPR
	Christine Smart	(CS)	CU
Genomics/ bioinformatics	***Zhangjun Fei – Team Leader***	(ZF)	BTI
	Amnon Levi (watermelon)	(AL)	ARS-SC
	Mike Mazourek (squash)	(MM)	CU
	Pat Wechter (melon)	(PW)	ARS-SC
	Yiqun Weng (cucumber)	(YW)	ARS-WI
	Shan Wu	(SW)	BTI
Integrated Disease Management	***Lina Quesada - Team Leader***	(LQ)	NCSU
	Mary Hausbeck	(MH)	MSU
	Anthony Keinath	(AK)	CLU
	Shaker Kousik	(SK)	ARS-SC
	Jonathan Schultheis	(JS)	NCSU
	Christine Smart	(CS)	CU
	Daniel Tregeagle	(DT)	NCSU

[a]Institution abbreviations: ARS-CA (Salinas), SC (Charleston), WI (Madison); BTI-Boyce Thompson Inst; CLU-Clemson Univ; CU-Cornell Univ; MSU-Michigan St Univ; NCSU-North Carolina St Univ; UGA-Univ Georgia; UFL- Univ Florida; UPR-Univ Puerto Rico; WVSU-West Virginia St Univ

TEAM REPORTS

Genomics and Bioinformatics Team

Genomics and Bioinformatics Team

Team members:

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Objectives

Develop novel advanced bioinformatic, pan-genome, and genetic mapping tools for cucurbits.

1.1. Develop genomic and bioinformatic platforms for cucurbit crops.

1.1.1. Development of high-resolution genotyping platforms for cucurbits.

Genome resequencing of the cucumber (388 accessions) and the squash (207 *Cucurbita pepo* accessions) core collections has been completed. The average depths of cleaned sequences of cucumber and squash cores are 49.7× and 49.9×, respectively. For melon (384 accessions) and watermelon (372 accessions) cores, genome sequencing of 313 and 301 accessions, respectively, has been completed. In addition, we have also completed genome resequencing for 26 *C. maxima* and seven *C. moschata* accessions.

The sequence data of cucumber, squash, melon and watermelon cores have been processed for SNP and small indel calling using the Gy14 genome (v2.1), the MU-CU-16 genome (v4.1), the 97103 genome (v2.5) and the DHL92 genome (v4) as the references, respectively. Statistics of called variants are summarized in **Table 1**. Raw sequencing data and called variants have been distributed to our industry partners who have requested access to the data. Biallelic variants with MAF>0.01 of cucumber and squash core collections are available for mining publicly at CuGenDBv2 (http://cucurbitgenomics.org/v2/genotype). The remaining accessions in the melon and watermelon cores are currently under sample collection and DNA preparation and will be sequenced. Currently, of the remaining 71 accessions in the watermelon core, DNA has been prepared for 45 accessions while the other 26 accessions did not germinate. Variants will be updated for the watermelon and melon cores once new sequences are available.

Table 1 Summary of genome sequencing of cucurbit core collections

	cucumber	squash	melon	watermelon
No. accessions	388	207	384	372
No. DNA prepared	388	207	313	346
No. sequenced	388	207	313	301
Average sequencing depth	49.7	49.9	54.1	47.7
No raw SNPs	5,332,225	5,007,376	21,022,493	11,042,203
No. biallelic SNPs with MAF>0.01	2,513,882	4,104,452	10,294,239	6,057,627
No raw indels	1,385,149	2,008,251	4,481,966	1,732,023
No. biallelic indels with MAF>0.01	490,882	1,264,224	1,540,406	1,195,826

We recently found that a total of 58 accessions in the cucumber core contain large numbers of missing SNPs (5-35%) due to the poor quality of the sequencing libraries. These libraries were constructed during CucCAP1 using a cheap protocol. Sequencing of these accessions are being redone. DNA has been prepared for 45 accessions, while the remaining 13 accessions did not germinate. Variants will be updated with new sequences when available.

1.1.2. Development of novel, advanced genome and pan-genome platforms for cucurbit species.
For cucumber, we have selected 25 accessions including five wild *Cucumis sativus* var. *hardwickii*, four semi-wild Xishuangbanna and 16 cultivated cucumbers for PacBio HiFi sequencing. Ten of these 25 accessions are from the core collection. HiFi sequences have been generated for all the 25 accessions, with an average depth of 33.4×.

For watermelon, we selected a total of 135 accessions for reference-grade genome development, including one *Citrullus naudinianus*, one *C. rehmii*, two *C. ecirrhosus*, five *C. colocynthis*, 16 *C. amarus*, seven *C. mucosospermus*, five *C. lanatus* var. *cordophanus*, seven landraces, and 82 cultivars and nine interspecific hybrids. HiFi sequences have been generated for all 135 accessions, with an average depth of 30.3×.

For melon, a total of 27 representative accessions have been selected for HiFi sequencing, including 14 *C. melo* ssp. *melo* and 13 *C. melo* ssp. *agrestis* accessions, among which 13 from India/Pakistan, two from Turkey, three from Americas, and two from Africa, four from Central/West Asia, two from East Asia, and one from Europe. HiFi sequences have been generated for 22 of the 27 accessions, with an average depth of 33.7×.

For squash, three accessions, two from *Cucurbita pepo* ssp. *texana* (also known as ssp. *ovifera*) and one from *C. pepo* ssp. *pepo*, have been selected for HiFi sequencing. HiFi sequences of these three accessions have been generated. We have also generated HiFi sequences for *C. maxima* Rimu and *C. moschata* Rifu.

1.1.3. De novo genome assembly and pan-genome construction
We have finished the assembling of chromosome-scale genomes of the 25 cucumber accessions. The assembled genome sizes of the 25 accessions range from 259.0 Mb to 302.3 Mb (average: 287.43 Mb) and N50 contig sizes from 5.25 Mb to 22.98 Mb (average: 15.46 Mb). BUSCO completeness rate of these genome assemblies ranges from 96.4% to 98.8%, with an average of 98.4%. An average of 95.5% of the contigs (ranging from 90.3% to 97.8%) are assigned to the seven cucumber chromosomes. Protein-coding genes have been predicted in these genomes, as well as an additional of 11 previously published chromosome-scale cucumber genomes (seven cultivated, one Xishuangbanna and three wild *hardwickii*). The number of predicted genes ranges from 21,347 to 22551, with an average of 21,870. BUSCO completeness rate of genes predicted from each of these 36 cucumber genome assemblies ranges from 93.0% to 97.0%, with an average of 96.0%. Using the newly assembled WI7631 ('Chinese long') genome as the reference/backbone, large structural variants (SVs) have been called and for the other 24 assembled genomes and the 11 previously published genomes (**Table 2**). A graph pan-genome has been constructed using the WI7631 genome and the called SVs and used to Genotype these SVs in the core collection using the resequencing short reads.

For watermelon, we have finished chromosome-scale genome assemblies and gene prediction for all 135 accessions. The assembled genome sizes range from 368.6 Mb to 406.7 Mb (average: 377.5 Mb) and N50 sizes are all greater than 20 Mb (20.37-35.64 Mb; an average of 30.49 Mb). BUSCO completeness rate of these genome assemblies ranges from 93.9% to 99.2%, with an average of 99.0%. An average of 99.2% of the contigs (ranging from 96.2% to 99.9%) are assigned to the 11 watermelon chromosomes. The number of predicted protein-coding genes ranges from 20,834 to 23,330 (average: 21,785). BUSCO completeness rate of genes predicted from each of these 135 watermelon genome assemblies ranges from 91.6% to 96.6%, with an average of 95.9%. Using the newly assembled '97103' genome as the backbone, SVs are being called in the other 134 watermelon accessions, as well as three previously published long read assemblies (**Table 2**). The final SVs and the '97103' genome have been used to construct a *Citrullus* graph pan-genome, which has been used to genotype these SVs in the core collection and other accessions using the resequencing short reads (a total of 756 accessions, including 436 cultivars, 114 landraces, 13 *cordophanus*, 39 *mucosospermus*, 120 *amarus*, 33 *colocynthis* and 1 *rehmii*).

Table 2 Summary statistics of SVs identified in cucumber and watermelon across 36 and 138 genome assemblies, respectively.

SV size	Cucumber				Watermelon			
	Insertion		Deletion		Insertion		Deletion	
	Number	Total (bp)	Number	Total (bp)	Number	Total (bp)	Number	Total (bp)
20-50 bp	62,420	1,917,907	55,044	1,669,281	281,414	8,245,901	240,223	7,041,136
50-100 bp	24,113	1,658,511	20,318	1,398,601	62,573	4,254,586	55,405	3,789,248
100bp-1 kb	46,543	17,836,477	39,954	15,124,808	85,402	27,285,463	74,247	23,213,992
1-10 kb	36,341	130,392,254	27,822	99,874,704	63,348	222,301,442	36,663	118,733,794
>10 kb	8,278	192,479,460	7,156	171,750,044	10,959	304,306,832	1,492	20,001,261
Total	177,695	344,284,609	150,294	289,817,438	503,696	566,394,224	408,030	172,779,431

For melon, we have finished the chromosome-level assemblies of 22 accessions. The assembled genome sizes range from 355.7 Mb to 387.0 Mb (average: 371.7 Mb) and N50 contig sizes from 9.41 Mb to 19.60 Mb (average: 13.85 Mb). BUSCO completeness rate of these genome assemblies ranges from 93.7% to 97.9%, with an average of 97.3%. An average of 97.2% of the contigs (ranging from 92.4% to 99.5%) are assigned to the 12 melon chromosomes. Protein-coding genes have been predicted in 21 of the 22 assembled genomes, and the number of genes predicted in each genome ranges from 23,108 to 27,678 (average: 24,570). BUSCO completeness rate of genes predicted from each of these 21 melon genomes ranges from 95.5% to 97.6%, with an average of 96.6%.

For *Cucurbita* species, we have finished genome assemblies and gene predictions of three squash (*C. pepo*) accessions, and *C. maxima* Rimu and *C. moschata* Rifu (**Table 3**).

Table 3 Statistics of *Cucurbita* genome assemblies.

	***C. maxima* Rimu**	***C. moschata* Rifu**	***C. pepo* ssp. *texana* C31**	***C. pepo* ssp. *texana* C38**	***C. pepo* ssp. *pepo* C39**
Assembly size (bp)	350,631,597	311,872,014	349,507,311	351,024,241	378,453,046
Genome BUSCO completeness (%)	98.7	98.36	98.45	98.57	98.57
N50 (bp)	12,573,384	9,281,623	7,690,470	9,175,707	10,726,264
Chromosome size	333,304,442	294,885,981	331,481,260	325,708,589	361,045,115
Chromosome size %	95.06	94.55	94.84	92.79	95.40
No. genes	30,540	30,857	31,528	30,412	31,327
Gene BUSCO completeness (%)	98.76	98.51	97.77	97.83	97.89

1.1.4. Breeder-friendly web-based database for phenotypic, genotypic and QTL information

We have updated CuGenDB to version 2 (CuGenDBv2) and officially released CuGenDBv2 in April 2022. CuGenDBv2 currently hosts 34 reference genomes from 27 cucurbit species/subspecies belonging to 10 different genera. Protein-coding genes from all these 34 genomes (total: 919,903; average: 27,056) have been comprehensively annotated, and the annotated genes can be queried and extracted in the database. Genomic synteny blocks and syntenic gene pairs have been identified between any two and within each of the 34 cucurbit genome assemblies (595 pairwise genome comparisons). A total of 391,379 synteny blocks and 12,130,719 syntenic gene pairs (average: 31 per synteny block) have been identified between the 34 cucurbit genomes. The 'Synteny Viewer' module have been re-implemented in CuGenDBv2 to improve the efficiency in processing and displaying the large-scale synteny data.

A 'Genotype' module has been newly developed in CuGenDBv2. The module provides a suite of functions that allow users to mine, analyze, extract, and download variants including SNPs and small indels from large-scale population genome sequencing projects. Currently variants (SNPs and small indels) called for cucumber and squash core collections and watermelon resequencing panel, and SNPs called from the GBS data generated under CucCAP1 for watermelon, melon, cucumber, *C. pepo*, *C. maxima* and *C. moschata* are available in the database for query and mining.

The 'Expression' module in CuGenDBv2 has been redesigned to provide a complete cucurbit gene expression atlas, using the publicly available cucurbit RNA-Seq datasets. Currently raw RNA-Seq data of a total of 221 projects, 1,513 distinct samples and 3,560 runs (or libraries) have been downloaded from NCBI and processed to derive expression values, which can be queried in CuGenDBv2 to display expression profiles of specific interesting genes in different tissues, development stages, and under different treatment conditions.

Phenotype data have been generated for melon and cucumber core collections. A total of 33 vegetative, flower and fruit characters and two disease resistance traits have been evaluated for the melon core collection, and for the cucumber core collection a combination of 15 external and internal characteristics have been collected for immature and mature fruit of plants grown in 2019 and 2021. A tool to display the fruit images of cucumber core accessions has been developed (http://www.cucurbitgenomics.org/cgi-bin/core?pid=P04). Additional tools to visualize and analyze the phenotypic data will be developed in CuGenDBv2.

1.2 Perform seed multiplication and sequencing analysis of core collections of the four species, provide community resources for genome wide association studies (GWAS).

1.2.1. Seed multiplication of core collections

For cucumber, seed increases of the 388 accessions in the core collection were carried out by five participating seed companies. As of March 2024, seeds for 310 accessions with more than 1000 seeds per accession have been received.

For watermelon, HM.Clause is increasing the seeds for 293 accessions in the core collection given to them by USDA-ARS. HM.Clause have already shipped to the USDA, ARS, U.S. Vegetable Laboratory S3 seeds of 177 accessions (with about 1,000 seed/accession) and will ship during 2024 the S3 seeds of the other 116 accessions they committed to increase. S2 seed of additional 39 accessions will be sent by University of Georgia to HM.CLAUSE for increase. During 2024, S2 seeds of additional 167 PIs (mainly Citrullus amarus) will be increased at the USDA, ARS, U.S. Vegetable Laboratory to reach 500 S3 seeds per accession.

Three companies assisted in advancing the melon core set in 2023: 259 of the 384 melon core lines were sent to three seed company cooperators; seed was obtained from 180 of those lines. United Genetics advanced 13 S0 lines to S1 and three S1 lines to S2. Nunhems advanced 13 S0 lines to S1 (**Table 4**). Sakata advanced 151 S2 lines to S3, with seed counts per line ranging from 21 to 3,100, based on seed weight; only 57 lines produced 1,000 or more S3 seed (**Table 5**).

Table 4 Seed multiplication status of melon core

Company	Stage	No. lines
United Genetics	S0->S1	13
	S1->S2	3
Nunhems	S0->S1	13
Sakata	S2->S3	151

Table 5 Estimated number of seeds per S3 Melon core lines (based on seed weight) by Sakata

No. seeds	No. lines
< 100	14
100-199	9
200-299	7
300-399	14
400-499	10
500-599	11
600-699	5
700-799	4
800-899	11
900-999	9
> 1,000	57
Total	151

For the *C. pepo* squash core increase, we expect to receive the last of the seed this summer. All of the squash core will be increased by a professional nursery, Villa Plants and have robust phytosanitary documentation. One line may have some IP restrictions and may be dropped from the core.

1.2.2. Population genetics and phenotype-genotype association analysis

Phylogenies of accessions in the cucumber, melon, squash, and watermelon cores have been inferred using the LD-pruned SNPs at four-fold degenerate sites. The phylogenies of cucumber and melon core accessions are largely consistent with their geographic origins and the phylogeny of watermelon accessions is consistent with their species classifications, while no clear separations were observed for squash accessions related to their geographic origins or improvement status.

Phenotype-genotype association analysis has been performed for the cucumber core. The cucumber core accessions were grown in the field at the Michigan State University Horticulture Teaching and Research Center in 2019-2022. Young and mature fruits were harvested at ~5-7 and 30-40 days post pollination, respectively. The following traits were measured for mature fruit: fruit length, diameter, fruit shape index, carpel number, seed cavity, flesh thickness, hollowness, curvature, tapering, skin color, flesh color and netting; and the following for young fruit: fruit shape index, curvature, tapering, skin color, and spine density. Genome-wide association studies (GWAS) were performed on these fruit traits using different models including FarmCPU, BLINK, MLMM, and MLM (**Fig. 1**). Chromosomal locations of the detected significantly associated SNPs are illustrated in **Fig. 2**. QTLs for some of the traits were closely clustered. For example, SNPs for several highly correlated fruit size and shape traits, including mature fruit length, young fruit shape index, carpel number, and seed cavity size, were closely located on chromosome 1 at ~10 Mb. Multiple external fruit traits were also mapped to the same region on chromosome 1, such as netting, spine density, young fruit color R/G values. Several significant SNPs identified by GWAS were also in close vicinity (within 1Mb) to prior identified fruit trait QTL and candidate genes.

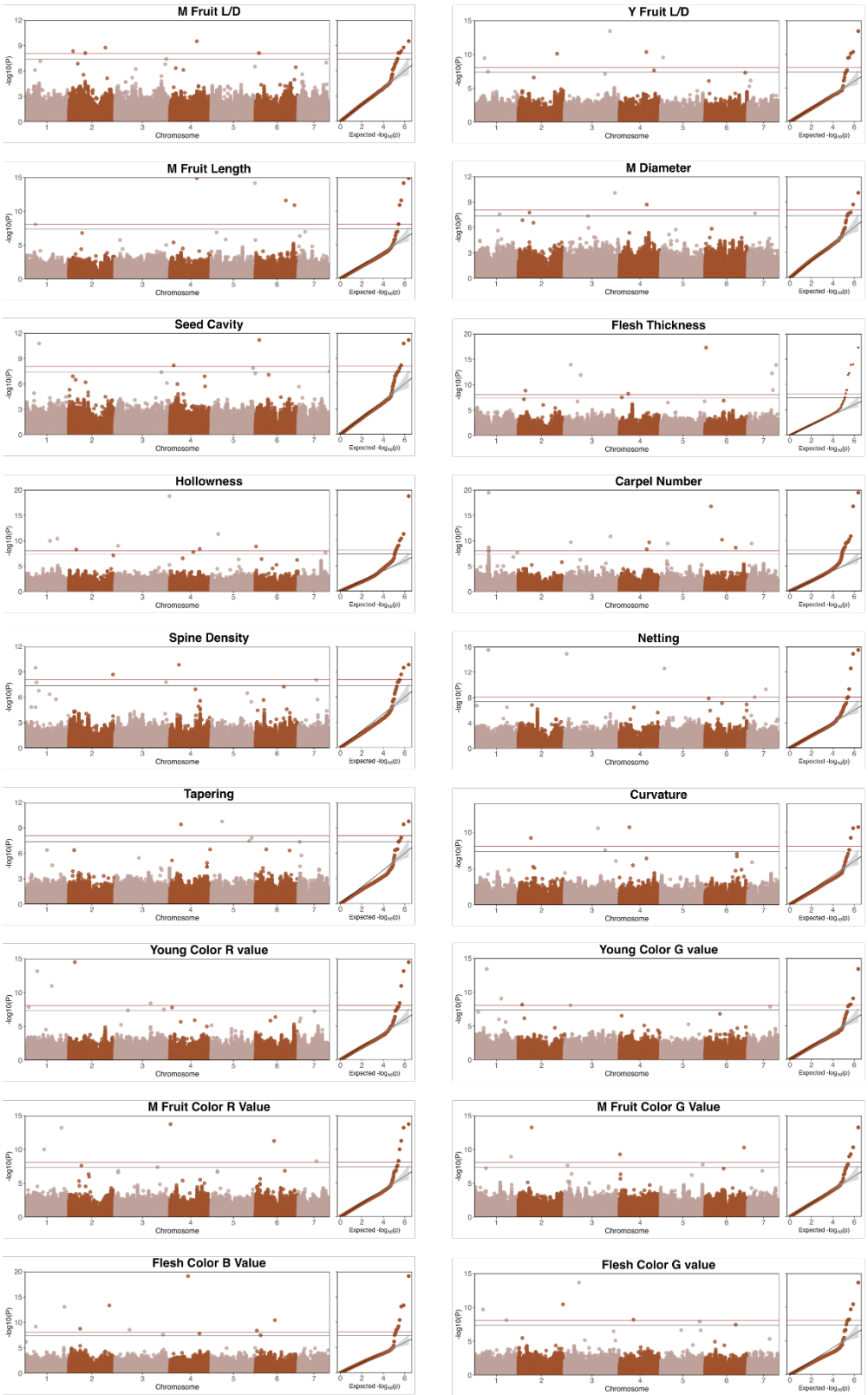

Fig. 1. Manhattan and quantile-quantile (QQ) plot of GWAS analysis of the morphological traits observed in the cucumber core collection using the FarmCPU model. The gray and red horizontal lines represent Bonferroni corrected p-values of 0.05 and 0.01, respectively.

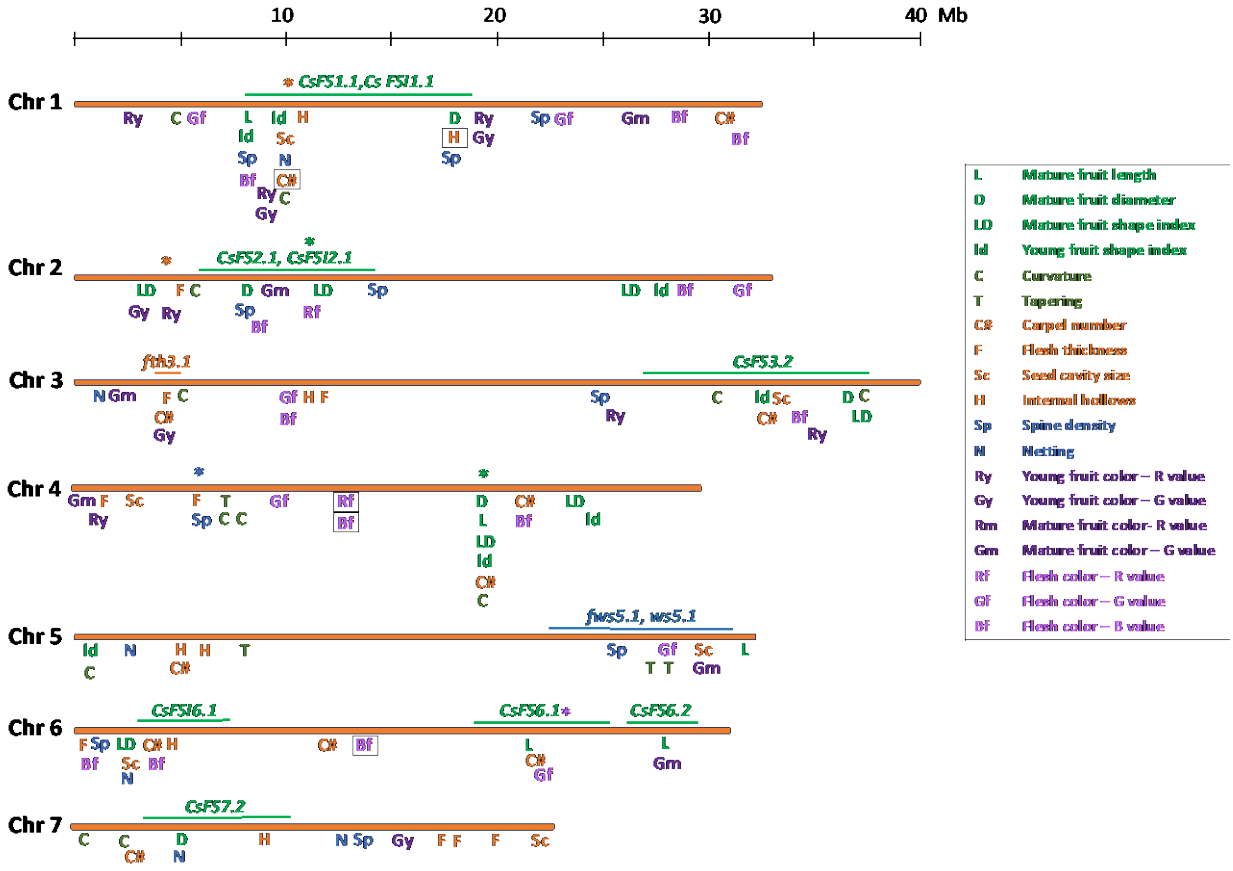

Fig. 2. Chromosomal locations of significant SNPs identified by GWAS analysis (FarmCPU) for the morphological traits measured in the cucumber core collection. Chromosomes are indicated by brown bars. SNPs identified in this study are located beneath the chromosomes. SNPs explaining >10% variance are boxed. QTLs and asterisks above the chromosomes indicate consensus QTLs and candidate genes from the literature. Asterisks above the chromosomes indicate proposed candidate genes identified from this study.

Watermelon Team

Team members:

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Cecilia McGregor (University of Georgia)
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Cecilia McGregor -UGA

1.2.1 Seed multiplication of core collections

Thirty-nine S_2 accessions (31 *C. amarus*, 7 *C. lanatus and 1 C. mucosospermus*) have been sequenced and seed are ready to be sent foe increase (waiting for LOU to be executed).

2.1 Map resistances and identify QTL for key cucurbit diseases

The WPop GSB1 (PI 482276 x Crimson Sweet) $F_{2:3}$ population used for identification of Qgsb5.1 (syn. *ClGSB5.1*; Gimode et al., 2020) and Qgsb7.1 (syn. *ClGSB7.1*; Gimode et al., 2020) is being advanced to a RIL population. This RIL population will be used to identify QTL associated with stem resistance to *S. citrulli*.

F_2 population WPop GSB3 from a cross between Crimson Sweet and PI 482379 (resistant) has been phenotyped. Khufu (Korani et al., 2021) will be used to identify QTL associated with resistance in this population. PI 482379 has not been previously used for QTL mapping or breeding.

2.2. Develop and verify markers for MAS

We previously developed and/or validated KASP marker assays for selection of *Qgsb5.1* (syn. *ClGSB5.1*; Gimode et al., 2020), and *Qgsb7.1* (syn. *ClGSB7.1*; Gimode et al., 2020). These QTL are currently being introgressed into Crimson Sweet. We evaluated 8 BC_2F_3 introgression lines as well as 6 genotypes obtained from North Carolina State University (Rivera-Burgos et al., 2021) in the field. Sugar Baby, Crimson Sweet and Fiesta were included as susceptible controls and UGA11 (selection from PI 482379), UGA81 (selection from PI 189225) and UGA 1081 (selection from PI 482276) were included as resistant controls. Plants were artificially inoculated with an *S. citrulli* isolate and leaf surfaces were kept wet using a mist system.

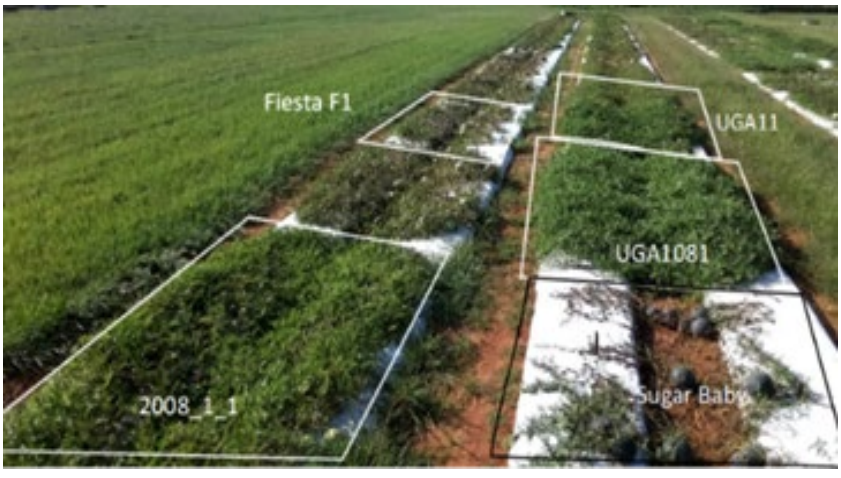
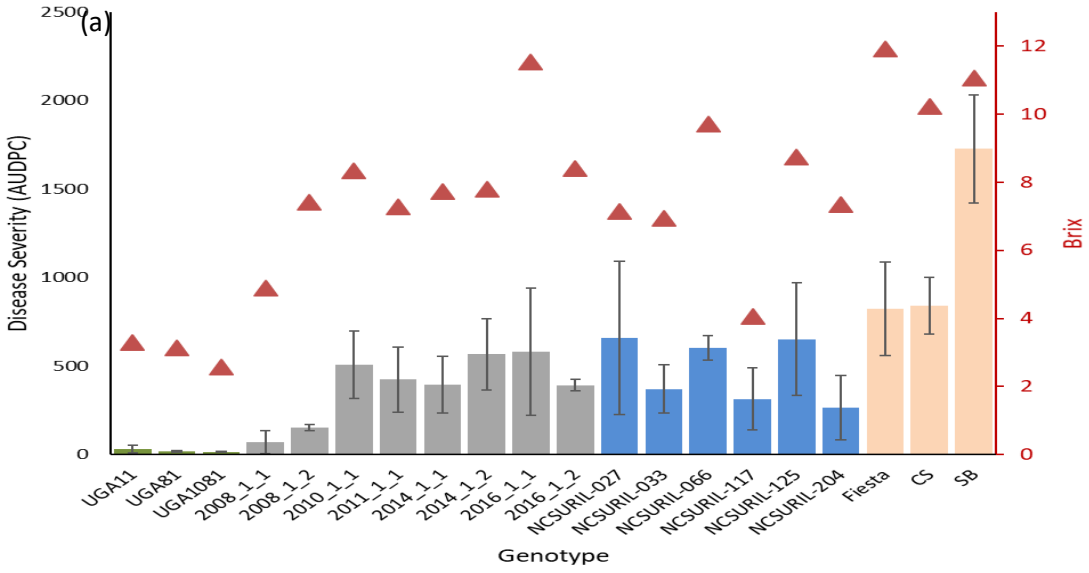


Fig.1 (a) Disease severity and Brix of introgression lines (grey) and genotypes form NCSU (blue) inoculated with *S. citrulli* in the field in summer 2023. Red triangles are average Brix for each line. (b) Disease severity of introgression line 2008_1_1 compared to controls.



Introgression lines 2008_1_1 and 2008_1_2 and NCSU-RIL-117 and NCSU-RIL-204 had significantly lower gummy stem blight disease severity (AUDPC) than susceptible controls. The average brix of these lines was 4.9, 7.4, 4.1 and 7.3, respectively. The BC_3F_3 for lines 2008_1_1 and 2008_1_2 are complete and will the evaluated in the field in 2024.

References

Gimode, W., K. Bao, Z. Fei and C. McGregor (2021) QTL Associated with Gummy Stem Blight Resistance in Watermelon. Theor Appl Genet 134:573–584.

Korani W, O'Connor D, Chu Y, Chavarro C, Ballen C, Guo B, Ozias-Akins P, Wright G, Clevenger J (2021) De novo QTL-seq Identifies Loci Linked to Blanchability in Peanut (Arachis hypogaea) and Refines Previously Identified QTL with Low Coverage Sequence. Agronomy 11:2201

Rivera-Burgos, L.A., E. Silverman, N. Sari and T.C. Wehner. (2021) Evaluation of Resistance to Gummy Stem Blight in a Population of Recombinant Inbred Lines of Watermelon × Citron. HortScience 1:1–9.

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Obj 2. Map and develop markers for disease resistance

2.1: Developing populations (P), phenotyping (Ph), QTL mapping (Q), Fine mapping (F)
-*Fon* race 2, Ph/Q

- Completed QTL mapping in the USVL246-FR2xUSVL114 RIL population and narrowed the QTL intervals found in the F2:3 population from the same cross. Developed KASP markers for four QTL and validated them in an independent interspecific (USVL246x'Sugar Baby') population. Manuscript accepted with revision in *Theoretical and Applied Genetics*.

- Completed disease screening (two replicated tests) of the *C. amarus* core collection for response to inoculation with *Fon* race 2 and used the phenotypes for GWAS and genomic predictions. Ganaparthi et al. 2023 *Plant Disease*.

-Downy mildew, Ph/Q

Completed disease screening (two replicated tests) of the *C. amarus* core collection for DM resistance and used the phenotypes for GWAS. Katuuramu et al. 2022.

2.2: Develop marker (M), verify (V)

-Fon race 2, M/V

- KASP markers for Fon race 2 resistance were developed in the C. amarus 246x114 RIL population. An F2:3 interspecific population of USVL246-FR2 by 'Sugar Baby' was evaluated for response to inoculation with Fon race 2 in two replicated tests and the phenotypes used for QTL mapping with the KASP markers. Manuscript accepted with revision in *Theoretical and Applied Genetics*.

-Powdery mildew race 2w, M/V

- XP-GWAS of powdery mildew race 2 resistance was completed for the USDA Citrullus core collection using historical data. KASP markers were designed for three regions of the genome with a significant signal. They were validated in two hundred accessions from the extremes of the distribution. Manuscript in preparation.

Obj. 3A. Introgress, pyramid/stack resistances into advanced breeding lines

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

-*Fon* races 1 and 2, VG/AB/PW/AL/SB

- Phenotypic and marker-based selections for Fon race 2 resistance were made from the interspecific population of USVL246-FR2 by 'Sugar Baby' and selfed and backcrossed to 'Sugar Baby'. We are currently making seed for the F5 and BC2F2 generations from this cross. We have also crossed USVL246 to 'All Sweet' and 'Crimson Sweet' to begin introgression into a variety of elite backgrounds (Table 1).

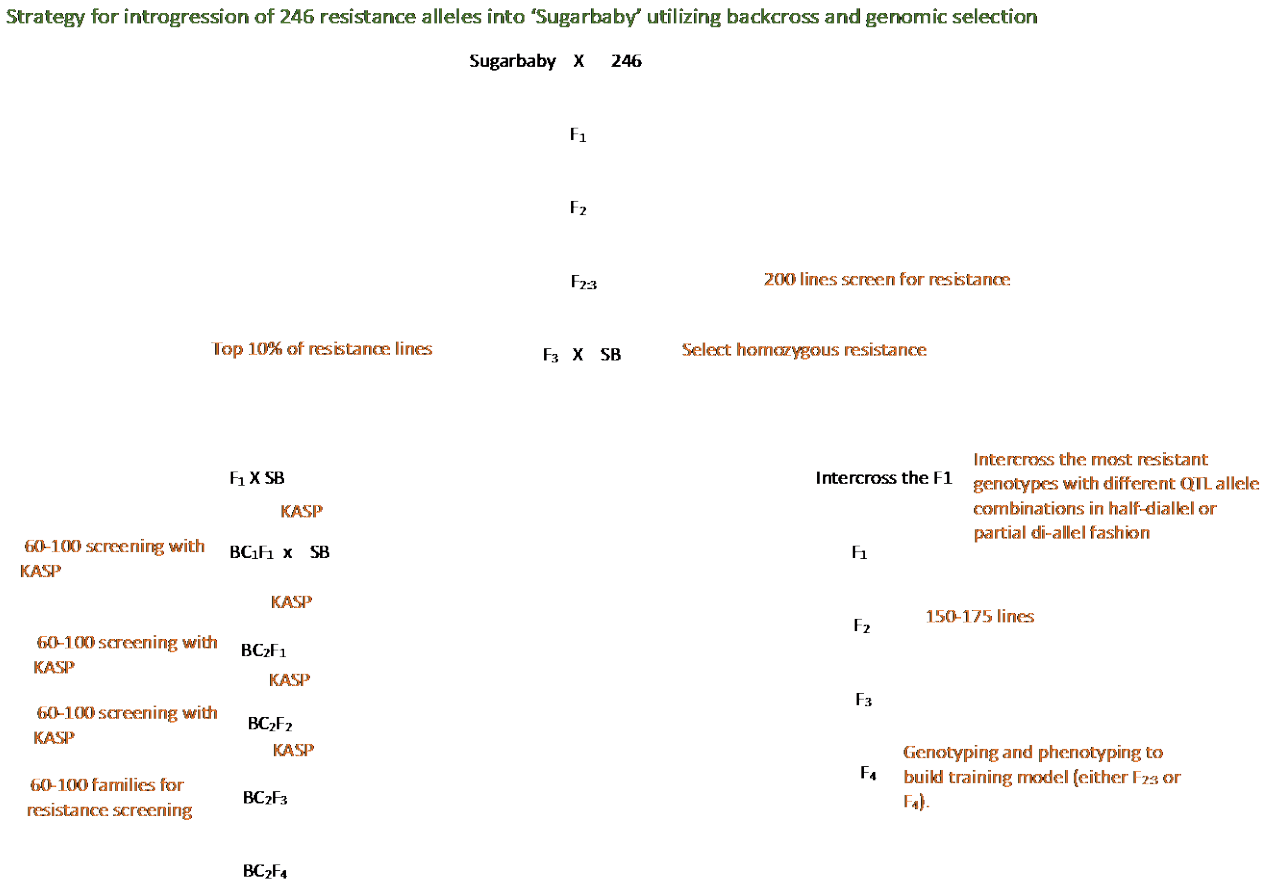

Table 1. Progress of introgression of *Fon* race 2 resistance into elite breeding lines

Crop	Current seed being generated	Number of plants (selections)
watermelon	F6 (Charleston Gray x 246)	5
watermelon	F5 (Sugarbaby x 246)	5
watermelon	F2 (Crimson Sweet x 246)	5
watermelon	F2 (All Sweet x 246)	5
watermelon	BC1F2 & BC2F1 (Charleston Gray x 246) x Charleston Gray	5
watermelon	BC2F1 (Sugarbaby x 246) x Sugarbaby	5

Figure 1. A BC1F1 genetic population (Charleston Gray) Charleston Gray] showing resistance to Fusarium wilt race 2.

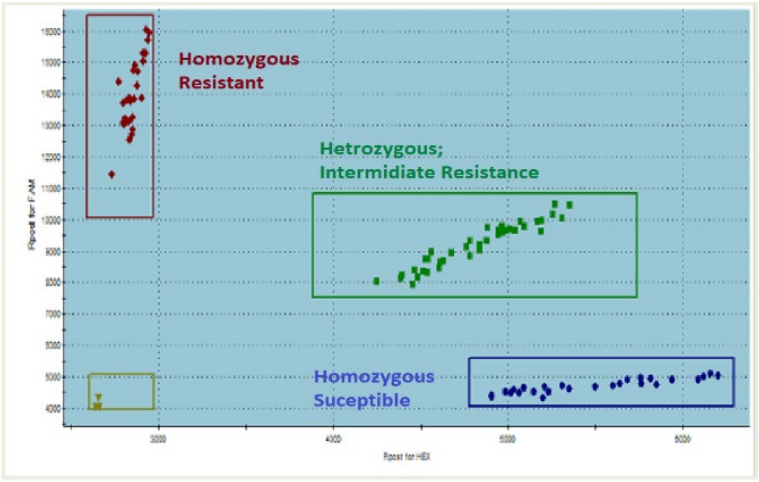

Figure 2. A KASP marker for a QTL associated with Fusarium wilt race 2-resistance. Dual color scatter plot of a KASP marker differentiating genotypes into three clear clusters.

<u>Obj. 3B. Using 'genomic selection' approach to incorporate Fusarium wilt race 2 resistance into watermelon cultivars</u> AB/VG/PW/AL/SB

Constructing and utilizing training populations for 'genomic selection' experiments

USVL246 x USVL114 (RIL), USVL252 x USVL119 (F3)

USVL246 x Sugar Baby (F3), USVL 252 x Sugar Baby (F3; F4; BC1;F2;F3)

Fig. 3. Genomic selection experiments for Fusarium wilt race 2-resistance and fruit quality traits at the USDA, ARS, U.S. Vegetable Laboratory and Clemson-Costal Research and Education Center (CREC), Charleston, SC (Summer 2023).

Seven genomic selection (GS) models were evaluated. G-BLUP and Random Forest performed best, achieving prediction accuracies. Genomic estimated breeding values (GEBV) effectively identified superior families at different selection intensities and captured all *Fon* race 2-associated QTLs at 30% intensity.

Developing small-seed lines with multiple disease resistance (FW races 1, 2 and Potyviruses, including ZYMV and PRSV) (BC6F1 and BC5F2 lines).

Brix =10.8 (SD = 0.71)

Cooperative Research and Development Agreement (CRADA)-Partnerships for constructing MAGIC Populations and Speed the Use of CucCAP Germplasm and Genomic Data by Seed Companies (Users)

CRADA was established and signed between USDA-ARS and BASF/Nunhems

Additional CRADAs for constructing the MAGIC populations are underway with Origene Seeds, Enza Zaden, Rijk Zwaan, Takii Seeds, and Sakata Seeds.

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USDA, ARS, U.S. Vegetable Laboratory

Cucurbits project summary report (2023- 2024). Watermelon resistance to CGMMV

Cucumber green mottle mosaic virus (CGMMV) is an emerging tobamovirus in North America. The control of this virus through breeding for natural resistance requires the identification of a new source of genetic resistance. In screening of the USDA watermelon germplasm, we have identified a source of resistance to CGMMV in a wild watermelon relative (*Citrullus colocynthis* L.). A segregating population of F2 libraries was generated through a cross between resistance (USVL#157) and susceptible (USVL#138) *C. colocynthis* lines. Phenotypic analysis through mechanical inoculation of the F2 population revealed a genetic segregation, suggest the existence of two gene model controlling the CGMMV resistance. Bulked segregant analysis (BSA) analysis was conducted by bulking two extreme phenotypes of the F2 generation as an effort to identify the SNPs associated with CGMMV resistance. A total of four SNPs and several candidate genes have been identified to be associated with CGMMV resistance in watermelon. These SNPs are now being validated by using the genome of the *C. colocynthis* line PI 537300. The mapping percentage ranged from 68% to 98% when mapped to the PI 537300 genome and 80% - 85% for the reference genome 'Charleston gray'. The identified SNPs spanned the region at chromosome 3, 5, 6 and 10. Upon validation using the DEEPBSA tool, the SNP at chromosome 10 dropped below threshold. Therefore, to confirm our results we mapped it to a second genome, PI 537300. The mapping quality was improved. The BSA sequence analysis revealed a total of 27 genes surrounding these identified SNP regions. Four of these genes were potential candidate genes based on the genomic position and functional annotation. These genes encoded for the U-box domain-containing protein, NB-ARC domain containing protein, Serine hydroxymethyltransferase and ABC transporter D family member, respectively.

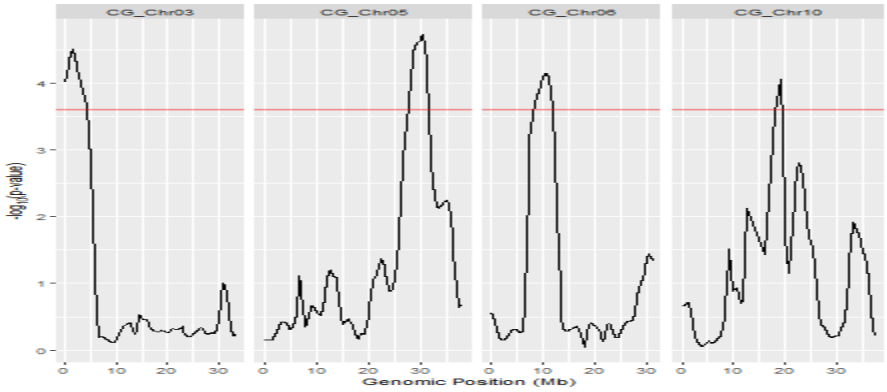

Figure 1. Genomic position of significant SNPs associated with resistance to the CGMMV pathogen in Watermelon.

Table 1 Summary of sequencing data

Samples ID	Reads generated	Mapping percentage to reference genome Charleston gray	Mapping percentage to the genome of line PI 537300
S157	112651760	80.05%	**98.73%**
S138	244145664	80.90%	**68.84%**
S0	109931089	85.49%	**98.32%**
S2	113195007	84.45%	**98.70%**

Table 2. Identified SNPs identified with Reference genome Charleston Gray associated with resistance to the CGMMV infection in Watermelon.

CHROM	QTL	start	end
CG_Chr03	1	27314	4343536
CG_Chr05	2	27669515	31552144
CG_Chr06	3	8372143	11716243
CG_Chr10	4	19244439	19244439

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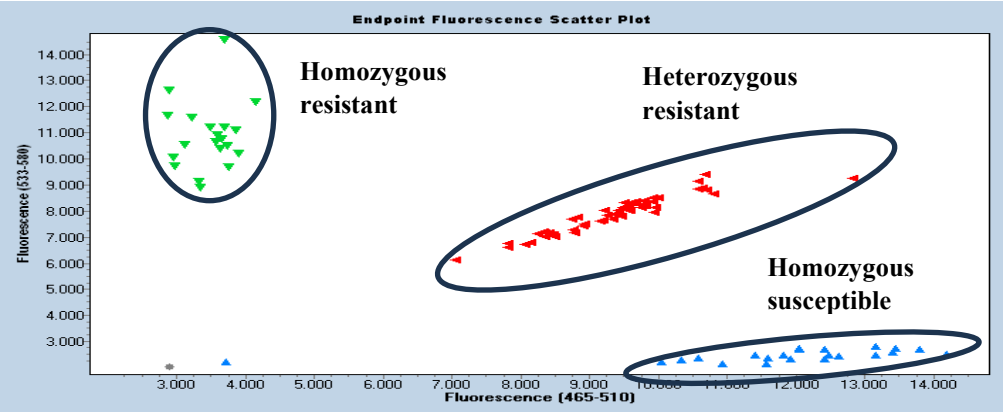
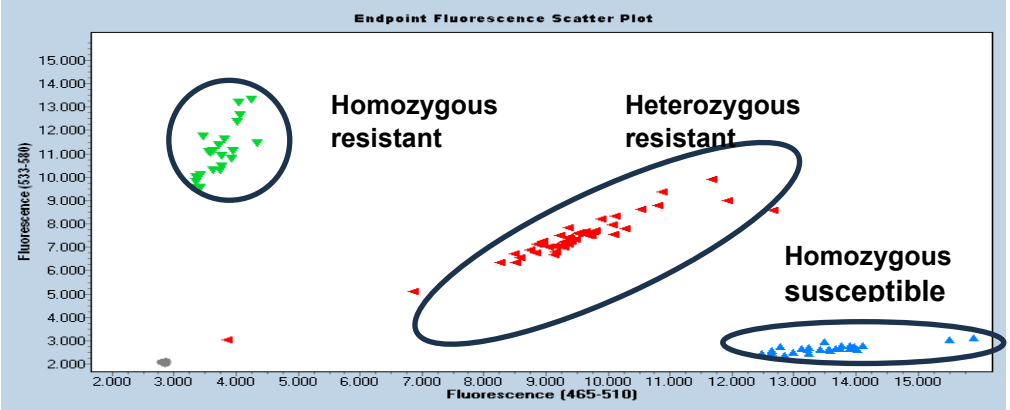
Powdery mildew of watermelon.

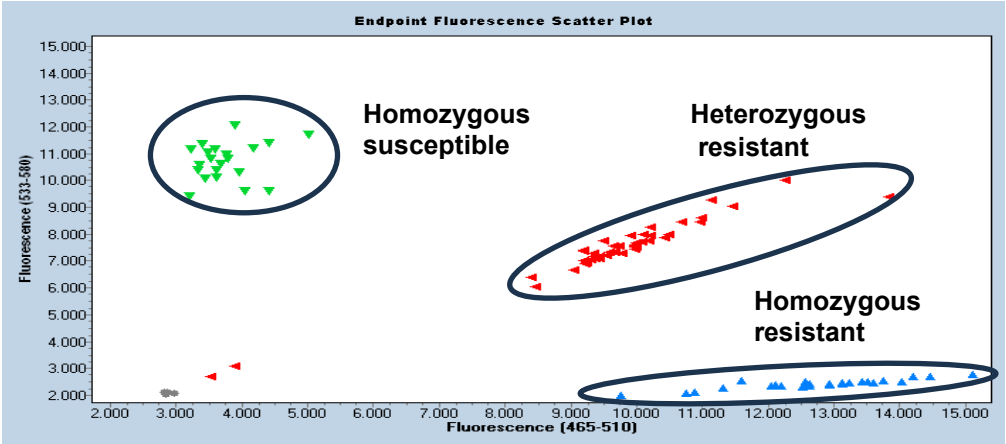
- Completed evaluation of commercial seedless varieties for resistance to powdery mildew in 2021 and 2022. Several resistant seedless watermelon lines were identified, and detailed results are presented in Extension section of the report in 2023.
- Publicly released USVL531-MDR. Manuscript was published in HortScience. The cover Page for the April 2023 HortScience issue displays resistance to Powdery mildew in USVL531-MDR compared to USVL677-PMS.
- A F_2 population **(**USVL608-PMR X USVL677-PMS) was used for the QTLs mapping using the bulk segregant analysis method and a genomic region was mapped on the chromosome 2 and KASP markers were designed to find linked markers.
- Three F_2 populations (USVL608-PMR X USVL677-PMS, USVL608-PMR X Sugar baby, USVL608-PMR X Dixie Lee) were phenotyped for resistance to powdery mildew in a growth chamber after inoculation. Data on powdery mildew development on each F_2 progeny was collected.
- KASP markers were used for the genotyping of F_2 progenies (96 for each population) and homozygous resistant progenies were selected.
- Different KASP Markers worked well with the different susceptible parent.
- The KASP markers were tightly linked with the Powdery mildew phenotypic rection.
- F_3 progenies were produced from the selected homozygous resistant plants for the confirmation linkage with markers and phenotype.
- All the F_3 progenies of three populations showed resistance to powdery mildew and genotyping of plants confirmed the presence of homozygous resistant alleles in all progenies.
- Advanced lines are being developed for the public release from each of the three crosses.
- Evaluated and collected data on powdery mildew development on RIL lines in the field (summer 2022, Summer and Fall 2023). Five plants of each RIL line were planted per plot and each RIL line had two replications. Data has been collected over three field season and is being analyzed.
- DNA was extracted from all the 187 RIL Lines. DNA bulk of powdery mildew resistant and susceptible lines were sent for sequencing to North Carolina State University Genomics Center. DNA of all the RIL Lines will be sequenced in collaboration with University of Ilinois.
- Advanced RIL lines with PM resistance and red flesh to develop useable resistant germplasm lines. The advanced RIL lines had the KASP marker based on the NBS-LRR gene in watermelon Chr02, *ClaPMR2* that is tightly linked with PM resistance.
- Publicly released USVL531-MDR. Manuscript submitted to HortScience has been accepted and was published in the April 2023 issue. The cover Page for the April 2023 issue displays resistance to Powdery mildew in USVL531-MDR compared to USVL677-PMS.



USVL608-PMR X Sugar baby F_2

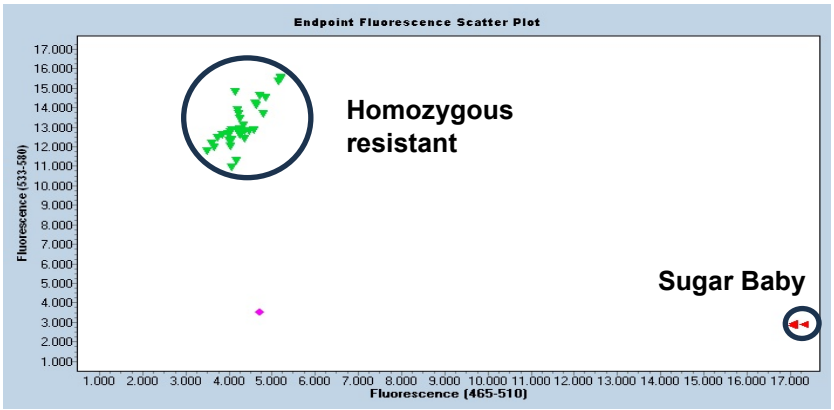


USVL608-PMR X Dixie Lee F_2

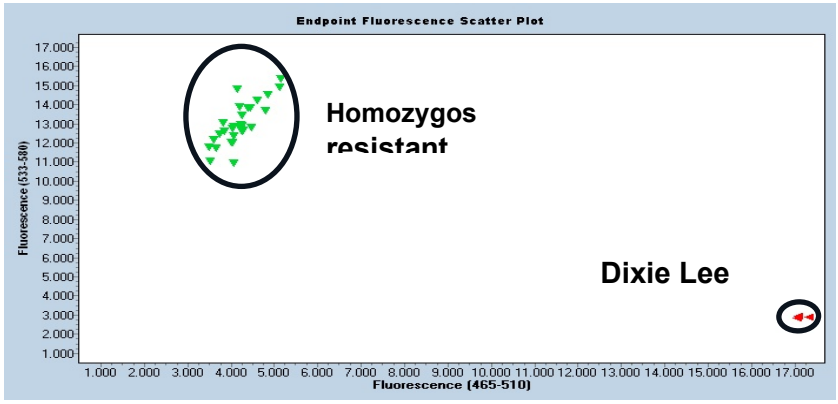


USVL608-PMR X USVL677-PMS F_2

Figure 1. Genotyping of three F_2 populations USVL608-PMR (resistant) X USVL677-PMS (susceptible), USVL608-PMR X Sugar baby (susceptible), USVL608-PMR X Dixie Lee (susceptible) using KASP markers. KASP markers were developed from one major QTL in watermelon Chr02. There was a common KASP marker for USVL608-PMR X Sugar baby, USVL608-PMR X Dixie Lee population and another marker for USVL608-PMR X USVL677-PMS population.



USVL608-PMR X Sugar Baby F3



USVL608-PMR X Dixie Lee F_3

Figure 2. Genotyping of three F_3 populations of USVL608-PMR X Sugar baby (susceptible), USVL608-PMR X Dixie Lee (susceptible) using KASP markers. Phenotyping and genotyping confirmed that all the progenies were homozygous resistant.

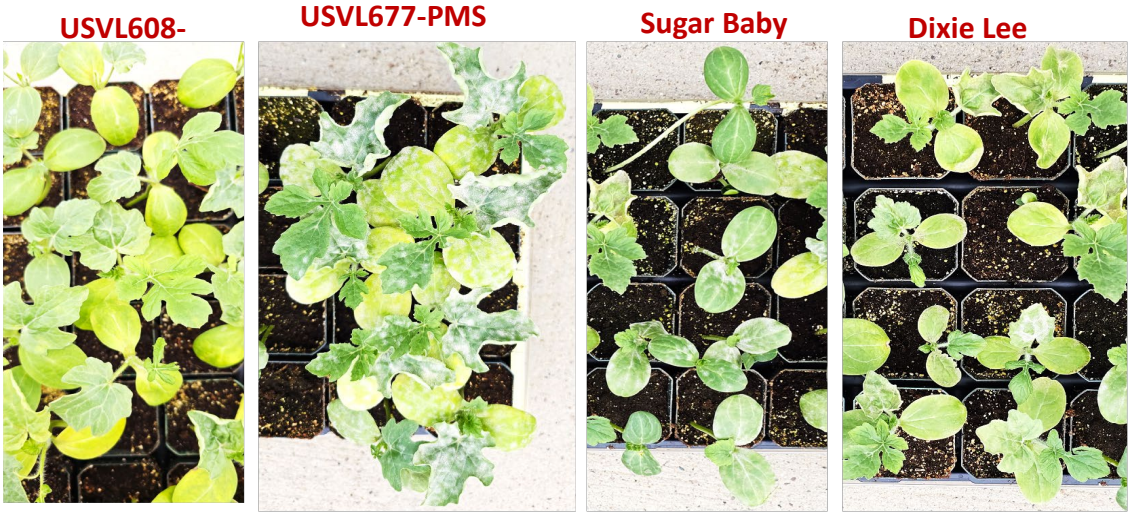


Figure 3. Phenotypic reaction of parents used in the crosses to develop powdery mildew resistant watermelon lines with high quality. The varieties Sugar Baby and Dixie Lee with high Brix and good fruit quality were crossed with USVL608-PMR to develop F_2 and F_3 lines.

Phytophthora fruit rot of watermelon

Figure 4. Phytophthora fruit rot resistant RIL Lines with red flesh selected from the cross of USVL531-MDR X USVL677-PMS based on evaluation in Fort Pierce, FL. June 2023. Fruit on left from USVL-677-PMS displays significant development of Phytophthora fruit rot compared to the fruit on right which is resistant.

- Three advanced lines (F_{11}) lines developed from the cross between USVL531-MDR (resistant) and USVL677-PMS (susceptible) were evaluated at in Fort Pierce, FL for phytophthora fruit rot resistance and horticultural traits (Brix value, flesh color, rind thickness, fruit color, and weight.
- One hundred eight seven advanced RIL lines were evaluated at the two different locations in Charleston for the phytophthora fruit rot resistance and horticultural traits during summer and fall of 2023.
- Fruits were harvested at maturity and envaulted for phytophthora fruit rot resistance under control conditions in a large walk-in-growth chamber.
- Fruits were evaluated for fruit color, rind thickness, flesh color, fruit firmness, taste (bitter or sweet), brix value, seed color, and seed type (egusi or non-egusi).
- Under field conditions, RILs were evaluated for leaf type, flowering date, and fruiting date.
- DNA was extracted from 187 advanced RIL lines for whole genome sequencing.
- Bulks were made from the phytophthora fruit rot and powdery mildew resistant and susceptible progenies for the QTLs mapping. Bulks have been sequenced and QTLs mapping analysis is being done.
- KASP markers will be developed for phytophthora fruit rot and powdery mildew resistance.
- Evaluated F_2 and $F_{2:3}$ population of USVL003-MDR (*Citrullus mucosospermus*) X Dixie Lee (*C. lanatus*, cultivated type with good horticultural traits). QTLseq analysis indicated significantly associated QTLs with Phytophthora fruit rot resistance in Chr04, Chr07 and Chr10.

- Advanced, red-fleshed resistant Phytophthora fruit rot resistant lines (USVL003-MDR x Dixie Lee) after screening and selection.

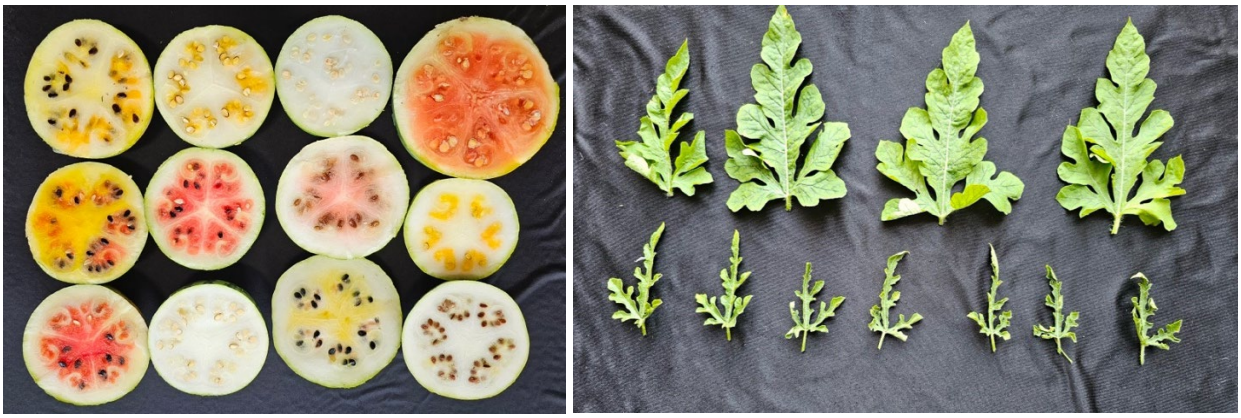


Figure 5. Extreme variability in fruit flesh color, seed type (Egusi or non-egusi), rind thickness, flesh firmness and fruit size, and leaf type were observed among the RIL lines (F_{11}) developed from the cross of USVL531-MDR and USVL677-PMS. Data has been collected on all these traits and is being analyzed along with the genotypic data. Geneotype data is being developed whole genome sequencing of all 187 RIL Lines.

Umesh Reddy, West Virginia State University

1. Brief progress report

Report: Whole genome re-sequencing and analysis of 161 diverse watermelon accessions

The watermelon whole genome re-sequencing project, conducted on 161 watermelon accessions, has produced a vast dataset of high-density genetic variants by employing state-of-the-art genomic techniques. Each accession was subjected to deep sequencing, achieving over 30x coverage, which ensures a comprehensive representation of the genomic diversity within the sample population. Leveraging high-throughput sequencing technologies, the study aimed to capture a broad spectrum of genetic variation by mapping sequenced reads to two reference genomes. This genomic insight is pivotal for genome-wide association studies (GWAS) and marker-assisted breeding programs.

Methodology

The experiment began with the germination of seeds in a controlled greenhouse environment, leading to the collection of leaf material that was snap-frozen in liquid nitrogen. This

immediate preservation is crucial to halt any enzymatic processes that could degrade the nucleic acids. Genomic DNA was subsequently extracted using the DNeasy Plant Pro kit (Qiagen, USA). Agarose gel electrophoresis and Qubit fluorimeter were used to assess the quality and quantity of the genomic DNA, respectively, ensuring that only high-quality genomic DNA proceeded to the library preparation stage for Illumina sequencing. The Illumina DNA sequencing libraries prepared from the genome DNA were sequenced on the NovaSeq platform (Illumina, USA), yielding a massive data trove of raw reads. For each accession, the depth of sequencing exceeded 30x coverage, far surpassing the standard required for accurate variant calling. The raw reads were subjected to stringent quality control before being aligned to two distinct reference genomes: Charleston Grey (*Citrullus lanatus*) and USVL246 (*Citrullus amarus*), using the BWA-MEM algorithm. SAMtools facilitated the subsequent sorting and indexing of reads, and duplicate reads were marked using Picard tools.

Variant calling was accomplished using the GATK Haplotype Caller, which is widely recognized for its robustness in detecting genomic variants. Following this, a series of quality filters were applied, tailored to exclude any variant not meeting the stringent quality parameters as follows: for SNPs, a Quality by Depth (QD) less than 2.0, Fisher Strand (FS) greater than 60.0, Mapping Quality (MQ) less than 40.0, MQRankSum less than -12.5, and ReadPosRankSum less than -8.0; for indels, a QD less than 2.0, FS greater than 200.0, and ReadPosRankSum less than -20.0. These thresholds are critical in ensuring that only the most reliable variants are carried forward in the analysis. Following the hard filtering step, the SNPs underwent further filtration to retain only biallelic forms. These biallelic SNPs were then subject to additional filtering based on minor allele frequency (MAF) of 0.05 and a call rate of 70%, which are criteria indicative of reliable and informative genetic markers for downstream analysis.

Results

The project's sequencing efforts resulted in a staggering number of raw reads (11.9 billion), specifically 11,970,459,092 in total for 161 accessions, which after quality filtering, retained an impressive total of 11,423,749,213 reads (as shown in Table 1). Remarkably, the percentage of these reads uniquely mapped to the reference genomes was more than 99% for both, indicating the high fidelity of the data and reflecting the high precision of the alignment process.

Table 1 presents a comprehensive summary of the sequencing and variant calling efforts. In the table, it is noteworthy that the USVL246 genome mapping revealed a greater number of variants compared to the Charleston Grey genome, which may indicate a broader genetic diversity within the *Citrullus amarus* species or reflect the genetic diversity of the accession set.

Table 1. Summary of watermelon re-sequencing, genome mapping and genomic variants.

Particulars	Mapping to Charleston Grey (lanatus) genome	Mapping to USVL246 (amarus) genome
Total number of samples	161	161
Total number of raw reads	11970459092	11970459092
Total number of quality filtered reads	11423749213	11423749213
Percentage of uniquely mapped reads	99.8	99.8
Total number of Variants	12991397	18585670
Total number of SNPs	10774296	15621573
Total number of INDELs	2217101	2964097
Total number of quality filtered SNPs	8455555	9512704
Total number of quality filtered INDELs	2104199	2746997
Total number of quality filtered biallelic SNPs	8249596	9134054
Total number of quality filtered biallelic SNPs with MAF 0.05 and Call rate 70%	47220	484314
ts/tv	2.05	2.06
ts	7390561	10990744
tv	3611101	5332990

The total number of variants identified was higher when aligned to the USVL246 genome, with 18,585,670 variants, compared to 12,991,397 for the Charleston Grey genome. Notably, the number of SNPs called from the USVL246 genome was approximately 4.7 million more than from the Charleston Grey genome. Similarly, indels identified in the USVL246 genome exceeded those in the Charleston Grey by nearly 750,000. Quality filtration of these variants resulted in 8,455,555 high-confidence SNPs for Charleston Grey and 9,512,704 for USVL246. The number of quality-filtered indels was 2,104,199 for Charleston Grey and 2,746,997 for USVL246. Post filtration, 8,249,596 biallelic SNPs for Charleston Grey and 9,134,054 for USVL246 were retained. When considering biallelic SNPs that met the stringent MAF and call rate thresholds, 47,220 were identified in the Charleston Grey genome and an impressive 484,314 in the USVL246 genome.

The transition/transversion ratio (ts/tv), an indicator of the genetic variation quality, was marginally higher in the USVL246 mapping (2.06) compared to the Charleston Grey genome (2.05). The number of transitions (ts) stood at 7,390,561 for Charleston Grey and significantly increased to 10,990,744 for USVL246. Conversely, the number of transversions (tv) was also higher in the USVL246 genome, with 5,332,990 compared to 3,611,101 for Charleston Grey.

Future Directions

The variants identified through this comprehensive and high-coverage approach will be further utilized for genome-wide association studies (GWAS) in conjunction with various

phenotypic data. This will enable the investigation of the genetic basis of important traits in watermelon, facilitating the identification of candidate genes and markers for breeding programs.

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Developing a Multi-Parent Advanced Generation Intercross (MAGIC) Population Useful for Enhancing the Watermelon Germplasm and for identification of gene loci associated with Disease Resistance

Watermelon CucCAP2 Team in Collaboration with Seed Companies

Amnon Levi, Shaker Kousik, Cecilia McGregor, Sandra Branham, Patrick Wechter, Zhangjun Fei, Umesh Reddy, and Kai-shu Ling

Table 1. MAGIC Population-Founder Lines (United States Plant Introduction; PIs and USVL lines) with disease, potyvirus (PRSV, ZYMV, SqVYV) or root-knot nematode resistance.
R-resistant (tolerant), MR-moderate resistance (tolerance) S-susceptible

Accession	*Citrullus* Sp.	FW R1	FW R2	GSB	PM	Phyto-phtora	PRSV	ZYMV	SqVYV	BFB	Root-knot Nematode	Fruit/Seed Quality
UVL246	CA	R	R	MR						MR	R	
UVL252	CA	R	R								R	
PI 244019	CA						R	R			R	
UGA1081	CC			R							R	
UVL531	CM				R	R						
PI 392291	CL								MR			
PI 595203	CM				R		R	R				
PI 189225	CA			R							R	
PI 279461	CL			R								
PI 269677	CL			S								
NHM	CL											
Jenny	CL											Micro seeds
Sugar Baby	CL											small globular
Calhoun Gray	CL	R										Elongated
Jubilee	CL											Elongated
Hungarian Line	CL											Orange Flesh
Mickeylee	CL											Globular fruit
Klondike Black Seeded	CL											
Dixie Lee	CL											
Crimson Sweet	CL											High quality/brix

Two MAGIC populations are under construction

MAGIC populations are at F2 stage and will be continued to F8/F9 generations in collaboration with seed companies, with the objective to have 500 F8/F9 RIL lines for each population.

MAGIC-1 (22005) Pedigree

21171 x 21159

21058-1♂ x 21122-1♀ 21171
X
21061-2♂ x 21015-1♀ 21159

[USVL 531 x PI 269677]-2♂ x [Crimson Sweet x UGA 1081]-5♀ 21058
X
[USVL 246 x Sugar Baby]-6♂ x [Calhoun Gray x PI 595203]-4♀ 21122

[Hungarian x USVL252]-4♂ x [PI 392291 x Mickylee]-3♀ 21061
X
[Klondike Black Seeded x NH Midget]-3♂ x [Dixie Lee x PI 244019]-1♀ 21015

MAGIC-2 (22002) Pedigree

21154 x 21168

21090-1♂ x 21086-3♀ 21154
X

21056-3♂ x 21047-1♀ 21168

[PI 392291 x Mickylee]-3♂ x [Crimson Sweet x PI 244019]-3♀ 21090
X
[NH Midget x Calhoun Gray]-1♂ x [Hungarian x USVL252]-3♀ 21086

[USVL 246 x Sugar Baby]-2♂ x [USVL 531 x PI 269677]-1♀ 21056
X
[Jenny x PI 595203]-1♂ x [PI 189225 x PI 279461]-1♀ 21047

Collaboration With Seed Companies in Developing the MAGIC Populations for Watermelon

Cooperative research and development agreement (CRADA)-Partnerships to speed transfer of germplasm and technology to Users (Seed Companies)

Cooperative Research and Development Agreement (CRADA) was signed between USDA, ARS and BASF- Nunhems (December 2023).

Additional CRADAs are underway with Origene Seeds, Enza Zaden, Rijk Zwaan, Takii Seeds, and Sakata Seeds.

Melon Team

Team members:
Jim McCreight (USDA, ARS), Shaker Kousik (USDA, ARS), Pat Wechter (USDA, ARS), Bill Wintermantel (USDA, ARS)

CucCAP Affiliated Postdocs and Graduate Students
Shaonpius Mondal, USDA-ARS, Salinas (McCreight, Wintermantel)
Prabin Tamang, postdoc, USDA-ARS, Salinas (McCreight, Wintermantel)

Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances
2.1.2. Melon
-Powdery mildew, Ph, Q, F

- The F2:3 PI 313970 x Top Mark developed in CA will be planted in a greenhouse at Charleston in November to evaluate for resistance to powdery mildew.
- Completed QTL mapping in the MR-1xAY RIL population and narrowed the QTL intervals by adding KASP markers to the GBS data. Developed KASP markers for two major QTL and validated them in a set of unrelated cultivars. Branham et al. 2021.

-Fusarium, Ph, Q, F

- Completed QTL mapping in the MR-1xAY RIL population and narrowed the QTL intervals by adding KASP markers to the GBS data. Developed KASP markers for the single major QTL for races 1 and 2 and validated them in a set of unrelated cultivars. population. Branham et al. 2018.

-Downy mildew, Ph, Q, F

- Completed QTL mapping in the MR-1xAY RIL population and narrowed the QTL intervals by adding KASP markers to the GBS data. Developed KASP markers for the major QTL. Toporek et al. 2023.

- CYSDV: Two QTL for resistance in were found in F2:3 PI 313970 x Top Mark, on chromosomes 3 and 5. The QTL on chromosome 5 was observed in naturally infected field tests in 2018 and 2019, and explained 16 % and 35 % of the variation in CYSDV titer, respectively. The QTL on Chromosome 3 explained 20 % of virus titer variation in 2018 but was undetected in 2019 (Tamang et al. 2021). Single gene recessive, Mendelian resistance was previously reported in PI 313970 (McCreight and Wintermantel, 2011). One or both of the two markers flanking the gene on Chromosome 5 were present in six of 10 other putative CYSDV resistance sources. Eight F2:3 lines with low virus titer resembled PI 313970 for the two flanking markers, which can, therefore, be utilized in marker assisted breeding of CYSDV-resistant melons.

One of the eight F2:3 lines with low virus titer in 2019 has been evaluated to date for resistance reaction in a controlled inoculation, growth chamber test. Plants will be selfed and backcrossed with CYSDV-susceptible ‘Top Mark’. The other seven F2:3 families are in preparation for screening. Testing was initially delayed due to a permit modification issue, but is now in

progress. QTL mapping of these lines will be evaluated concurrently with evaluation of resistance reactions.

New virus threat to Cucurbit production: It should be noted that during the spring and fall of 2023, changes were observed in the prevalence of CCYV during the spring melon season in the lower desert region of Arizona. CCYV has been the dominant yellowing virus in spring melons since its establishment in the desert, whereas CYSDV has been the dominant yellowing virus during the fall season (Mondal et al., 2023). In contrast to recent years, CYSDV dominated spring yellowing of melons grown in the Yuma region in 2023, whereas the adjacent Imperial Valley production region had a more typical pattern, with infections dominated by CCYV. This was followed by the identification of a new virus, watermelon chlorotic stunt virus (WmCSV) in fall 2023 in the Yuma production region as well as in adjacent Imperial Valley, CA. Virtually all fall melon plants infected with WmCSV were co-infected with CYSDV, but again, very few plants were infected with CCYV. These changing dynamics in desert production regions warrant further monitoring to determine impact of WmCSV on virus epidemiology, and how this may influence the need for advancement of resistance to this new virus. WmCSV is known to infect all cucurbits, and is particularly severe on watermelon. A source of resistance has been identified in watermelon (Ali-Shtayeh et al., 2024) and advancement of this material into watermelon germplasm may be warranted. No resistance to WmCSV is known in other cucurbit crops.

2.2 Marker development and verification
2.2.2. Melon
-Powdery mildew, M, V

- KASP markers for powdery mildew race 1 resistance were developed in the MR-1xAY RIL population and were validated with a set of commercial cultivars.

-Fusarium, M, V

- KASP markers for Fusarium wilt races 1 and 2 resistance were developed in the MR-1xAY RIL population and were validated with a set of commercial cultivars.

-Downy mildew, M

- KASP markers for downy mildew mating groups 1 and 2 resistance were developed in the MR-1xAY RIL population. Toporek et al. 2020 and 2023

- CYSDV: see 2.1.2 above

Obj. 3. Introgress, pyramid/stack resistances into advanced breeding lines
3.2. Melon
- powdery mildew: see 2.1.2 above
Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R)
-Fusarium wilt races 1 and 2/Powdery mildew race 1, B,I,A
Over the past 12 months we have continued with selections of more advanced lines from an initial four-way cross of MR-1 (resistant to Powdery mildew race 1, Fusarium oxysporum f. sp. melonis race 1 and race 2 and sulfur) x Top Mark x Charentais (Figure 1). Selections have been made following two breeding paths, one as backcrossed into Top Mark and on as backcrossed into Charentais. Selections have been made using a combination of KASP markers and

phenotyping. We currently have BC2F1 possessing resistance alleles in the heterozygous state for all traits. Additionally, we have just finished generating BC2F2 seed. The BC2F1 individuals were also selected for horticultural traits such as fruit shape, flesh color, brix, flesh texture and netting. We have also made selections from the Charentais breeding path for autonomous selfing and growth habits in Controlled Environment Agricultural (CEA), hydroponic-based systems. Current generations of all families (Table 1) will be evaluated for horticultural traits in replicated field trials summer 2024.

- CYSDV: see 2.1.2 above

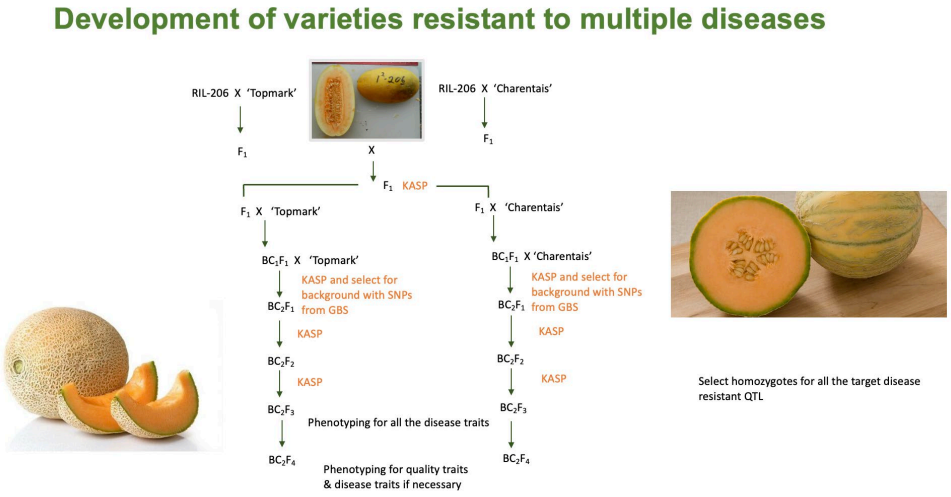

Table 1. Breeding progress for development of multi-disease resistant cantaloupe

Crop	Current seed being generated	Number of families (selections)
Melon	BC2F4 (Charentais x RIL-206)	60
Melon	BC2F4 (Topmark x RIL-206)	8
Melon	BC1F5 (Charentais x RIL-206)	5
Melon	F_1 [BC2F4 (Charentais x RIL-206) x BC2F4 (Topmark x RIL-206)]	5

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Toporek S.M., S.E. Branham, A.P. Keinath, and W.P. Wechter. 2023. QTL Mapping of Resistance to Pseudoperonospora cubensis Clade 2, Mating Type A1, in Cucumis melo and Dual-Clade Marker Development. Theoretical and Applied Genetics. Accepted.

Cucumber Team

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Objectives and timeline

Objectives	Personnel	Timeline			
		2021	2022	2023	2024
Obj. 1. Develop genomic, bioinformatic, mapping approaches and tools for cucurbits (ZF, BTI)					
1.1. Genomic and bioinformatics					
Provide seeds of core collection for re-sequencing, pan-genome analysis					
***1.2.* Seed multiplication of core populations - cucumber**					
PI line purification, seed increase.					
Obj. 2. Map and develop markers for disease resistance					
2.1 QTL mapping of resistances (P/Ph: population/Phenotyping; Q: QTL identification; F: fine mapping)					
- DM	YW, AK	P, Ph	Q, F	Q, F	F
- Phytophthora	RG	P, Ph	Q	F	Q
- CGMMV	KL, YW	Ph, P	P, Q	Q	
***2.2 Marker development (M) and verification* (V)**					
- DM	YW, AK	M	MV	V	V
- Phytophthora	RG	M	MV	V	
Obj. 3. QTL introgression (I) into breeding (B)/advanced lines (A), and release to breeders (R)					
- DM	YW, AK	B, I	I, A	I, A	R
- Phytophthora	MG	B	B, I	I, A	A, R
- DM + Phytophthora	YW, RG, AK	B	BI	I, A	R
Obj. 4. Economic impact analyses, disease control information					
Provide extension team advanced breeding line for field trials					

Obj. 1.2 Seed multiplication of cucumber core collection (YW & industry collaborators)

Seed increase of 388 accessions from diverse taxonomic groups, geographic origins, and market groups was continued in 2023 by industry collaborators. As of February 27, 2024, seed increase of 372 accessions has completed with 310 having >1000 seeds each and 62 with 100-1000 seeds per line. The remaining 16 failed for seed increase.

Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances

2.1.1 Downy mildew (YW & AK)

We collected phenotypic data for downy mildew (DM) responses to natural infection in field conditions in North and South Carolinas (multiple years), as we all as Hancock, Wisconsin fields (2022 only). We are performing association analysis using the data. Here is an example of GWAS with data from Hancock field in 2022 when an epidemic of DM occurred unexpectedly. In total 207 lines were grown in the trial with two replications. DM symptoms were scored with necrosis (Nec), chlorosis or yellowing (Yel), and general impression (GI). Frequency distribution of mean GI scores is shown in **Figure 1**. Nearly half of the lines are highly susceptible. The majority of lines, and less than 1% (17 of 207) showed moderately or highly resistant.

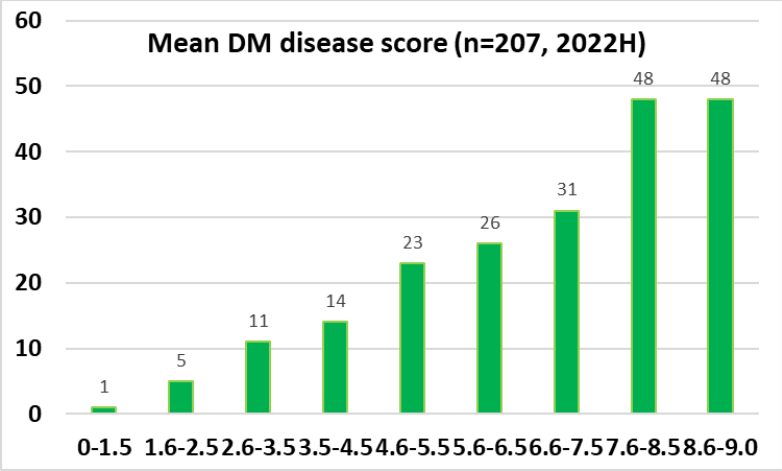

Figure 1. Frequency distribution of mean DM disease scores (GI) of 207 cucumber accessions upon natural infection of *P. cubensis* pathogen from 2022 field trial at Hancock, WI.

GWAS was conducted with mean disease scores and nearly half million high quality SNPs among 197 lines. No significant association was detected when GI or Nec were used. However, when Yel (chlorosis) ratings were employed, a SNP 2,0328,829 position (Gy14v2.1) on Chromosome 5 was detected to be significantly association with Yel in this panel. (Gy14v2.1) (**Figure 2**). This is only ~150 kb away from the *dm1/CsSGR* (staygreen, *CsGy5G003280*; 2,148,563-2,150,155) locus that confers broad-spectrum disease resistances including pre-20024 *P. cubensis* field strain(s). In the same filed, DM data from a population of Gy14 x 9930 cross with 112 RILs were also collected including Yel, Nec and GI. QTL analysis in this population revealed a major-effect QTL that is consistent with *CsSGR/dm1* for DM resistance in Gy14 (**Figure 3**). These observations suggest CsSGR still plays a critical role in DM resistance, especially anti-chlorosis in the field.

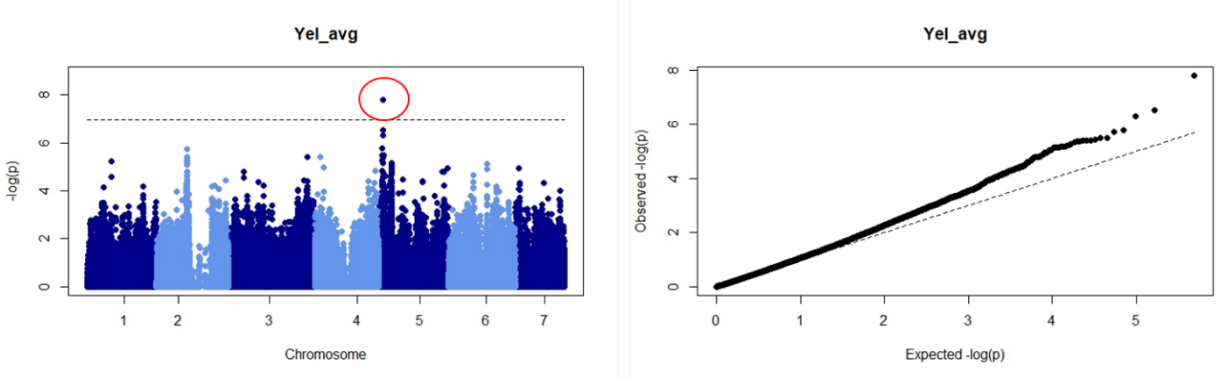

Figure 2. A significant association of DM resistance was detected near the *dm1/CsSGR* locus on cucumber chromosome 5 using 2022 Hancock mean Yel data and kinship + PC1.

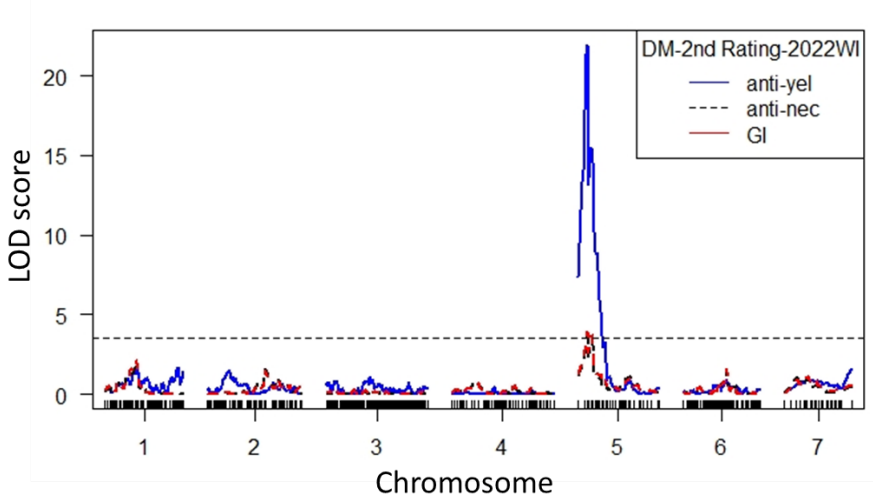

Figure 3. LOD curve from QTL analysis of DM disease scores in the Gy14 x 9930 RIL population grown in 2022 Hancock field.

2.1.2 Phytophthora fruit rot (RG and Ying-Chen Lin)

GWAS

The cucumber core collection (370 accessions for which data were collected) was screened from 2019 to 2021; 70% of the accessions had at least two years of data. The score for each accession is the mean of 30-50 fruits/year. Disease scores for the population were normally distributed. The correlation between years ranged from 0.48-0.80.

GWAS was performed using BLUE values calculated from disease scores from 2019-2021 using MLM, FarmCPU, BLINK and MLMM models (**Figure 4, Table 1**). SNP data for the core collection was downloaded from CucGenDBv2 (Yu et al., 2023). SNPs were filtered using BCFtools (Danecek et al., 2021) and GATK (Van der Auwera et al., 2013) with the following criteria: bi-allelic, GQ scores >20, maximum read depth within two standard deviations of the mean read depth, minor allele frequency > 0.1, missing rate <20%, resulting in 1,168,270 SNPs for analysis. Eleven significant SNPs (Bonferroni-corrected genome-wide significance threshold at $\alpha = 0.05$) were identified from the different models. The phenotype variance explained (PVE) ranged from 0.38-24.49%.

Table 1. Significant SNPs identified in multiple GWAS models (FarmCPU, Blink, MLMM, and MLM) for young fruit resistance in the cucumber core collection.

SNP	Chr	Position (bp) [1]	p-value / PVE (percent phenotypic variation explained) (%)			
			FarmCPU	BLINK	MLMM	MLM
S1_21117743	1	21,117,743	-	5.15E-13 / 9.01	-	1.73E-8 / 7.74
S2_10199046	2	10,199,046	-	8.31E-10 / 3.63	-	-
S2_10226744	2	10,226,744	3.85E-5 / 3.60	-	9.76E-10 / 24.49	5.95E-9 / 1.44
S3_18480786	3	18,480,786	1.06E-8 / 0.38	-	-	-
S3_37752706	3	37,752,706	3.37E-11 / 1.53	4.49E-10 / 2.09	-	-
S4_8024257	4	8,024,257	-	2.64E-9 / 2.25	-	-
S5_23563699	5	23,563,699	1.73E-8 / 3.23	-	-	-
S6_29086459	6	29,086,459	2.70E-8 / 2.03	-	-	-
S6_29175300	6	29,175,300	3.31E-11 / 5.48	-	-	-
S7_3391182	7	3,391,182	9.52E-9 / 0.79	-	-	-
S7_17739386	7	17,739,386	-	6.04E-9 / 1.35	-	-

[1]Genomic locations are according to Gy14 v. 2.1 (CuGenDB v.2; http://cucurbitgenomics.org/v2/)

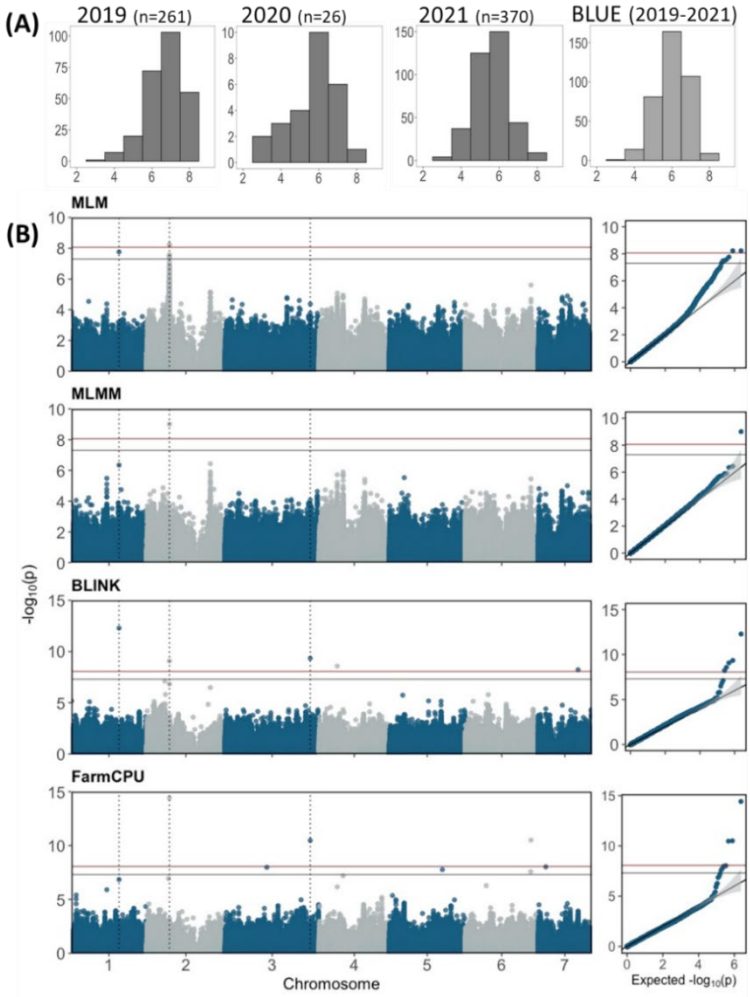

Figure 4. (A) Disease score distribution for young fruit resistance to *Phytophthora capsici* from the cucumber core collection, and BLUE distribution of combined data 2019-2021. The score for each accession is the mean of 30-50 fruits/year. (B) Manhattan plots and quantile–quantile plots of the genome-wide association study analyses for young fruit resistance in the cucumber core population. The horizontal blue and red lines represent significance thresholds of p = 0.05 and p = 0.01 based on Bonferroni correction, respectively. The dotted vertical lines show the locations of SNPs that were significant in at least two models.

Phenotypes were significantly different between accessions carrying homozygous reference vs. alternate alleles for all significant SNPs except S3_18480786. Of the nine SNPs, five alternate alleles led to increased resistance (lower disease scores) and four to increased susceptibility. Of the alternate alleles conferring increased resistance, only two were present in PI

109483-derived breeding line 'A4-3' (SNP S1_21117743 and S3_37752706), suggesting that the other alleles identified by GWAS may provide additional sources of resistance. When the alternate alleles associated with lower disease scores were rare in the core collection (< 10%, i.e., < 38 accessions), the majority of accessions (64%-81%) carrying the alternate allele originated from the India/South Asia region (**Table 2**). Conversely, four of the five SNPs associated with increased resistance were very uncommon in the East Asian accessions (0-3%). For S2_10226744, where the rare alternate allele was associated with increased susceptibility, 77% of the accessions were from East Asia. When the alternate alleles occurred frequently in the germplasm (>50%) (e.g., S3_37752706 and S7_3391182), the origins were widely distributed across regions.

Table 2. Geographical origin of accessions carrying the alternate alleles for the significant SNPs for young fruit resistance to *P. capsici* as identified by GWAS.

SNP		Region of origin[a]								
	Effect[b]	Africa	Europe	East Asia	Central/ West Asia	India/ South Asia	North America	Turkey	Other	Total
S1_2111743	↓	-	-	-	2	9	1	2	-	14
S3_37752706	↓	7	28	82	13	24	13	30	0	197
S4_8024257	↓	4	24	1	11	18	4	32	2	96
S5_23563699	↓	-	1	1	1	26	3	-	-	32
S6_29175300	↓	-	2	1	-	27	4	-	-	34
S3_18480786	-	1	2	23	2	5	1	-	-	34
S2_10226744	↑	1	2	23	-	2	2	-	-	30
S7_3391182	↑	5	43	79	30	47	37	32	2	275
S7_17739386	↑	1	17	17	7	15	10	17	-	84

[a] The regions are as defined in Wang et al. (2018).

[b] The effect of alternative alleles compared to reference alleles in disease score. ↓ - decreased disease score (more resistant); ↑ - increased disease score (more susceptible)

XP-GWAS

Extreme phenotype (XP) GWAS was performed to enable additional replication of phenotypic data. Weighted disease scores from 2019-2021 data were used to select the 29 most resistant and susceptible accessions that were then tested again in 2022. The clear difference in disease scores between the bulks was reproduced in the replicated trial in 2022 (**Figure 5A,B**), verifying accuracy of the bulk selection for XP-GWAS analysis. Correlations for the selected resistant and susceptible bulks among 2019, 2021, 2022 were 0.755-0.912. SNP data from the selected accessions were combined via in-silico bulking. XP-GWAS analysis identified significant SNPs distributed across the seven chromosomes (Bonferroni corrected p=0.05 threshold) including 39 significant SNPs located on chromosomes 1 and 5 (**Figure 5C**). The XP-GWAS SNP identified on chromosome 5 overlapped with the QTL previously identified by QTL-seq.

The QTL peaks detected on chromosome 1 were located at 21.17 Mb by GWAS and 24.75 Mb by XP-GWAS. The QTL on chromosomes 5 and 6 were consistently identified by QTL-seq, GWAS and XP-GWAS methods (on chromosome 5 all were located within 7 Mb, and on chromosome 6 all were within 3 Mb). The signal on chromosome 5 was stronger in QTL-seq and XP-GWAS compared to GWAS, possibly due to the use of the resistant line 'A4-3' in the bi-parental QTL-seq analysis, and the enrichment of rare alleles in the resistant bulk for XP-GWAS

analysis. On chromosome 3, the peak SNP was located at 37.75 Mb in GWAS and at 39.29 Mb in XP-GWAS. Both fall within the QTL region previously identified from QTL-seq analysis for age-related resistance.

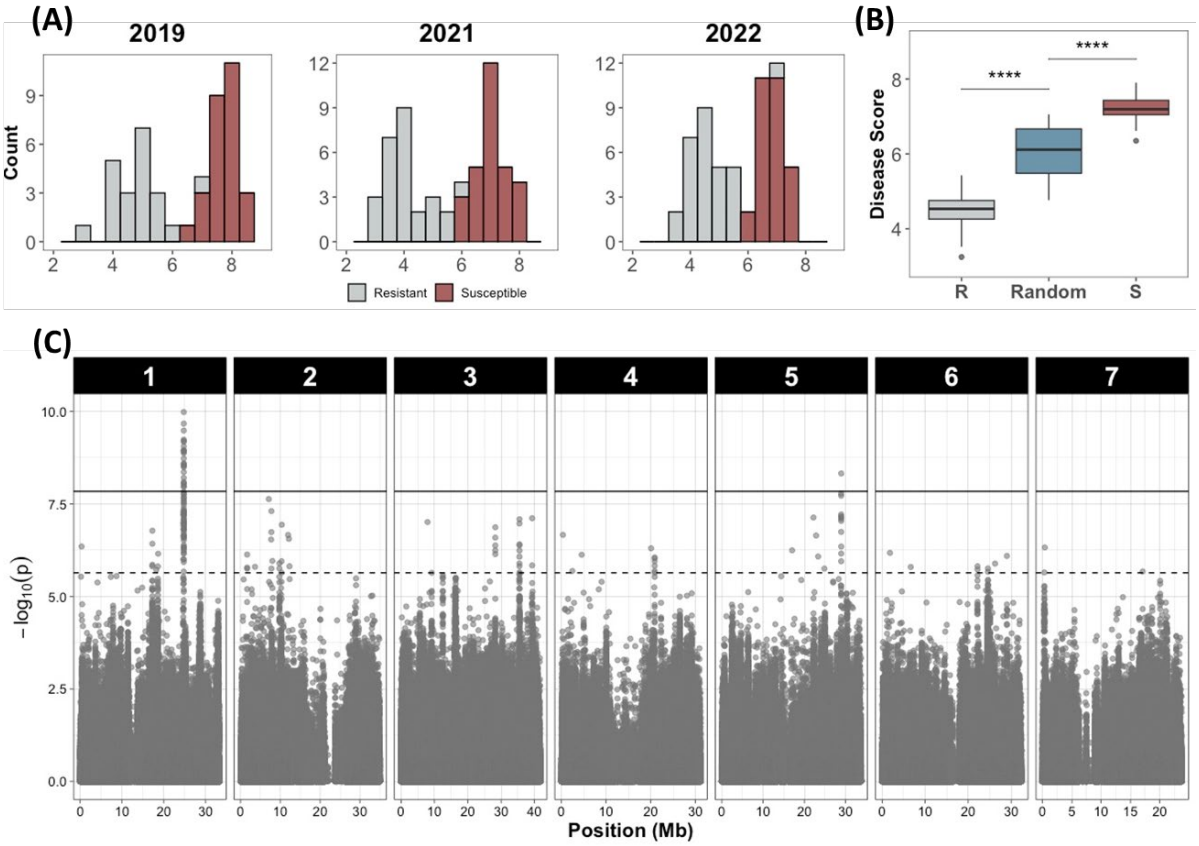

Figure 5. Disease score distribution and Manhattan plot of the XP-GWAS analysis to identify SNPs associated with young fruit resistance. (A) Disease score distribution of the resistant and susceptible bulks in different years. (B) Disease score values of the resistant (R), susceptible (S), and random bulks (**** indicates P<0.0001, Wilcoxon test). (C) Manhattan plot of the XP-GWAS analysis. The dashed line indicates the 5% FDR threshold; the solid line indicates significance threshold of p = 0.05 based on Bonferroni correction.

In most cases, the QTL identified for Phytophthora fruit rot co-localized with prior identified disease resistance hot spots (Wang et al. 2020) for resistances to downy mildew, powdery mildew, fusarium wilt, and gummy stem blight (**Figure 6**). The aggregation of these QTL suggests that these genomic regions play an important role in disease resistance to fungal and oomycete pathogens.

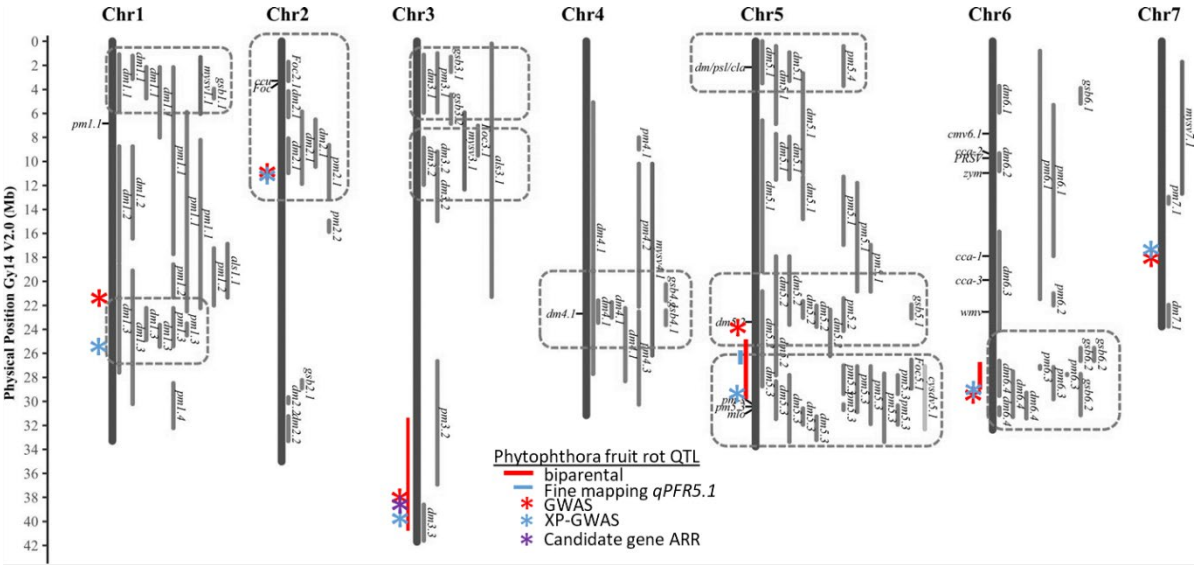

Figure 6. Chromosomal locations of QTL identified for Phythophthora fruit rot of cucumber in relation to prior QTL identified for resistances to other cucumber diseases. The indicated PFR QTL were identified from multiple analyses: red bar – biparental QTL-seq; blue bar – fine mapping of biparental populations; red asterisk GWAS of cucumber core collection; blue asterisk XP-GWAS; purple asterisk – candidate gene identified by RNAseq analyses. Figure is adapted from Wang et al., 2020 *Horticulture Research* under Creative Commons license http://creativecommons.org/licenses/by/4.0/. Abbreviations: DM, downy mildew; PM, powdery mildew; ALS, angular leaf spot; Foc, fusarium wilt; GSB, gummy stem blight; MYSV, melon yellow spot virus; CYSDV, cucurbit yellow stunting disorder virus.

2.1.3 CGMMV (KL and YW)

Cucurbit green mottle mosaic virus (CGMMV) is an emerging seed-borne virus in North America. CGMMV causes serious disease symptoms and losses in cucurbits, particularly cucumber and watermelon. As an effort to combat CGMMV in cucumber and watermelon, genetic resources were explored to develop genetically resistant/tolerant cucumber and watermelon lines in this study. Initially a total of 50 cucumber lines were screened to assess phenotypic reactions to the CGMMV infection (**Figure 7**). As a result, three lines were identified as tolerant with no phenotypic symptoms but intermediate serological reactions. The three tolerant lines identified seems to have a common origin ('Chinese Long'). The selected tolerant lines were crossed with susceptible cucumber lines to develop F_1, F_2 and RILs. The F_2 populations developed from the tolerant and susceptible lines will be evaluated further for SNP genotyping.

In another experiment, a GWAS panel of 177 cucumber accessions were evaluated for their resistance to CGMMV. The GWAS panel showed diverse responses to CGMMV infection

resulting in 12 lines in tolerant, 24 being intermediate and 137 with susceptibility (**Figure 8**). Phenotyping results are being validated by genotypic analysis to obtain the associated QTLs for resistance to CGMMV.

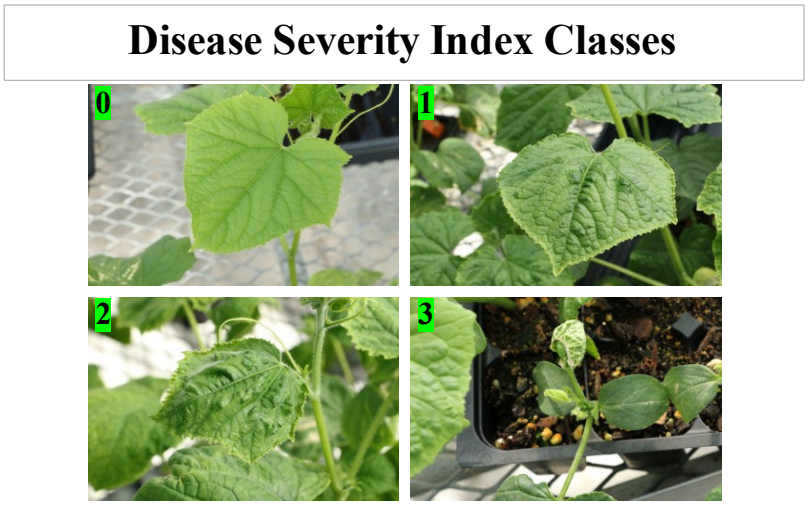

Figure 7. Rating classes of cucumber infected by CGMMV, rating 0: no symptom, rating 1: mild mosaic symptom, plant recovery; rating 2: severe mottle mosaic and rating 3: severe mottling and plant stunting.

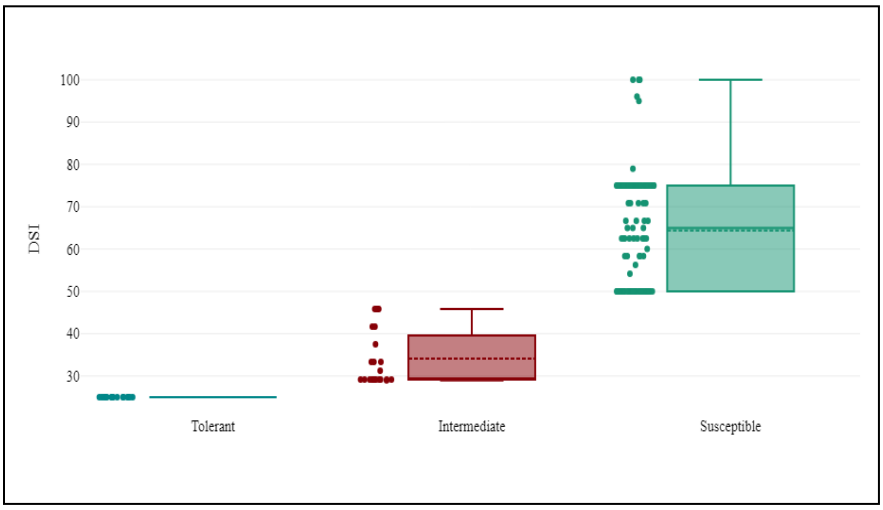

Figure 8. Phenotypic distribution of accessions to CGMMV testing among the GWAS panel. Phenotypic screening was conducted using 6 plants/line and symptom reading scoring of 0-3, with 0: no symptom, rating 1: mild mosaic symptom, plant recovery; rating 2: severe mottle mosaic and rating 3: severe mottling and plant stunting. Tolerant is lines with a disease severity index (DSI) = <25; Intermediate is lines with a DSI = >25 - <50, and Susceptible is lines with a DSI = >50.

2.2 Marker development and verification

We conducted fine mapping of the major-effect DM QTL, *dm4.1,* and *dm5.3*, and introgress them into different genetic backgrounds through marker-assisted QTL pyramiding. We found that there are actually four sub-QTL at the *dm4.1* locus that are present in both WI7120 and PI 197088

including *dm4.1.1*, *dm4.1.2A*, *dm4.1.2B,* and *dm4.1.3*. The candidate genes for *dm4.1.2A* and *dm4.1.3* in PI 197088 have been identified previously (Berg et al. 2020, 2021). So we focued on cloning of the *dm4.12B* QTLfrom WI7102 which was delimited into a 36.2 kb region on Chromosome 4. With multiple lines of evidence, we show that the gene for the L-2-hydroxyglutarate dehydrogenase (L-2HGDH) is a candidate for *dm4.1.2B* (**Figure 9**).

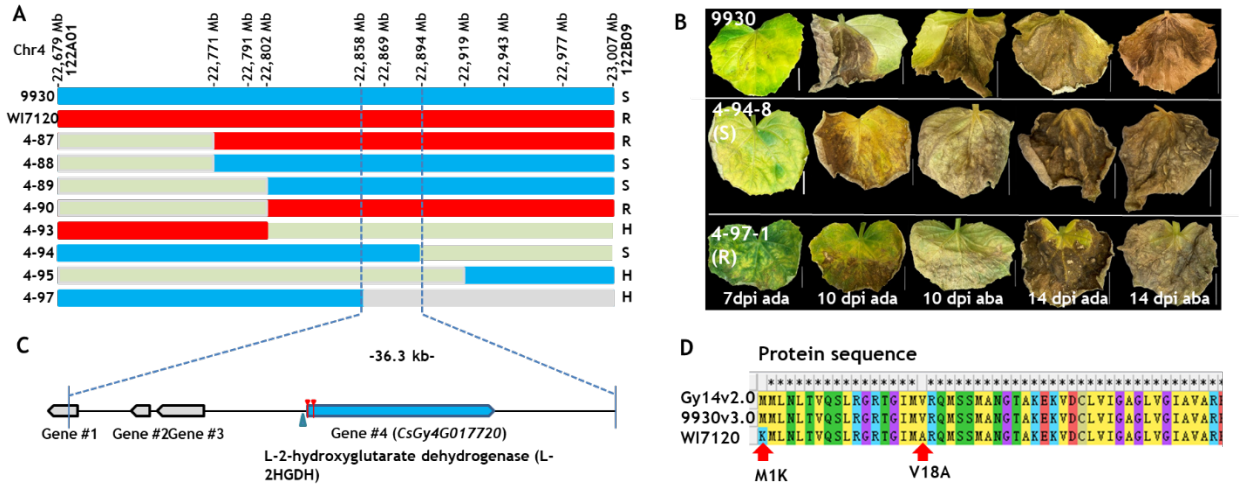

Figure 9. Map-based cloning of the *dm4.1.2B* sub-QTL. **A**. Eight *dm4.1.2B* NIL-F2 recombinants defined by 11 marker loci delimit *dm4.1.2B* into a 36.3 kb interval on Chr 4. **B**. DM symptoms of two critical recombinants 4-94 and 4-97 scored at 7, 10 and 14 days post inoculation (dpi) at either adaxial (ada), abaxial (aba) or both sides**. C.** The 36.6kb region contains four predicted genes with gene #4 as the best candidate of *dm4.1.2B*, which encodes L-2HGDH. There are two non-synonymous SNPs in the coding region (red triangles) and a 53 bp insertion (blue triangle) in WI7120 that is located at -79 bp upstream of the translation start (TSS) of *L-2HGDH*. **D**. Alignment of deduced amino acid sequences (partial) of L-2HGDH among Gy14, 9930 and WI7120. The 1st and 2nd SNPs would result in M1K (change of start codon) and V18A amino acid substitutions, respectively.

There are multiple polymorphisms inside the promoter region of *dm4.1.2B* between PI 197088 and susceptible 9930 parental lines including a 52-bp insertion in PI 190788. DM resistance conferred by two other sub-QTL, *dm4.1.2A* and *dm4.1.3* have been previously shown to be due to insertion of a 551bp and a 7,688 bp transposon insertion, respectively (Berg et al. 2020, 2021). We examined the allelic diversity of three the Sub-QTL among cucumber accessions with high resistance to DM (**Figure 10; Table 3**) which revealed commonalities and differences of alleles carried by these cucumber lines.

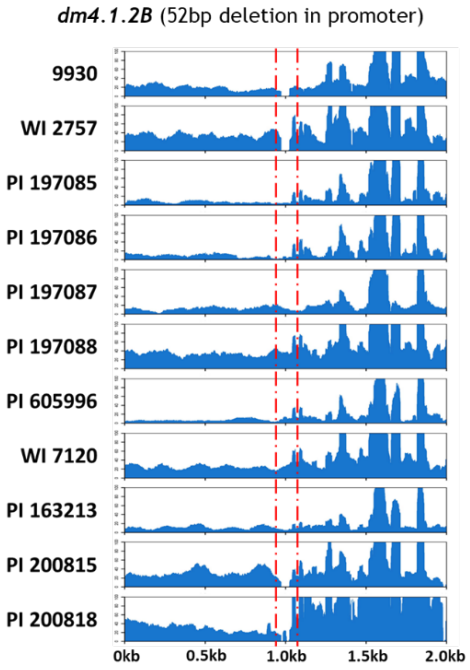

Figure 10. Sequence alignment of *dm4.1.2B* locus revealed association of the 52-bp deletion in the promoter region and DM resistance among cucumber lines with DM resistance.

Table 3. Correspondence of alleles at *dm4.12A, dm4.1.2B* and *dm4.1.3* Sub-QTL with DM resistance among different cucumber lines.

Lines	Origin	DM	*dm4.1.2A* (551bp insertion)	*dm4.1.2B* (52bp insertion)	*dm4.1.3* (RT insertion)
9930		S	S	S	S
WI 2757	US	MR	S	S	S
PI 197085	India	HR	R	R	R
PI 197086	India	HR	R	R	R
PI 197087	India	MR?	R	R	R
PI 197088	India	HR	R	R	R
PI 605996	India	HR	R	R	R
WI 7120	Pakistan	HR	R	R	R
PI 163213	Pakistan	R	R	R	S
PI 200815	Myanmar	R	S	S	R
PI 200818	Myanmar	S	S	S	S

Obj. 3. QTL introgression into breeding or advanced lines (YW, RG and AK)

One objective of this project is to develop inbred lines with both DM and PFR (Phytophthora fruit rot) resistances through marker-assisted QTL pyramiding. So far we have completed introgression of three DM QTL (*dm4.1, dm5.2, dm5.3*) into Gy14 (pickle), 9930 (Asian Long), and WI7204 (mini) backgrounds, which were named Gy14Q3, 9930Q3 and WI7204Q3, respectively. We further introduced the major-effect QTL for PFR resistance, *qPFR5.1* into Gy14, which were in repulsive phase with *dm5.2* and *dm5.3*. We took a revised marker-assisted backcrossing strategy

and identified ideal recombinants carrying alleles for all four resistance loci (*dm4.1, dm5.2*, *qPFR5.1*, and *dm5.3*) in homozygous states.

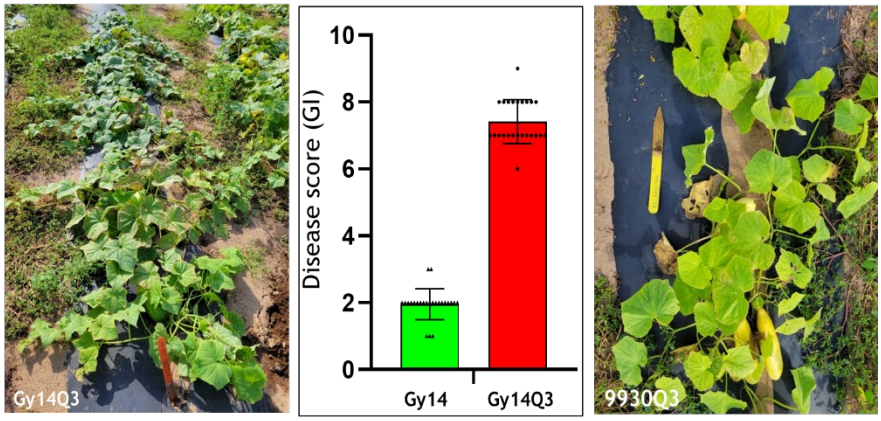

Figure 9. DM resistance of Gy14Q3 (left and middle) and 9930Q3 under heavy *P. cubensis* infection in Hancock field in 022 summer season.

DM resistances of these introgression lines was observed in 2022 and 2023 filed trials. In 202 Hancock field with heavy DM infection, both 9930Q3 and Gy14Q3 showed much higher resistance than lines donor lines (**Figure 9**). However, in 2023 Spring field trial at Clemson, South Carolina, Gy14Q3 and 9930Q3 only shoed slightly high resistance than their respective donors that also depend on the time after infection (Table 4). Overall, compared with the original donor of these QTL (PI 197088), the resistance conferred by the three QTL does not seem to satisfactory). In particular, from multiple years' observation, WI7024 was highly susceptible to DM. We also tested responses to infection by the PFR pathogen in Gy14Q4, which did not show significantly higher resistance than the donor (Gy14 or Gy14Q3). Whether this is due to wrong genotyping or negative genetic background effects (linkage drag) needs further investigation.

Table 4. Disease scores of DM of selected introgression lines and controls under natural infection (2023 Clemson)

	Rating1			Rating2		
Lines	**Yel**	**Nec**	**GI**	**Yel**	**Nec**	**GI**
9930	4	4	7	4	5	7
9930Q3	4	2	5	4	3	6
WI7204	3	2	4	3	3	4
WI7204Q3	4	2	4	3	2	4
Gy14	3	3	4	3	3	4
Gy14Q3	3	3	4	3	3	4
Gy14Q4	2	3	4	3	2	4
PI 197088	2	1	2	2	1	2
Straight 8	4	4	6	4	4	7
WI2757	3	3	5	3	3	6
WI7362A-89_RIL	3	2	3	3	2	3
9930 dm4.1_NIL	4	3	6	4	3	5

For the fine mapped/cloned DM/PFR resistance QTL (*dm1/CsSGR, dm4.1, dm5.2*, dm5.3 and *qPFR5.1*), we are developing SNP assays adapted to high throughput SNP genotyping. Figure 10 is an example for *dm5.3/CsSIB1* with the KASPar genotyping platform.

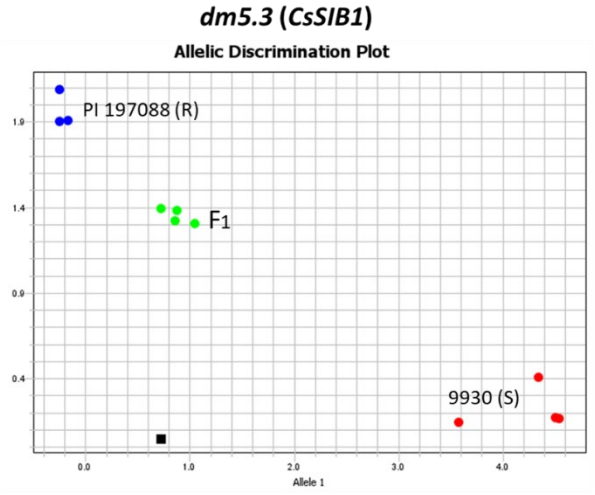

Figure 10. Development of KASPar markers for SNP genotyping using *dm5.3* as an example.

Squash Team

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Obj. 2. Map and develop markers for disease resistance
2.1 QTL mapping of resistances
2.1.4 Squash
- Powdery Mildew – *C. pepo* Geoffrey Meru, Prerna Sabharwal, and Yuqing Fu & Michael Mazourek and Gregor Inzinna

In 2023, screening of 207 USDA germplasm accessions of *C. pepo* for PM resistance was conducted at the University of Florida (greenhouse) and Cornell University (field) XP GWAS. Seeds of each accession (n=15) were grown in three replicates in a randomized complete block design. Success PM (carrying *PM-0*) and Early Prolific Straightneck cultivars were used as resistant and susceptible checks, respectively. Pathogen inoculum was provided through naturally infected plants and by spraying spore suspension on the foliage. At the 6th true-leaf stage, symptom severity data were recorded on a scale of 0-100%, based on visible pathogen sporulation on the surface of top 4th leaf, bottom 4th leaf, stem above 4th leaf, stem below 4th leaf and the whole plant level (**Figure 1**).

Figure 1 Greenhouse screening at the University of Florida for powdery mildew resistance in the USDA *C. pepo* collection (n= 207).

Across locations, Success PM/ 209 (R check) and Early Prolific/208 (S check) were consistently tolerant and susceptible, respectively. On the other hand, wide phenotypic variation was observed across the 207 *C. pepo* accessions (**Figure 2**).

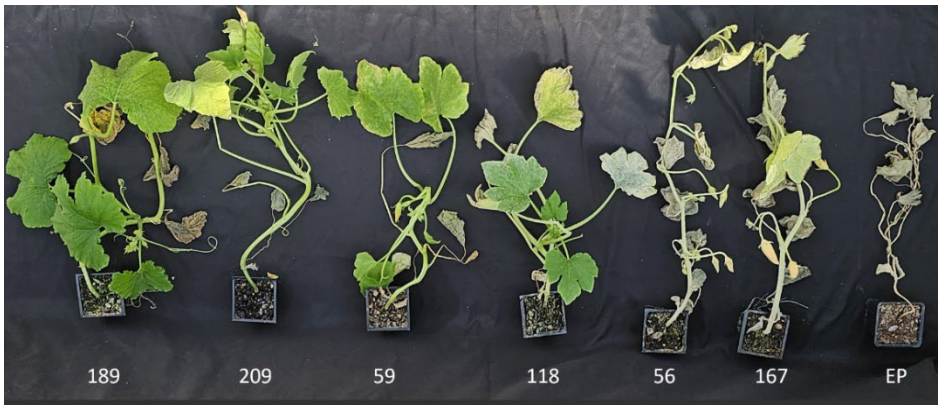

Figure 2 Variation in powdery mildew symptoms in a few USDA accessions (189, 59, 56 & 167) and the resistant (209) and susceptible (EP) checks.

The greenhouse trial in Florida revealed the most resistant USDA accessions across each disease severity parameter (**Table 1**): top 4th leaf (68), bottom 4th leaf (188), stem above 4th leaf (188, 189), stem below 4th leaf (125, 188, 189) and whole plant (188, 189).

Table 1 The top 10 *C. pepo* accessions tolerant to powdery at the greenhouse trial in Florida. Genotypes denoted by an asterisk (*) were tolerant across all the recorded parameters.

Access-ion	Top 4th leaf	Access ion	Bottom 4th leaf	Access ion	Stem above 4th leaf	Access ion	Stem below 4th leaf	Access ion	Whole plant
68	30.0	209*	3.3	209*	14.2	125	0.0	188*	0.0
189*	42.5	188*	7.5	189*	21.1	188*	0.0	189*	3.8
25	45.3	193	26.3	188*	22.5	189*	0.0	209*	6.7
209*	48.3	40	30.0	125	25.0	209*	7.5	125	10.0
144	50.0	190	33.3	144	25.0	68	10.0	144	20.0
188*	55.0	192	36.3	68	40.0	144	10.0	68	25.0
62	59.5	179	42.1	72	40.0	114	12.5	193	33.8
114	60.0	114	42.5	187	40.8	40	20.0	114	35.0
48	64.6	125	42.5	16	42.5	38	24.8	16	38.8
169	65.7	189*	42.5	55	44.8	111	26.3	6	40.0

Powdery mildew disease severity data (top 4th leaf, bottom 4th leaf, stem above 4th leaf, stem below 4th leaf and the whole plant) was used for GWAS analysis. The phenotype data was derived from PM screening trials in Florida (greenhouse- 2023), NY (greenhouse-2022), and Michigan (field-2022). Corresponding genotype data for approximately 4 million SNPs was obtained for the 207 *C. pepo* accessions from the Boyce Thompson Institute (Fei Lab). The SNP dataset was first converted from its original Variant Call Format (.vcf) to a numeric text (.txt) format, followed by the execution of the Genome Association and Prediction Integrated Tool (GAPIT) through the University of Florida HiPerGator computing infrastructure. Three statistical models (MLM, FarmCPU and Blink) were deployed to detect genomic loci significantly associated with powdery mildew resistance. GWAS analysis using greenhouse data (Florida) revealed significant genomic loci associated with PM resistance (**Figure 3**) for top 4th leaf (Chr 11 and Chr 20), stem above 4th leaf (Chr 4, Chr 14 and Chr 16) and whole plant (Chr 13, Chr 15, Chr 18 and Chr 20). Resistance

loci for 'top 4th leaf' and 'whole plant' co-located on Chr 20, suggesting linkage/ pleiotropy for the two traits.

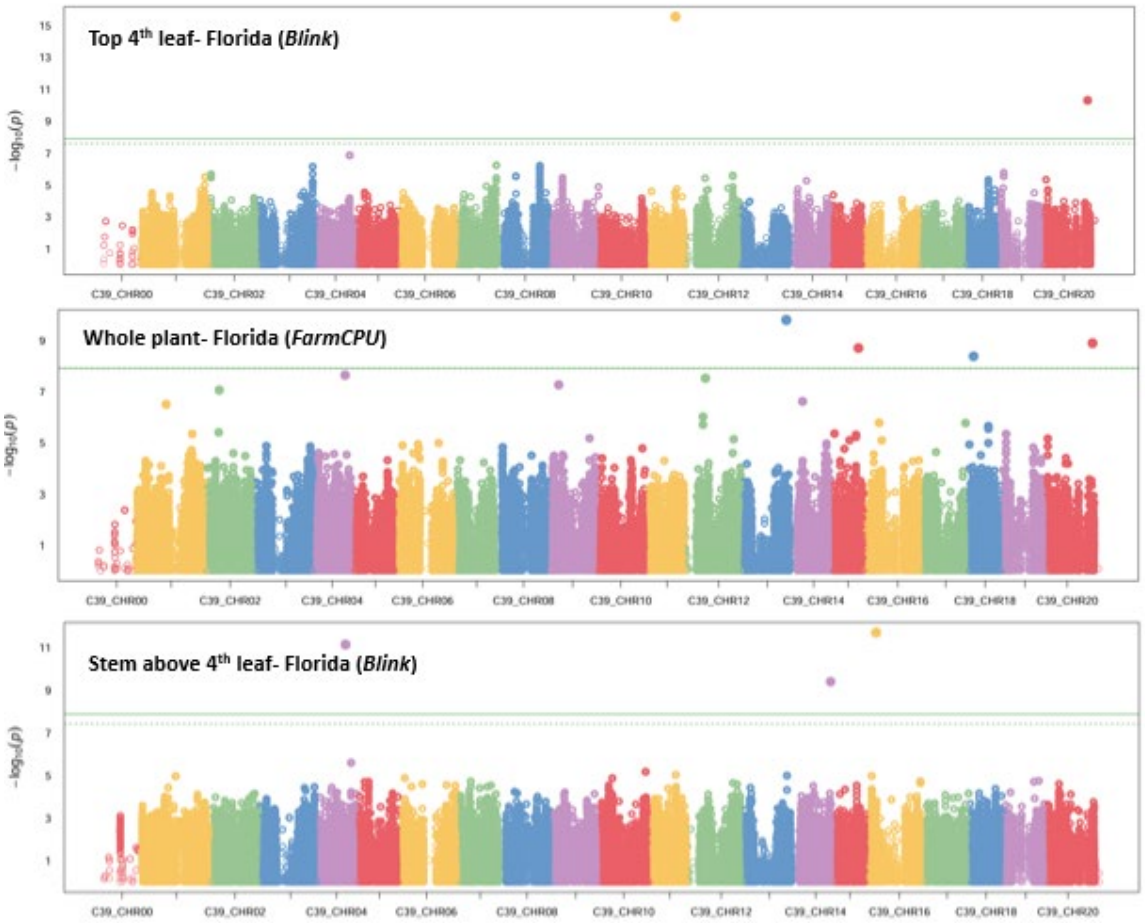

Figure 3 GWAS for PM resistance using phenotypic data collected in Florida (greenhouse). Significant loci were detected for top 4th leaf, stem above 4th leaf and the whole plant phenotype. On the other hand, GWAS analysis using phenotypic data collected in NY (greenhouse) revealed significant hits for PM resistance (**Figure 4**) for top 4th leaf (FarmPCU Chr 2, Chr 4, Chr 7, Chr 13 ad Chr 19//Blink: Chr 3, Chr 4, Chr 5 and Chr 19), bottom 4th leaf (Chr 6, Chr 14 and Chr 19) and stem above 4th leaf (Chr 12). The genomic loci on Chr 4 and Chr 19 for 'top 4th leaf' parameter were consistently detected using both the FarmCPU and Blink models.

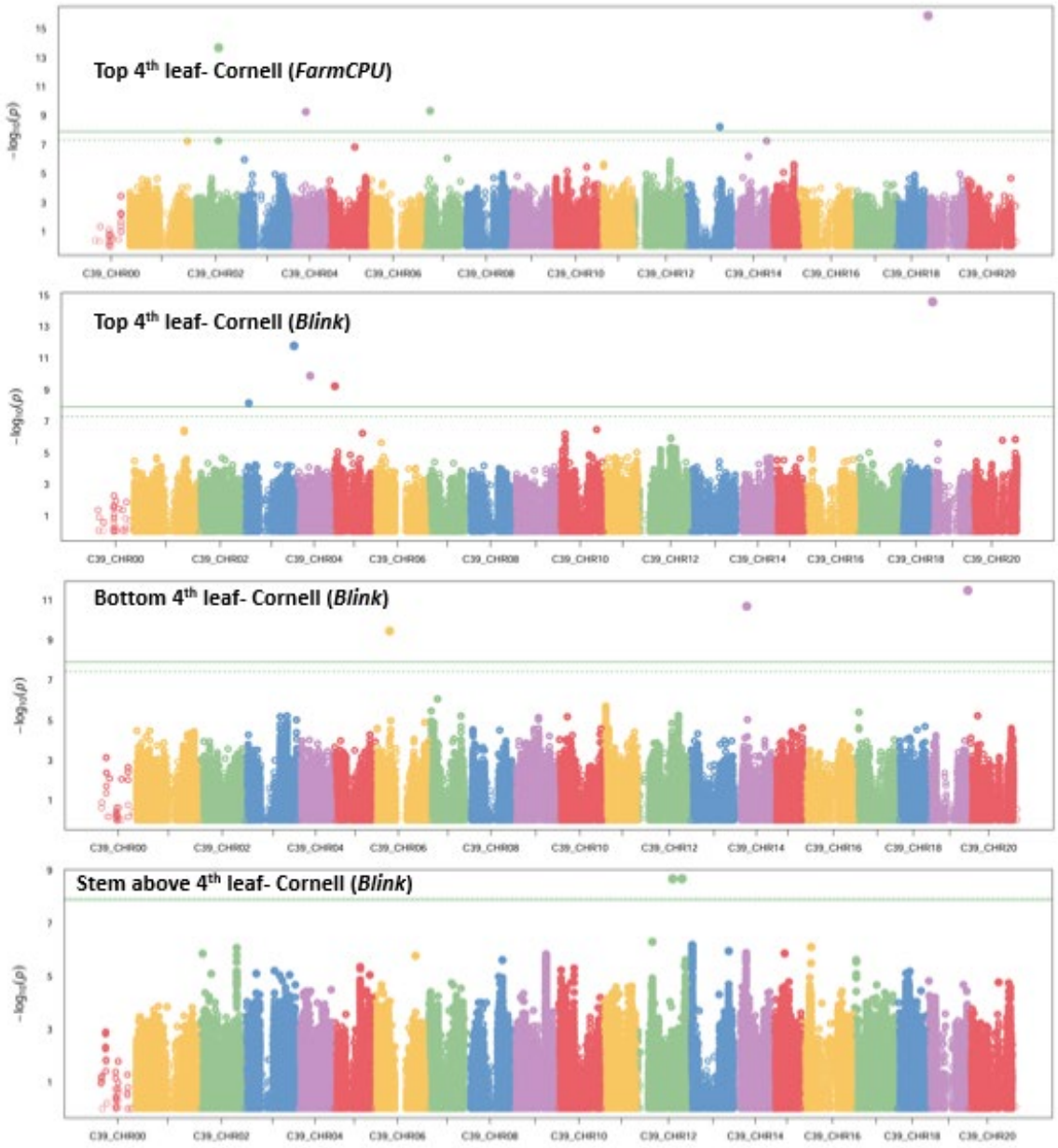

Figure 4 GWAS for PM resistance using phenotypic data collected in Florida (greenhouse). Significant loci were detected for top 4th leaf, stem above 4th leaf and the whole plant.

The lack of overlapping resistance loci between Florida and NY is not surprising because of the low correlations *(r* = 0.1 to 0.25) observed between the phenotypic data recorded at the two locations. This disparity may be a result of differences in powdery mildew strains in NY and FL, as well as contrasting experimental conditions. GWAS analysis using phenotypic data collected in Michigan (Field) did not yield any significant hits.

PM was very slow to develop in NY resulting in plants being very large and overgrown in the field. Acting on helpful advice from our colleagues in Charleston, XP-GWAS was conducted where we were able to identify extreme phenotypes where plants were either completely covered with mildew or surprisingly clean. Using the resequencing data aligned to the C39 reference genome, XP-GWAS revealed highly significant hits on chromosome 2 and 6 (**Figure 5**).

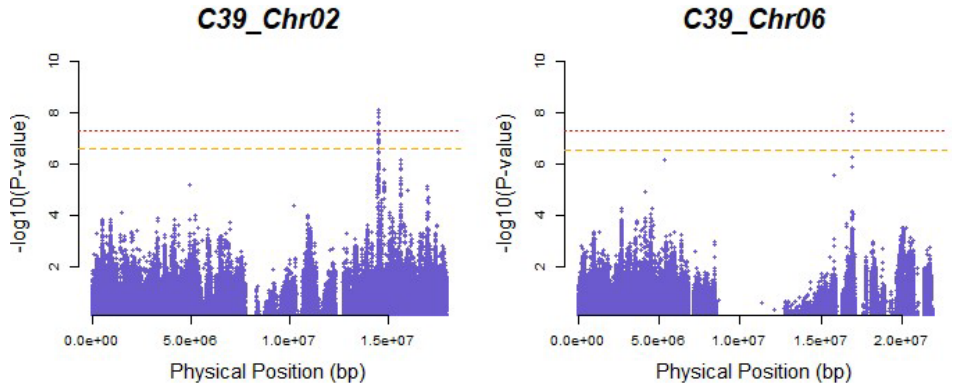

Figure 5. Manhattan plots illustrating XP-GWAS hits on C. pepo chromosomes 2 and 6

- Phytophthora – *C. pepo* Geoffrey Meru, Prerna Sabharwal, and Yuqing Fu

In 2023, we continued population advancement for the introgression of crown rot resistance from 181761-36P (*C. pepo*) and 394-1-27-12 (*C. moschata*) into elite germplasm of various *C. pepo* market groups through backcrossing. More than 1,600 seedlings from 90 segregating families were screened for crown rot resistance in the greenhouse (**Figure 6**). A majority (68%) of which were susceptible (DS >4 out of 5), some (18%) showed moderate resistance (DS of 2-3.9 out of 5) while 14% showed high resistance (DS <2).

Figure 6 Segregation in *Phytophthora* crown resistance was observed among the ninety *C. pepo* families screened in the greenhouse at the University of Florida.

Among the promising families, SS2503, SS2528, 69-52 and SS2722, SS2523 and SS2636 were the most resistant (**Table 2**). These lines will be advanced to the next generation for further selection. As previously reported by Hausbeck's group, we noted that backcrosses or crosses involving subspecies *pepo* were more tolerant than those involving subspecies *ovifera*. Crosses involving the latter will necessitate an additional selfing step to eliminate a potential susceptibility gene in subspecies *ovifera*.

Table 2 Out of the 90 segregating families screened in 2023, eighteen *C. pepo* and bridge lines [*C. moschata* **x** *C. pepo*] families were most resistant or tolerant to *Phytophthora* crown rot.

Cross	**Female parent**	**Male parent**	**Disease severity (0-5)**
SS2503	SS2273-7-1	SS69-52-8-7^{R}&11^{R}	0
SS2528	SS853-3	SS69-52-8-7^{R}	0.5
SS69-52-2self	SS69-52-2^{R}	SS69-52-2^{R}	0.75
SS69-52-6sib8	SS69-52-6^{R}	SS69-52-8^{R}	0.8
SS2722	SS1531-10	SS69-52-8	0.9
SS2533	SS2273-5sib1-1	SS69-52-8-2^{R}	0.9286
SS2636	SS2411-2	SS2535-19^{R}	1
SS69-52-17self	SS69-52-17^{R}	SS69-52-17^{R}	1.3
SS2520	SS853-2	SS69-52-8-9^{R}	1.4
SS2567	SS69-52-8-3^{R}	SS2234-2	1.65
SS2483	SS2273-5sib1-( )	SS69-52-8-3^{R}	1.7273
SS2535	SS2236-2	SS69-52-8-3^{R}	2.1
PI181761-36P@	PI181761-36P@	PI181761-36P@	2.2667
SS2078	Black beauty	PI181761-36P-6-2	2.3333
SS2594	Black beauty	SS1531-10	2.4
SS2535-21sib	SS2535-21^{R}	SS2535- ( )^{R}	2.45
SS2534	SS2455-5-5	SS69-52-8-9^{R}	2.4828
PI181761-36P-6-1	Tolerant check	-	2.7
Yellow Crookneck	Susceptible check	-	5

2.2 Marker development and verification

-Identifying Genomic Regions Associated with the Novel Powdery Mildew Resistance Native to *Cucurbita moschata* Michael Mazourek and Gregor Inzinna

Currently, squash growers are relying on one gene for powdery mildew resistance (*Pm-0)* introgressed from *Cucurbita okeechobeensis martinezii* into *C. moschata* over 50 years ago by Henry Munger at Cornell. Towards building a more robust resistance package for squash growers, we have identified a native PMR in *C. moschata.* This novel resistance protects the leaf surface, stem and petiole, while the dominant *Pm*-0 gene primarily confers resistance to the stem

and petiole. However, the physiological mechanisms and segregation patterns of this new resistance were unknown.

As previously reported, in the winter of 2021-2022 we genotyped resistant and susceptible bulks of an F_2 population of a cross between Waltham and this novel resistance source and found regions associated with this trait by genotyping the 22 most resistant, 22 most susceptible individuals and the parents and identified genomic regions associated with the resistance using a BSA approach. Initial results indicated chromosome 13 was the most significantly associated region of the genome (Rifu_version1); segregation ratios for the resistance indicated a recessive resistance at the outset and we used this to guide our path forwards. Over the course of 2022 we created markers that spanned the 2MB region on chromosome 13 and differentiated the resistant and susceptible parents (**Table 2**).

Table 2. Fine mapping Chr 13 QTL

GH_ID	GH SCORE	M07	M52	M11	MW2	M98	PEDIGREE	Gen
R-01	SEG	R	R	R	R	H	Sus x Res	F3
R-03	SEG	R	R	R	R	H	Sus x Res	F3
R-12	SEG	H	H	H	H	R	Sus x Res	F3
R-05	SEG	H	H	H	H	R	Sus x Res	F3
R-08	SEG	H	H	H	R	R	Sus x Res	F3
R-16	SEG	H	R	R	R	R	Sus x Res	F3
S-Parent	S	S	S	S	S	S	Susceptible	OP
HET	S	H	H	H	H	H	Sus x Res	F1
R-Parent	R	R	R	R	R	R	Resistant	OP
Bugle	S		S	S	S	S	Bugle-*Pm-0*	OP

The position of the gene contributing to resistance is indicated by a vertical line

In the past year, we have worked to refine the resistance locus. In the spring of 2023, 190 F2, 90 F2 derived from the reciprocal cross (rF2), 120 BC1F2, and relevant controls were grown and genotyped for crossovers using a combination of HRM and PCR markers that were identified from the BSA. DNA was extracted using a CTAB method and SNP calls confirmed using Sanger sequencing. The resulting 60 individuals with crossovers were transplanted into the field for PM phenotyping and seed increase of F2:3 families. During the field season, we were able to self-pollinate 48 of these individuals and had enough seed for further greenhouse PM screens of 39 resulting F3 families.

Using the same method in our 2021-2022 winter greenhouse screen, 30 plants each of the 39 F3 families were screened in the greenhouse during the winter of 2023 to 2024. We also evaluated 300 susceptible(S) by resistant(R) F2, 300 (RxS) revF2, 300 ([SxR]xS)BC1F1, and 450 ([RxS]xR)revBC1F1 plants to identify resistant individuals for further genotyping and calculating more significant segregation ratios for the phenotype. In the greenhouse screen of the F3 familes, only 2 families showed a strong resistance phenotype, and 4 segregated for both leaf and petiole resistance. Based on the genotyping data of the crossovers in the F3 familes, it

became obvious that 2-3 more loci may be required to reproduce the PM leaf resistance of the parent.

This winter, we were also able to rerun the BSA-S using the original 22 resistant and susceptible individuals and a new *C. moschata* reference genome provided by Zhangjun Fei. This new reference genome was much more complete. This BSA run increased the significance of other regions of the genome and indicated as many as 4 other regions of the genome may be associated with this resistance (**Figure 5**). We have developed a new marker set to clarify which of these regions is necessary for the resistance and have extracted DNA from resistant and susceptible individuals from these individuals from the 2023-24 winter greenhouse screen. We hope to run these markers of the next few weeks to clarify our findings and create marker sets for introgressing this trait into common cultivars.

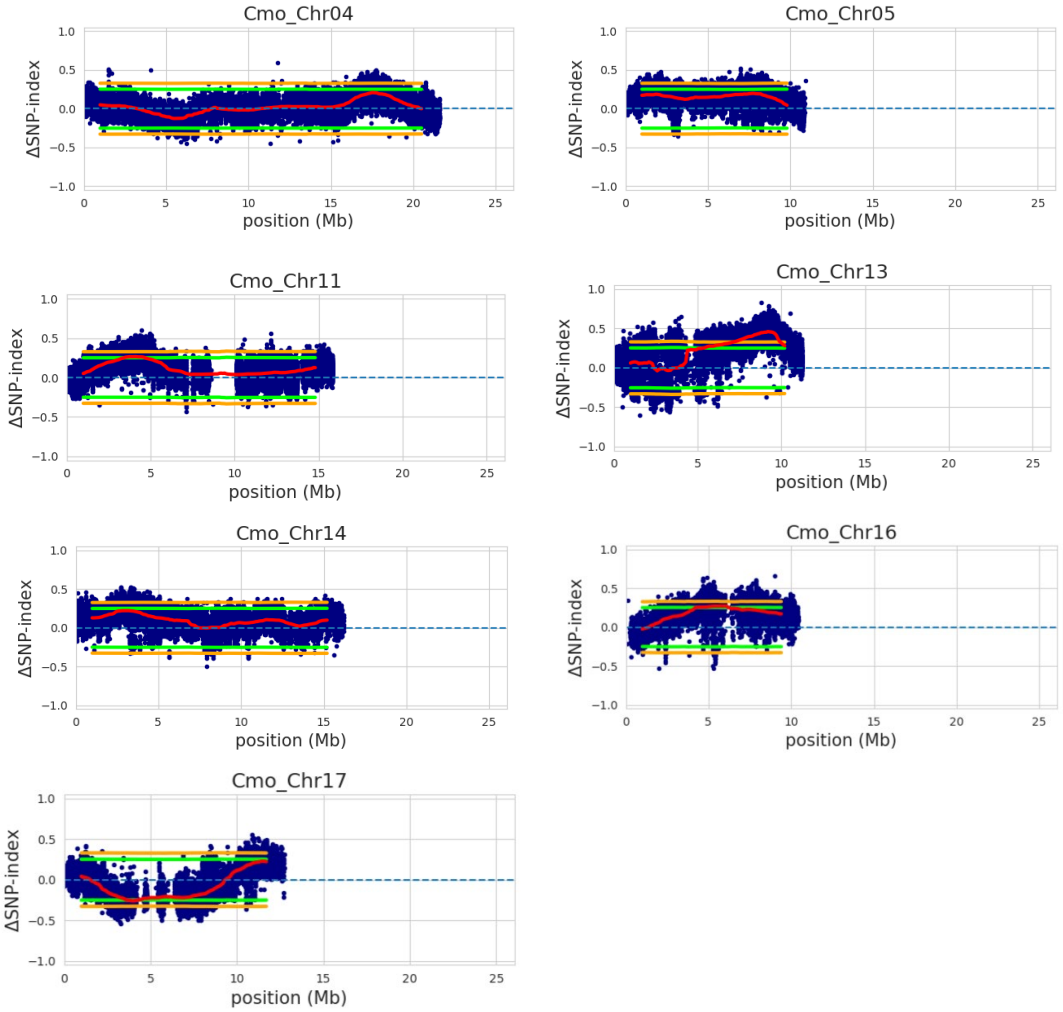

Figure 5. BSA-Seq results for native PMR in *C. moschata* with updated reference genome

Obj. 3.4. Introgress, pyramid/stack resistances into advanced breeding lines

-Evaluate the susceptibility of squash to Phytophthora crown rot under greenhouse conditions, 2023. Carmen Medina-Mora and Mary Hausbeck with Michael Mazourek and Gregor Inzinna

A greenhouse trial at Michigan State University Plant Science Greenhouses in East Lansing, MI was established to evaluate the susceptibility of *Cucurbita* species to *Phytophthora capsici*. The trial consisted of 16 winter squash accessions (PIs) and 1 *C. maxima* Hubbard type, 'Golden Delicious' provided by Dr. M. Mazourek (Cornell Univ., NY). On 8 Sept, 18 seeds per line were directly seeded onto 3x3 in plastic containers containing SureMix soilless medium. On 10 Oct, when seedlings were at 3-4 leaf stages, all seedlings were inoculated with 5 mls of a zoospore suspension ($1x10^5$/ml) consisting of a 1:1 mixture of 2 strains of *P. capsici* (M. Hausbeck *P. capsici* collection, strains SP98 and 12889). Seedlings were distributed in a randomized complete block design with three blocks and six replications per block. Disease ratings based on a 0-5 categorical scale (0= healthy, 1=small lesion at crown, 2= water-soaked lesions beyond cotyledons, 3=wilt and plant partially collapsing, 4= severe wilt and plant completely collapsing, and 5=plant death) were conducted 7 days after inoculation on 16, 23, and 30 Oct. Data were analyzed using SAS PROC GLIMMIX procedure (ver.9.4; SAS Institute, Cary, NC) with maximum likelihood estimation method and Kenward-Rogers degrees of freedom as options. Differences among treatments were analyzed using Least-Square Means comparisons (P=0.05).

	Average disease rating (0-5)[z]		
Variety name (accession)	**16 Oct**	**23 Oct**	**30 Oct**
Zapallo de Tronco (PI.458698)	3.0 ab[y]	4.9 a	5.0 a
Plomo ruso (PI.458702)	2.3 bcd	3.7 bc	4.5 ab
Kestane (PI.176527)	2.1 cd	3.2 cd	3.8 b
No. 7488 (PI.143274)	1.7 de	2.4 de	4.1 ab
Amzibegovska (PI.357898)	2.7 ab	4.6 ab	5.0 a
Golema (PI.370454)	2.6 abc	4.6 ab	5.0 a
Buttercup Burgess Strain (G.23449)	0.4 gh	0.8 fg	2.1 c
Mayo Blusher (G.30147)	2.9 ab	4.6 ab	4.9 a
Alayo (G.23726)	3.2 a	4.7 a	5.0 a
Vistalba (PI.458683)	2.7 ab	4.8 a	5.0 a
Fipushi (PI.500529)	3.0 ab	4.4 ab	5.0 a
Queensland Blue (PI.234608)	0.7 fg	1.5 ef	2.9 c
Autumn Cup F1	0.2 gh	0.5 g	1.1 d
Thunder F1	0.1 gh	0.2 g	1.1 d
Dickinson	0.0 h	0.5 fg	0.8 d
Golden Delicious (GWAS-197-1)	1.1 ef	3.2 cd	4.2 ab
Analysis of Variance (P value)	<0.0001	<0.0001	<0.0001

[z]Average disease ratings based on a 0-5 categorical scale (0= healthy, 1=small lesion at crown, 2= water-soaked lesions beyond cotyledons, 3=wilt and plant partially collapsing, 4= severe wilt and pant completely collapsing, and 5=plant death).

[y]Least squares means with the same letter are not significantly different (P=0.05).

The disease progressed rapidly; 7 days after inoculation (on 16 Oct) susceptible accessions were partially collapsing. On 30 Oct, 21 days after inoculation, at the end of this trial a high number of seedlings were severely diseased or dead. Overall, 10 out of the 12 accessions tested were as susceptible as the susceptible standard 'Golden Delicious'. The remaining 2 accessions ('Queensland Blue' and 'Buttercup Burgess Strain') were less susceptible than 'Golden Delicious' but not as resistant as the *C. maxima* Kabocha type included in this trial.

-Evaluate Age-Related Resistance (ARR) of squash breeding lines to fruit rot caused by *Phytophthora capsici*, 2023. Carmen. Medina-Mora, Matthew Uebbing, and Mary Hausbeck with Michael Mazourek and Gregor Inzinna

A pollination plot was established at Michigan State University Plant Pathology Farm, East Lansing, MI; the Capac loam soil was plowed and disced on 9 May and 15 May, respectively, and amended with 130 lb Urea and 130 lb Potash on 16 May. On 13 Jun, 4-week-old seedlings were transplanted every 18-in onto raised beds (rows 100-ft X 16-ft center to center) covered with black polyethylene plastic. Until fruits were harvested, 28% fertilizer (1gal/A) was applied weekly and non-target diseases and insects were controlled. Starting on 5 Jul, Admire Pro (10.5 fl oz) was delivered through drip tape to control cucumber beetle and squash bug. In addition, on 28 Jul, squash plants were sprayed with Warrior (1.9 fl oz) to control beetles. Starting on 13 Jul, a fungicide program including chlorothalonil, quinoxyfen, and cyflufenamid [Bravo (3pt/A), a mixture of Quintec (6 fl oz) and Bravo (3 pt/A), a mixture of Torino (1.6 fl oz) and Bravo (32 fl oz), Torino (1.6 fl oz), and Quintec (6 fl oz)] was applied, as needed, to control the incidence of powdery mildew. To facilitate fruit set and reduce natural flower abortion, flowers at anthesis were hand-pollinated using an artist's paint brush and marked at the petiole using colored-flagging tape. Because flower development is asynchronized among breeding lines, the presence of flowers at anthesis was monitored daily for 32 days and flowers were hand-pollination every other day. A total of 500 flowers were hand-pollinated to harvest a maximum of 8 fruits corresponding to 21 days post-pollination (dpp) and 8 fruits corresponding to 28dpp per line. A 9mm mycelial plug of a 7-day-old culture of *P. capsisci* (strain SP98) was placed onto the skin of each disinfected fruit (10% bleach and rinsed with water) on same day each fruit was harvested. Fruit rot was evaluated 5 days post-inoculation (dpi) and disease assessment included: 1) lesion size, 2) incidence of hyphae beyond inoculation point, and 3) disease severity based on a 0-4 categorical scale (0= healthy, 1=water-soaked tissue, 2= light visible mycelial growth, 3=moderate mycelial growth, 4= dense mycelial growth). Incidence of fruit rot symptoms was recorded as binary data, where evidence of water-soaked tissue or mycelia was considered 100% incidence and the absence of these symptoms as 0% incidence. The size (width and length) of fruit lesions were recorded in cm and the area of an ellipse was calculated for each fruit lesion as follows: Area = 3.14*1/2 width *1/2 length. Data for fruit lesion size were analyzed SAS PROC GLIMMIX (ver.9.4; SAS institute, Cary, NC) with Least-Square Means (P=0.05) for pairwise comparisons. The incidence of water-soaking in the pulp was recorded as binary data, where evidence of water-soaked tissue was considered 100% incidence and the absence of water-soaking as 0% incidence.

Fruits of 'Buttercup Burgess Strain' and 'Thunder F1' that developed at 28dpp had lower fruit rot incidence (12.5% and 0.0%), smaller fruit size lesions (0.39 and 0.00 cm^2), and lower incidence of water-soaked tissue in the pulp (13.0% and 13.0%) than fruits that developed at 21 dpp, indicating age-related resistance. The incidence of fruit rot and water-soaked tissue in the pulp for fruits of 'Dickinson' that developed at 28dpp were unexpected; might be an effect of mechanical injury to the skin of the fruit. Fruits of 'Queensland Blue' and 'Kestane' did not demonstrate age-related resistance and were as susceptible as the susceptible control 'Golden Delicious'.

	incidence fruit rot		area of fruit lesion		incidence ws (pulp) (%)	
Pedigree	21dpp	28dpp	21dpp	28dpp	21dpp	28dpp
Buttercup Burgess	87.5	12.5	6.29	0.39 c	100.0	13.0
Kestane	100.0	100.0	2.84	3.63 ab	88.0	88.0
Queensland Blue	100.0 [y]	100.0	6.48	6.09 a	100.0	75.0
Golden Delicious	100.0 [x]	100.0	3.50	2.04 bc	100.0	88.0
Thunder F1	50.0	0.0	0.82	0.00 c	75.0	13.0
Dickinson	0.0 [w]	12.5	0.00	0.00 c	0.0	13.0
P-value			0.0634	0.0068		

[z]Average area of fruit lesions were calculated using formula for the area of an ellipse. Pairwise comparisons were performed using Least-Square Means.
[y]Average incidence is based on 4 fruits harvested at 21dpp.
[x]Average incidence is based on 6 fruits harvested at 21dpp.
[w]Average incidence is based on 4 fruits harvested at 21dpp.

-Improved Dickinson (*Cucurbita moschata* Duchesne) breeding lines and their impact on processing quality: Chris Smart, Libby Indermaur, Colin Day, Taylere Herrmann with Michael Mazourek and Gregor Inzinna

To expand on preliminary work from 2022, a protocol was developed in consultation with Olga Padilla-Zakour (Cornell University, Department of Food Science) and the Cornell Food Venture Center Pilot Plant in Geneva, NY to process fruit at an industrial scale. Accessions, including two *C. moschata* breeding lines, plus both parents, 'Dickinson' and 'Bugle', were grown in a research field in Geneva, NY. Representative images of fruit from each accession are shown in **Figure 6.** For processing, fruit were washed, seeds were removed, and fruit flesh with skin from 20 kg of representative squash were steam cooked for 20 minutes ('Dickinson', TR2-03, and TR2-06) or 40 minutes ('Bugle') in 100% humidity at 210˚F in three replications. Cooked squash was processed, also in triplicate, in a Bertocchi CX extractor at 1950 rpm. Squash purée was cooked in a kettle to 180˚F and subsequently filled in ten 14oz, 300×407 2pc tinplate cans with lacquered bodies and ends per replication. Cans were sealed with an atmospheric closing machine and products were pasteurized in an industrial retort with a chamber temperature of 250˚F for 62 minutes. Cans were stored at room temperature (approximately 68˚F) for one month for later analysis.

For each accession, three cans from each processing run (nine cans total) were evaluated for ˚Brix, color, consistency, moisture content, and pH. Purée from a single brand of commercially available canned pumpkin (Libby's) was used as a comparison. Color was assessed with an UltraScan VIS Spectrophotometer by measuring the CIE color space values of L*, a*, and b*.

Consistency was measured with a Bostwick consistometer at both 30 seconds and 1 minute, and is reported as distance flowed in cm. Moisture content (%) was measured with a MX-50 Moisture Analyzer. Representative images of opened cans are shown in **Figure 7.**

Table 1. Fruit and canning quality assessments. Means were calculated per nine samples. Statistically significant differences between accessions were determined with the Tukey's HSD test, at P=0.05. Numbers followed by the same letter are not significantly different.

Accession	Dry Matter (%)	˚Brix	Bostwick 30 S (cm)	Bostwick 1 Min (cm)	Moisture (%)	pH
'Dickinson'	7.54 a	6.09 a	1.79 c	2.92 b	92.1 d	4.98 a
TR2-03	9.25 b	8.08 b	0.06 a	0.13 a	88.5 b	5.06 b
TR2-06	8.29 ab	7.21 ab	1.34 b	2.20 b	90.7 c	4.97 a
'Bugle'	16.05 c	12.64 c	0.00 a	0.00 a	82.9 a	5.28 c
Libby's	--	4.72 a	0.00 a	0.00 a	90.2 bcd	5.16 bc
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Figure 6. Representative fruit from the accessions evaluated in this study.

Figure 7. Representative cans showing purées evaluated in this study.

Integrated Disease Management Team

Integrated Disease Management Team and Personnel Working on Project

- Lina Quesada-Ocampo (NC State)
    - Yara Rosado (technician)
    - Mary Lorscheider (extension communicator)
    - Mariana Prieto (PhD student)
    - Ziaur Bhuyian (Postdoc)
- Mary Hausbeck (MSU)
    - Cheryl Engfehr (Extension support specialist)
    - Carmen M. Medina-Mora (technician)
    - David Perla (Research Technician)
    - John Spafford (Graduate student)
- Chris Smart (Cornell)
    - Colin Day (lab manager)
    - Taylere Herrmann (technician)
    - Libby Indermaur (PhD student)
    - Emma Nelson (PhD student)
- Anthony Keinath (Clemson)
    - Sierra Zardus
    - Anna Mothersbaugh
- Shaker Kousik (USDA-ARS)
    - Jennifer Ikerd
- Jonathan Schultheis (NC State)
    - Stuart Michel (Technician/MS Student)
    - Brandon Parker (Research Associate)
    - Baker Stickley (Technical Support - Hourly)
    - Andrew Pfefferkorn (Technical Support - Hourly)
    - Kaleb Holder (Technical Support - Hourly)
- Daniel Tregeagle (NC State)
    - Alice Kilduff (PhD Student)

4.1. Disease management information and recommendations (Year 1-4).

CucCAP website: From Sept. 1, 2020 until Feb. 29, 2024, the CucCAP website was visited by 52127 users with 65868 sessions and 143713 pageviews. A total of 475 news items were posted on the website including 280 posts of news from the CucCAP researchers and 320 crop and disease reports from regional Vegetable specialists. These posts were shared in 35 monthly newsletters sent to 167 subscribers since October 2020. Posts on the CucCAP website are social media including Facebook with 199 followers, Twitter with 318 followers, and LinkedIn with 41 followers. Cucurbit disease factsheets and links to integrated pest management resources are maintained and updated annually on the website. The CucCAP website events calendar shared notices of 75 regional commodity meetings, 53 education sessions, and 28 Scientific meetings.

Quesada: Since the start of the project, Quesada has provided diagnostics and disease management recommendations for 28 cucumber, 52 watermelon, 12 melon, 20 squash, and 15 pumpkin

samples submitted to the NC State Plant Disease and Insect Clinic. Quesada has also been involved in providing disease management recommendations through oral presentations, social media (Twitter: 7,483 (lab) + 4,101 (Quesada) followers, LinkedIn: 3,341 followers), and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US Vegetable Crop Handbook.

Schultheis provides cultivar/advance line (cultigen) study results that are posted regularly on the North Carolina State University cucurbit portal and shared on the CucCAP website.

Keinath posts disease management articles during the growing season in the SC Grower blog and in the American Vegetable Grower online magazine.

Smart provided recommendations to growers and extension educators across NY. Between August 2023 – March 2024, she responded to 123 text messages and 47 email messages about cucurbit diseases. Additionally, she made 7 farm visits including farms on Long Island, Hudson Valley, Capital District, and Western NY.

Hausbeck maintains a dedicated downy mildew page and fact sheets for Phytophthora on cucurbits.

4.2. Perform Multi-location, multi-isolate trials and pathogen population analyses.

4.2.1 Evaluation of cucurbit cultivars and breeding lines for disease resistance (Year 1-2).

Watermelon powdery mildew (Lead: Kousik, Secondary Site: Quesada):

Kousik (2021, 2022): Powdery mildew (PM) of watermelon (*Citrullus lanatus*) caused by *Podosphaera xanthii* is a major factor limiting production in greenhouses and open fields. In recent years, occurrence of PM has been increasing on watermelon across the United States. The disease continues to be a constant problem throughout the southeast. Our survey of watermelon researchers also indicated that powdery mildew was considered an important priority for research across the U.S.A. Several seed companies have developed commercial seeded and seedless watermelon varieties with powdery mildew resistance. The objective of this part of the project was to evaluate commercial watermelon varieties from seed companies for their reaction to locally prevailing powdery mildew pathogen in South Carolina. Experiments were conducted in 2021 and 2022 at the U.S. Vegetable Laboratory farm in Charleston, SC. The soil at the farm was Yonges loamy fine sand. The experimental design was a randomized complete block with four replications for each variety. Watermelon varieties were seeded in 50-cell jiffy trays and four-week-old seedlings were transplanted onto 91-cm wide raised. Beds were spaced 4.6 meters apart and covered with white plastic mulch. Plants were irrigated weekly using subsurface drip irrigation using a drip tape placed 2.54-cm below the top of the plastic mulched beds. Each variety plot was a single row of 5 plants spaced 46-cm apart with 2.7 meters spacing between plots. Vines of the watermelon plants were regularly turned every week so as to keep the plants from growing into the neighboring plots. Plants of germplasm line USVL677-PMS and cultivar Mickey Lee were used as susceptible controls. A USDA, U.S. Vegetable Laboratory developed germplasm line, USVL608-PMR resistant to powdery mildew was used as the resistant control. After bedding but before planting, the row middles were sprayed with Roundup Pro (1 pt/A), Dual Magnum (1 pt/A) and Sandea (1 oz/A) for weed management. Weeds between beds were controlled during the season with spot application of Roundup and by hand weeding. Powdery mildew occurs naturally at this location every year and hence plots were not inoculated. Plant foliage for each variety plot was rated for powdery mildew in 2021 on 22, 29 June, 6, 13 and 20 July using a 0-10 rating scale similar to the Horsfall and Barrett rating scale of increasing disease severity

(0=no visible symptoms of disease observed, 1=trace <1-3% on foliage, 2=3-6%, 3=6-12%, 5=25-50%, 7=75-87%, and 10= 97-100% area of leaf covered with PM). In 2022 seven weekly ratings were taken from May severity. 26 to July 8 as done during 2021. During each rating period ratings were recorded on lower leaves in the canopy. The underside of five lower leaves for each plot was observed to provide rating for each plot. The ratings were converted to the mid percentage points for analysis. Area under disease progress curves (AUDPC) was calculated for each plot and means were separated using Fisher's protected LSD (α=0.05).

A significant difference ($P \leq 0.0001$) in the response of watermelon varieties to powdery mildew over time was observed in both years (Table 1). The appearance of powdery mildew on these varieties was confirmed by the presence of conidia of the pathogen on the leaf surface microscopically. Significant disease development was observed on the susceptible cultivars USVL677-PMS and Mickey Lee, especially during the third and fourth ratings taken on 6 and 13 July in 2021. Based on AUDPC, all commercial varieties and the germplasm line USVL608-PMR and SP-6 were significantly more resistant compared to the susceptible controls in both years. In 2022 powdery mildew development was a little more severe and was observed on all the commercial varieties by end of the season (Table 1).

Table 1. Reaction of commercial watermelon varieties to natural powdery mildew infection at the U.S. vegetable Laboratory Farm in Charleston SC in 2021 and 2022.

Variety / Germplasm[z]	Seed Company	AUDPC (2021)[y]		PM Severity (%, July 13, 2021)[x]		AUDPC (2022)[y]		PM Severity (%, July 8, 2022)[x]	
USVL677	USDA	2107	a[w]	77	a	3123.3	a	81.5	a
Mickey Lee	Clifton	1663	b	82	a	2134.5	b	65.75	ab
7197HQ	BASF	415	c	21	b	795.4	c	62.5	abc
Embasy	BASF	234	cde	14	bcd	621.9	cd	51.5	bcd
Summerlicious	Syngenta	175	de	11	bcd	502.3	cde	43.75	b-e
Expert	Hazera	181	de	11	bcd	484.3	cde	30.38	efg
Excite	Hazera	321	cd	16	bc	476.4	cde	49.13	b-e
Endless Summer	Syngenta	98	e	6	cd	460.3	cde	43.75	c-f
Hazera-50036	Hazera	-		-		402.1	def	36.63	def
Suprema	Origene	398	c	19	b	356.6	d-g	27.13	fgh
Essense	Origene	-		-		161.4	efg	13.88	ghi

USVL608	USDA	75	e	4	d	90.2	fg	5.63	i
ORS6406A	Origene	121	de	5	cd	76.1	fg	9	hi
SP-6	Syngenta	62	e	4	cd	31.5	g	3.75	i

[z]Commercial watermelon varieties were kindly provided by the seed companies. yUSVL677-PMS and USVL608-PMR were developed at the USDA, ARS, US Vegetable Laboratory in Charleston, SC.
[y] Areas Under Disease Progress Curves (AUDPC) are presented. AUDPC was calculated based on weekly powdery mildew ratings. Powdery mildew ratings were recorded on a weekly basis for 5 weeks in 2021 and 7 weeks ratings in 2022 using a 0-10 rating scale similar to the Horsfall and Barrett rating scale of increasing disease severity (0=no visible symptoms of disease observed, 1=trace <1-3% on foliage, 2=3-6%, 3=6-12%, 5=25-50%, 7=75-87%, and 10= 97-100% area of leaf covered with PM). The mid percentage points were used in the AUDPC analysis.
[x]Powdery mildew (PM) rating recorded on July 13 in 2021 and on July 8, 2022.
[w]Means followed by the same alphabet are not significantly different (P=0.05).

Quesada (2021): The experiment was conducted at the Cherry Research Farm in Goldsboro, NC. Plots were single raised beds on 10-ft centers covered with white plastic mulch; 14-ft long with 10-ft fallow borders on each end and a non-treated guard row on one side. The previous year the field was planted with cucumber. Watermelon was transplanted on 21 May (2-ft in-row spacing, 7 plants/plot). Irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Watermelon varieties were randomized into four complete blocks. Disease severity was assessed on 16, 23 and 30 Jul and 5 Aug as percentage of total area colonized by *P. xanthii*. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's Protected LSD test to separate means.

Powdery mildew was first detected on 7 Jul at approximately 1% disease severity in the field. Disease progressed throughout the course of the experiment. ORS6406A and Embassy had the lowest levels of *P. xanthii*. The varieties Suprema, USVL 608, 7197 HQ, Excite and Summerlicious all had low levels of disease as well. In the table, varieties are sorted by the final disease severity rating on 5 Aug.

	Disease Severity[z] (%)			
Varieties	Jul-16	Jul-23	Jul-30	Aug-5
ORS6406A	2.8cd[y]	4.8c	10.0e	24.3e
Embassy	2.3d	4.3c	11.8de	26.3ef
Suprema	3.8cd	7.3bc	15.0cd	29.3de
USVL 608	2.5d	4.8c	13.0cde	30.0de
7197 HQ	3.0cd	5.8c	12.8cde	30.3de
Excite	4.0cd	7.5bc	16.8c	30.5de
Summerlicious	5.0bcd	8.0bc	15.5cd	31.0de
Expert	4.5cd	8.0bc	17.0c	31.5c
Endless Summer	7.5b	10.5b	17.0c	32.8cd
Sp-6	2.3d	5.8c	16.3c	36.0c
Mickey lee	5.5bc	11.0b	33.3b	50.0b
USVL 677	24.3a	56.3a	81.5a	94.0a

[z] Disease rating scale based on percent of total leaf area colonized by *P. xanthii*.
[y] Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Fisher's Protected LSD).

Quesada (2022): This experiment was conducted at the Cherry Farm Research Station in Goldsboro, NC. Research plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 10-ft fallow borders on each end. Watermelon was seeded on 5 Jun in the greenhouse, thinned to one plant per cell after emergence (2 seed/cell), and transplanted to the field on 15 Jun (2-ft in-row spacing, 7 plants/plot). Irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Thirteen cultivars were evaluated in a randomized complete block design with four repetitions. Disease severity per plot was assessed on 7, 14, 19, 25 July and 2, 9, 19 and 25 Aug. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's protected least significant differences (LSD) test to separate means.

Powdery mildew was first detected on 19 Jun at approximately 1% disease severity in the field. At the disease severity data obtained on 25 Aug the varieties Embasy and 50036 were statistically different from the varieties Mickey Lee and USVL677-PMS (susceptible checks). All varieties were statistically better than the susceptible check. The disease summary for the season (AUDPC) showed that all the varieties were statistically different from the susceptible checks (Mickey Lee and USVL677-PMS).

Varieties	Disease Severity[z] (%) 25 Aug	AUDPC[y]
Embasy	0.8 d[y]	39.25 c
7197 HQ	10.5 bc	123.00 c
Excite	16.8 bc	288.00 c
Expert	12.3 bc	191.25 c
Endless Summer	13.1 bc	200.25 c
Summerlicious	19.4 b	287.00 c
SP-6	15.0 bc	143.25 c
USVL677-PMS	93.7 a	1845.00 a
Mickey Lee	59.5 a	646.50 b
USVL608-PMR	13.8 bc	268.00 c
ORS6406A	6.1 c	90.00 c
Essence	22.2 b	286.75 c
50036	0.3 d	13.00 c

[z] Disease rating scale based on percent necrotic foliage caused by *P. xanthii*. / 25 Aug.
[y] Area under disease progress curve for total of all the foliar diseases present. AUDPC = $\sum_{i=1}^{n-1} \frac{y_i+y_{i+1}}{2} x(t_{i+1} - t_i)$
[x] Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Fisher's Protected LSD).

Watermelon Fusarium wilt (Lead: Schultheis, Secondary Site: Keinath):

Schultheis (2021, 2022): North Carolina studies were conducted in a *Fusarium*-infested field at the Central Crops Research Station, in Clayton NC in 2021 and 2022. *Fusarium* races 1, 2 and a virulent race 2 or race 3 were reported based on differential testing conducted by Syngenta in 2021. Ten commercial triploid watermelon cultigens (cultivars or advanced lines) were evaluated in 2021 and 12 cultigens were evaluated in 2022; with eight common cultigens being tested each year. There was a 92% correlation

between plants with *Fusarium* wilt symptoms and yield (i.e., lower *Fusarium* wilt incidence resulted in higher watermelon yields). Ten weeks after transplant, nine of the 10 entries had 75 to 100% *Fusarium* wilt incidence. Disease incidence was lowest in the Fascination cultivar and disease occurrence lagged behind the other cultivars over time. No *Fusarium* wilt incidence occurred when the Fascination cultivar was grafted to Carolina Strongback rootstock. Grafted Fascination plants yielded 1.9 marketable size fruit per plant which was superior when compared with all cultivars. The second highest yielding cultivar was Fascination which yielded 0.6 fruits per plant while all other cultivars yielded similarly producing 0.1 to 0.4 marketable fruit per plant. In 2022, two advanced Syngenta lines and 2 advanced HM Clause lines were included in the study to see if better *Fusarium* wilt tolerance could be achieved than in 2021. Fascination grafted to Carolina Strongback had the lowest *Fusarium* incidence (8%) 9 weeks after transplant (WAT). The cultivars in 2022 that were common to those evaluated in 2021 had a similar high *Fusarium* incidence (80 to 100%) 9 WAT. The two Syngenta lines were susceptible to *Fusarium* wilt (>90%) 9 WAT. HMC633810 (Eleanor) had the lowest *Fusarium* incidence (50%) on non-grafted plants 9 WAT. Although fruit sizes in 2022 were smaller than in 2021, total fruit weight per plot was highest with grafted Fascination, followed by Eleanor and was lowest with Shoreline which had 100% *Fusarium* incidence 9.WAT. The R square value between yield and disease incidence was 0.84.

Keinath (2021, 2022): The South Carolina trials were done in a *Fusarium*-infested field at the Clemson Coastal REC, a field that has had *Fusarium* races 1 and 2 in it since 2005. Ten cultivars were tested in 2021 and 12 cultivars were tested in 2022; eight cultivars were tested both years. The correlation between marketable weight per acre and the percentage of wilted plants at the end of the season (10 weeks in 2021 and 11 weeks in 2022) was highly significant ($r = -0.97$, $P = 0.001$). Based on this correlation, Fusarium wilt was the main reason yields differed among the eight cultivars. Fascination grafted onto Carolina Strongback citron rootstock yielded 40,000 pounds per acre and had no plants wilted (0 of 96 plants examined in the 2 years). On the other hand, Shoreline had the lowest yields in both years and the highest percentage of wilted plants. Disease ratings were similar for each cultivar in the two years, although disease was more severe in 2022 than in 2021. For example, the final wilt percentage on Shoreline was 68% in 2021 and 94% in 2022. Fusarium wilt symptoms continued to appear in 2022 after harvest started, likely due to the extremely dry conditions in June and high temperatures. Yields did not differ significantly between years. Postharvest quality was measured, but, in general, there were few differences among the cultivars. Hollow heart was relatively low, and most fruit with hollow heart had small cracks. There were more seeds per fruit in 2021 than in 2022. In 2022 grafted Fascination had more seeds per fruit, an average of 0.9 seed, than all other cultivars. In 2021, cultivars with more diseased plants had lower Brix, while Brix was relatively high for all cultivars in 2022, likely due to the dry weather. Flesh firmness differed among cultivars in both years; however, firmness varied each year. In general, Shoreline had the firmest fruit both years, firmer than all other cultivars.

Cucumber downy mildew (Lead: Hausbeck, Secondary Site: Quesada):

Hausbeck (2021): To evaluate cucumber cultivars and breeding lines for downy mildew (DM) resistance under Michigan's field conditions, a total of 4 pickling cucumber cultivars and 5 breeding lines were included in a field trial located at Michigan State University Plant Pathology Farm (MSU-PPF) in Lansing, MI. Prior to planting, the field was prepared following commercial production standards; the Capac loam soil was plowed and disced on 20-May and 1-Jun., respectively, and amended with 100 lb Urea and 45 lb Potash on 1-June. On 26-Jul., all seeds (80 seeds per cultivar or line) were directly seeded every 12 in onto raised beds (rows 20ft long, 8ft from center to center). Cultivars and lines were distributed in a complete randomized block design with four replications. To monitor natural DM infection, trap plants of the susceptible cultivar 'Valspik' were planted in an adjacent field. On 25-July, high levels of DM infection and typical DM symptoms were observed on trap plants. Starting on 15-August, Quadris (15.5 fl oz) and Torino (3.4 fl oz) were applied, as needed, to control the incidence of Alternaria and powdery mildew, respectively. Disease ratings (% foliar infection) were performed on 24-

and 29-Aug., and 5-September. The percentage of foliage with downy mildew symptoms and area under the disease progress curve (AUDPC) were calculated at the end of the season. Data were analyzed with SAS statistical software, version 9.4, using the PROC GLIMMIX procedure for a one-way ANOVA, with mean separation performed using Fisher's least significant difference (LSD) with a Tukey's adjustment.

On 24-August, disease severity in the susceptible check ('Straight-Eight') was 26.3% and increased significantly on 29-August (86.3%). On 29-August, 'Straight-Eight' was not different from 'Liszt', 'Gy14DH', and 'WI7822' but had more disease than all other cultivars. On 5 September, all cultivars had significantly less disease than 'Straight-Eight' except 'Liszt' and 'WI7822'; 'WI7088D' had significantly less disease (4.8%) than all other cultivars except 'Chaperon' and 'Peacemaker'. According to the area under the disease progress curve (AUDPC), 'WI7088D' was not different from 'Chaperon', 'Citadel', or 'Peacemaker'. Overall, this study shows that multiple cultivars evaluated ('WI7088D', 'Chaperon', 'Peacemaker', and 'Citadel') show moderate levels of resistance to the downy mildew pathogen, *Pseudoperonospora cubensis*, under natural infection conditions under high pathogen pressure in Michigan.

Cultivar	**Foliar infection (%)***							
	24-Aug		**29-Aug**		**5-Sep**		**AUDPC**	
Straight-Eight	26.3	a**	86.3	a	88.8	a	893.8	a
WI7088D	13.8	ab	8.0	d	4.8	e	99.0	d
Peacemaker	10.0	b	27.5	cd	23.8	de	273.1	cd
Chaperon	13.8	ab	26.3	cd	26.3	de	283.8	cd
Citadel	12.5	ab	31.3	cd	28.8	d	319.4	cd
WI7821	13.8	ab	48.8	bc	46.3	cd	488.8	bc
WI7822	13.8	ab	60.0	a-c	73.8	ab	652.5	ab
Gy14DH	17.5	ab	67.5	ab	65.0	bc	676.3	ab
Liszt	17.5	ab	70.0	ab	66.3	a-c	695.6	ab
P-value	0.0701		<0.0001		<0.0001		<0.0001	

*Based on visual estimation of foliage diseased (%).
**Column means with the same letter are not significantly different according to Fisher's Least Significant Difference (LSD Test; P=0.05) with Tukey's adjustment using ANOVA, SAS.

Quesada (2021): The experiment was conducted at the Horticultural Crops Research Station in Clinton, NC. Plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. This field was planted with cucumbers in 2020. Cucumber was directly seeded on 11 Aug (2-ft in-row spacing, 2 seed/hill) and thinned to one plant per hill after emergence (7 plants/plot). Irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Cucumber varieties were randomized into four complete blocks. Disease severity was assessed on 14, 23 and 29 Sep, 6 and 13 Oct as percent leaf area with necrosis per plot. Fruits were harvested on 22 and 27 Sep, 5 and 12 Oct. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's protected least significant difference (LSD) test to separate the means.

Downy mildew was first detected on 31 Aug at approximately 1% disease severity in the field and progressed throughout the course of the trial. PI-197088 had the lowest level of disease. All varieties were significantly better than the standard (Lizst) besides the Gy14. For the total marketable weight, the variety PI-197088 had the highest weight. For the total unmarketable, the variety Jumbo G/L had the most weight.

Variety	Disease Severity (%)[z] 14 Sep (Week 6)	Disease Severity (%)[y] 6 Oct (Week 9)	Total Marketable[x] (lbs/treatment)	Total Unmarketable (lbs/treatment)
PI-197088	5.0 g[w]	18.5 e	33.33 a	20.23 ab
Encounter	12.0 de	57.0 d	23.58 abc	12.6 bcd
Chaperon	9.5 ef	72.5 bc	22.5 a-d	14.4 a-d
Hyper C	16.5 c	57.0 d	28.95 ab	15.33 abc
Citadel	9.8 def	71.0 bc	21.55 a-e	13.28 a-d
Zircon	12.3 d	62.5 cd	18.98 b-e	13.1 a-d
Peacemaker	8.8 f	74.0 bc	18.13 b-e	14.28 a-d
Gy14Q2	16.8 c	71.5 bc	8.65 de	9.05 cd
Gy14	17.3 c	91.3 a	7.85 e	5.05 d
7204Q3	29.3 a	80.8 ab	9.9 cde	9.48 cd
Jumbo G/L	16.5 c	53.5 d	18.2 b-e	22.1 a
Liszt	20.3 b	86.0 a	13.05 cde	5.73 d

[z] Disease rating scale based on percent necrotic foliage caused by *P. cubensis* / Data point 14 Sep (Wk 6).
[y] Disease rating scale based on percent necrotic foliage caused by *P. cubensis*. / Data point 6 Oct (Wk 9).
[x] Marketable and non-marketable total yields (lbs./treatment).
[w] Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Fisher's Protected LSD).

Quesada (2022): The trial was performed at the Horticultural Crops Research Station in Clinton, NC. Experimental plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. Cucumber varieties were directly seeded on 21 Jun (2-ft in-row spacing, 2 seed/hill) and thinned to one plant per hill after emergence (7 plants/plot). Regular cultural practices like irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Twelve cultivars were tested in a randomized complete block design with four repetitions. Disease severity per plot was assessed on 20 and 29 Jul, 3, 10, 17 and 24 Aug. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's protected least significant differences (LSD) test to separate means.

Downy mildew was first detected on 18 Jul at approximately 5% disease severity in the field. The disease severity data obtained on 10 Aug, 7 weeks after planting the variety Peacemaker, was statistically different from the variety Liszt (susceptible control) but not for other varieties. The disease summary for the season (AUDPC) showed that all cucumber cultivars were statistically different from Lizst except the cultivar WI7821, being Peacemaker with the lowest disease value. Yields were assessed every week (4 data points) as marketable and non-marketable (summarized as total marketable and total non-marketable). For the marketable yields the variety Peacemaker and Chaperon were statistically better from the variety Liszt, but not for other treatments. For the non-marketable yields Gy14Q2 was the variety with more weight per treatment compared with Jumbo G/L that got the lowest weight per treatment.

Varieties	Disease Severity[z] (%) 10 Aug – Week 7	AUDPC[y]	Marketable Yields[x] (lbs./treatment)	Non-marketable Yields (lbs./treatment)
Hyper C	43.8 bc[w]	1118.50 bcd	43.75 ab	18.33 bcd
Encounter	35 cde	944.63 cd	44 ab	17.7 cd
Jumbo G/L	32.5 cde	952.75 cd	25.7 cd	8.5 e
WI7821	56.3 ab	1602.88 ab	14.28 e	29.8 a
WI7822	40 bcd	1190.00 bcd	18.15 de	13.75 de
PI 197088	30 cde	841.63 cd	35.55 bc	16.9 cd
Gy14DH	47.5 bc	1382.38 bc	18.55 de	17.9 cd
Peacemaker	20 e	625.25 d	52.7 a	16.15 cde
Citadel	47.5 bc	1306.13 bc	42.7 ab	22.25 abc
Chaperon	21.3 de	750.63 cd	51.55 a	20.75 bcd
Liszt	73.8 a	1941.75 a	30.7 c	13.1 de
Zircon	47.5 bc	1156.13 bcd	34.1 bc	26.5 ab

[z]Disease rating scale based on percent necrotic foliage caused by *P. cubensis*. / Week 7 after planting, 10 Aug.
[y]Area under disease progress curve for total of all the foliar diseases present. AUDPC = $\sum_{i=1}^{n-1} \frac{y_i+y_{i+1}}{2} x(t_{i+1} - t_i)$
[x]Marketable and non-marketable total yields (lbs./treatment).
[w]Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Fisher's Protected LSD).

Squash powdery mildew (Lead: Smart, Secondary Site: Hausbeck):

Smart (2022): Trial 1: To evaluate *C. pepo* accessions from the USDA germplasm collection, for PM resistance, Smart evaluated 198 lines (the same lines as the 201 that Hausbeck evaluated, but three accessions died in our hands). We evaluated the accessions in the greenhouse, with three replicates per accession and 5 plant plots in a randomized complete block design. Plants were rated once, two weeks after inoculation with *Podosphaera xanthii* conidia from leaves that were naturally infected in the field. Ratings were taken as % diseased leaf area on the surface of top 4th leaf, bottom 4th leaf, stem above 4th leaf, and stem below 4th leaf. Overall mean disease severity was highest in top 4th leaf (88.5%), followed by stem below 4th leaf (22.3%), bottom 4th leaf (9%), and least in stem above 4th leaf (6.5%). The accession Success PM was consistently resistant across reps, and accession 189 was also resistant.

Trial 2: The Smart lab also conducted a field trial with 6 *C. moschata* breeding lines for fruit processing quality, from Michael Mazourek's program. Plots were rated once for powdery mildew severity. This trial included 1 resistant parent, 1 susceptible parent, and 4 progeny. Representative fruit from each treatment were canned in fall 2022 to assess canning yield, dry matter, water separation, and color. Cultivars Butterfly and Bugle were more resistant than the others tested.

Hausbeck (2022): To evaluate squash breeding lines for powdery mildew (PM) resistance under Michigan's field conditions, a total of 201 entries from GWAS panel received from Cornell University (Mazourek) and University of Florida (Meru) were included in a strip field trial located at Michigan State University Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI. Prior

to planting, the field was prepared following commercial production standards; the sandy soil was plowed and disced on 20-May and 1-Jun., respectively, and amended with nitrogen (100lb/A), potassium (180lb/A), sulfur (25lb/A), and boron (2lb/A) on 31-May. On 23-August, all seeds (30 seeds per entry) were directly seeded every 6 inches onto raised beds (rows 15ft, 8 ft center to center) with no buffer rows. Each plot was divided into 3 "pseudoplots", where each pseudoplot consisted of a maximum of 10 plants. A field of squash (cv. 'Yellow squash') heavily PM infected and adjacent to the strip trial served as natural inoculum for this trial. To monitor natural PM infection, the development of PM colonies on susceptible lines were observed weekly, and disease rating was performed when at least 40 % of the foliage for the entire plot (i.e. max. 30 plants) of selected susceptible lines had PM colonies. On 10-October, 20 days after the first PM colonies were observed, the fourth leaf of one plant per pseudoplot was rated for % foliar infection at adaxial and abaxial surfaces and % coverage at the internodes below and above the fourth leaf. Overall, 63% (126 out of 201 entries) of the lines evaluated had an overall disease rating of less than or equal to 20% PM infection and 25% (50 out of 201 entries) of the lines evaluated had an overall disease rating of less than or equal to 40% PM infection. No single line evaluated had 100% PM infection, however 6% (13 out of 201 entries) of the lines evaluated had an overall disease rating of less than or equal to 80% PM infection. Lines with more than 40% PM should be considered susceptible while lines with 0% PM infection should be considered resistant to PM infection under the conditions of this trial. A total of 6 lines (3% of the total entries) had zero PM infection but the seed germination rate for these lines was reduced. A total of 6 lines (3% of the total entries) were not evaluated due to zero germination for these lines.

Hausbeck (2023) To Evaluate efficacy of biopesticides for control of powdery mildew on intermediately resistant acorn squash. The trial was established at the Michigan State University Plant Pathology Farm in Lansing, MI, in a field of Capac loam soil previously planted to squash. The field was plowed on 9 May and disced 15 May. Preplant fertilizer (130 lb/A urea and 130 lb/A potash) was applied and incorporated on 16 May. On 17 May, raised beds were formed in the field with black plastic mulch 12-ft apart with drip tape for irrigation and in-season fertilization. Biweekly mechanical cultivation and hand weeding was used for weed control. Planting occurred 31 May via transplanting Using three weeks olds seedlings. The cultivar used for this experiment was intermediately resistant 'Tiptop'. The treatments were arranged in a randomized complete block design with four replications. Each replication was 20 ft long with a 5-ft buffer between each plot in a row. Each week during the growing season the trial was fertilized with urea ammonium nitrate (28% N) at 1 gal/A through the drip tape. Admire Pro SC (10.5 fl oz/A) was applied through the drip tape on 5 and 24 Jul and Warrior CS (1.92 fl oz/A) was sprayed on 28 Jul for insect control. Spray treatments were applied on 19 and 27 Jul; 2, 9, 16, 24 and 31 Aug; and 7 Sep using a CO_2 backpack sprayer and a broadcast boom equipped with four XR8003 flat-fan nozzles spaced 18 in. apart, calibrated at 35 psi, and delivering 50 gal/A. Foliage was evaluated for disease severity (both upper and lower surface of the leaf) on 3, 14, 24 and 30 Aug; 7 and 14 Sep and for necrosis on 24 and 30 Aug; 7 and 14 Sep. Evaluations were conducted using a 0 to 100 % scale, with 0% = 0% foliar disease/necrosis and 100% = 100% foliar disease/necrosis. Area under the disease progress curve was calculated for the upper and lower leave surface and for necrosis using disease severity data. Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).

According to disease severity on the final rating date, there were no differences among treatments for either the upper or lower leaf surfaces. On the upper leaf surface, all treatments limited the area under the disease progress curve (AUDPC) compared to the untreated control except for Zonix L 500 ppm plus Kinetic L 6 oz/100 gal. No differences among treatments were observed for AUDPC on the lower leaf surface. According to the foliar disease severity on the final rating date, only treatments that included Sil-Matrix 3 qt./ 100 gal limited necrosis compared to the untreated control. According to AUDPC, only Zonix L 500 ppm plus Sil-Matrix SC 3 qt./ 100 gal had less disease than the untreated control.

Squash Phytophthora blight (Lead: Hausbeck, Secondary Site: Smart):

Hausbeck (2022): To evaluate squash cultivars and breeding lines for Phytophthora crown and rot resistance under Michigan's field conditions, a total of 30 squash breeding lines received from Cornell University (Mazourek: 16 entries) and University of Florida (Meru: 14 entries) were included in two independent trials (Trial 1 and Trial 2). Field plots were established at SWMREC, Benton Harbor, MI. Prior to planting, fields were prepared following commercial production standards: the sandy soil was plowed and disced, and amended with nitrogen (100lb/A), potassium (180lb/A), sulfur (25lb/A), and boron (2lb/A) on 31-May. For Trial 1 (Mazourek), on 7-Jul., thirty 3-week-old seedlings per line were transplanted onto raised beds covered with black polyethylene plastic at 18in apart from each. For Trial 2 (Meru), on 16-Aug., twenty 3-week-old seedlings per line were transplanted onto raised beds at 18in apart from each. Lines for each trial were distributed in a complete randomized block design with four (Mazurek) or three (Meru) replications. On 22-Jul. and 30-Aug. for Trial 1 and Trial 2, respectively, each plant was inoculated with 1 g of *P. capsici* (M. Hausbeck *P. capsici* collection, strains SP98 and 12889) at a 1:1 ratio) infested millet placed at the crown of each plant. On 12-Aug. and 12-Sept., the first symptoms of Phytophthora crown rot were observed in the susceptible control lines; 22-T1B-13 ('Golden Delicious') for Trial 1 and 'Early Prolific' for Trial 2. Plant death assessments were performed on; 12-, 19-, 26-Aug. and 1-, 6-, 13-Sept. for Trial 1, and 7-, 20-, and 27-Sept. for Trial 2. For both trials, the area under the disease progress curve (AUDPC) was calculated at the end of the season and data were analyzed with SAS statistical software, version 9.4, using the PROC GLIMMIX procedure for a one-way ANOVA, with mean separation performed using Fisher's least significant difference (LSD) with a Tukey's adjustment.

For Trial 1, on 12-August plant death in the susceptible line 22-T1B-13 ('Golden Delicious') was 1.9% and increased significantly on 13-September (75.3%). On 13-September, 5 lines (22-T1B-08, 22-T1B-06, 22-T1B-09, 22-T1B-10, and 22-T1B-11) were as susceptible as 'Golden Delicious'. Five lines (22-T1B-02, 22-T1B-04, 22-T1B-05, 22-T1B-07, and 22-T1B-12) were as resistant as the intermediate F1 lines 22-T1B-014 and 22-T1B-15 ('Autumn Cup' and 'Thunder', respectively) and the resistant line 22-T1B-16 ('Dickinson'). Resistance on 2 out of the 12 experimental lines tested (22-T1B-01 and 22-T1B-03) is still under segregation since plant death was not different from susceptible and resistant control lines. According to the area under the disease progress curve (AUDPC), 4 out of the experimental lines evaluated (22-T1B-02 22-T1B-04, 22-T1B-05, and 22-T1B-07) show promising levels of resistance to *P. capsici*.

For Trial 2, on 12-September plant death in the susceptible cultivar 'Early Prolific' was 44.4% and increased rapidly and significantly on 20-September (94.4%). On 27-September, one line (358-174) showed moderate resistance as moderate resistant controls (PI181761-36p-Lot 1 and PI181761-36p-Lot 3) and resistant control (SSS333-7). Three lines (SS2147, SS2071, and SS2078) were as resistant as the resistant controls (SS333-8 and SS69-72). According to the area under the disease progress curve (AUDPC), 3 out of the 10 experimental lines evaluated (SS2147, SS2071, and SS2078) show promising levels of resistance to *P. capsici* and 3 lines (358-195, SS792-2, and 358-164) are still under segregation.

Trial 1 (Seed lot supplied by M. Mazourek-Cornell, NY)

Line Evaluated 22-ID-CRR	Plant death (%)													
	12-Aug		19-Aug		26-Aug		1-Sep		6-Sep		13-Sep		AUDPC	
22-T1B-08	32.2	ab[z]	31.3	ab	30.2	ab	35.5	a-e	32.8	a-d	83.3	a	1211.3	ab
22-T1B-13	1.9	d	4.0	cd	4.0	b-d	55.5	ab	61.5	ab	75.3	ab	998.6	a-c
22-T1B-06	22.3	a-d	53.1	a	62.5	a	66.1	a	65.6	a	59.4	a-c	1821.2	a
22-T1B-09	53.6	a	32.1	a-c	38.8	ab	52.7	a-c	56.3	a	45.5	a-d	1451.6	ab
22-T1B-10	3.1	d	7.3	b-d	12.5	b-d	27.1	a-e	34.4	a-d	41.7	a-d	644.3	a-d
22-T1B-11	24.9	a-c	31.9	a-c	32.5	a-d	35.0	a-e	48.4	a-c	41.1	a-c	1148.7	a-c
22-T1B-03	10.0	cd	10.0	cd	33.9	a-d	31.7	a-e	36.9	a-d	37.2	b-e	851.4	a-d
22-T1B-01	20.7	a-d	25.3	a-c	18.1	a-d	34.8	a-d	29.9	a-d	26.8	a-e	831.2	a-c
22-T1B-05	9.4	cd	9.4	b-d	21.9	b-d	25.0	c-e	29.6	a-d	25.0	c-e	642.9	b-d
22-T1B-12	9.6	b-d	17.3	a-d	23.9	a-c	23.1	a-e	19.2	a-d	17.3	c-e	612.9	a-d
22-T1B-02	2.8	d	0.0	d	8.3	b-d	17.8	a-e	8.7	d	14.2	c-e	263.7	dc
22-T1B-04	6.8	cd	9.1	b-d	6.8	b-d	9.1	de	18.0	a-d	11.2	c-e	328.9	cd
22-T1B-15	4.6	d	6.5	b-d	4.2	b-d	4.2	e	4.2	d	4.2	de	151.4	cd
22-T1B-14	4.6	d	2.3	cd	2.3	dc	2.3	e	6.4	cd	4.2	e	112.3	d
22-T1B-16	5.8	d	3.9	cd	0.0	d	3.9	e	3.9	d	3.8	e	104.8	d
22-T1B-07	9.6	b-d	7.7	b-d	5.8	b-d	9.6	b-e	7.7	b-d	1.9	e	230.8	dc

[z] Letters in common within each column are not significantly different (LSD t-Test; *P*=0.05).

Trial 2 (Seed lot supplied by G. Meru-Univ. of Florida, FL)

	Plant death (%)							
Line Evaluated	**12-Sep**		**20-Sep**		**27-Sep**		**AUDPC**	
Early Prolific (S)	44.4 a	[z]	94.4	a	94.4	a	1216.7	a
358-174	27.8	a	27.8	b	27.8	bc	416.7	b
SS333-7 (R)	22.2	ab	22.2	bc	27.8	b	352.8	bc
PI181761-36p-Lot 3	16.7	a-c	22.2	b-d	22.2	b-d	311.1	b-d
PI181761-36p-Lot 1	16.7	a-c	16.7	b-d	16.7	b-d	250.0	b-d
SS2061	5.6	bc	11.1	c-e	11.1	c-e	144.4	c-e
358-195	0.0	c	0.0	e	5.6	de	19.4	de
SS792-2	11.1	a-c	5.6	de	5.6	de	105.6	de
358-164	5.6	bc	5.6	de	5.6	de	83.3	de
SS2147	0.0	c	0.0	e	0.0	e	0.0	e
SS69-72 (R)	0.0	c	0.0	e	0.0	e	0.0	e
SS2071	0.0	c	0.0	e	0.0	e	0.0	e
SS333-8 (R)	0.0	c	0.0	e	0.0	e	0.0	e
SS2078	0.0	c	0.0	e	0.0	e	0.0	e

[z] Letters in common within each column are not significantly different (LSD t-Test; *P*=0.05).

To evaluate breeding lines for Phytophthora fruit rot Age-Related Resistance (ARR), fruits from a total of 12 squash breeding lines and 4 squash cultivars were collected from the field and infected with *P. capsici* (strain SP98) under laboratory conditions. A pollination plot was established at MSU-PPF, East Lansing, MI and prepared following commercial production standards; the Capac loam soil was plowed and disced on 20-May and 1-Jun., respectively, and amended with 100 lb Urea and 45 lb Potash on 1-June. On 7-Jul., transplants (30 seeds per cultivar or line) were directly seeded every 18 in onto raised beds (rows 100ft X 16ft center to center) covered with black polyethylene plastic. Until fruits were harvested, 28% fertilizer (1gal/A) was applied weekly and non-target diseases and insects were controlled. Starting on 7-Sept., a mixture of Torino (3.4 fl oz) and Bravo (32 fl oz) was applied, as needed, to control the incidence of powdery mildew, and Admire Pro (10.5 fl oz) was delivered through drip tape to control insects. To facilitate fruit set and reduce natural flower abortion, flowers at anthesis were hand-pollinated using an artist's paint brush and marked at the petiole using colored-flagging tape. Because flower development among the lines was asynchronized, the presence of flowers at anthesis in each line was monitored daily for 30 days and hand-pollination was performed every other day during this time. A total of 790 flowers were hand-pollinated to harvest a maximum of 12 fruits corresponding to 21 days post-pollination (dpp) and 12 fruits corresponding to 28dpp per line. A 4 mm mycelial plug of a 7-day-old culture of *P. capsici* (strain SP98) was placed onto the surface/epidermis of each disinfected fruit on the same day each fruit was harvested. Fruit rot was evaluated 5 days post-inoculation (dpi) and disease assessment included: 1) lesion size, 2) incidence of hyphae beyond inoculation point, and 3) disease severity based on a 0-4 categorical scale (0= healthy, 1=water-soaked lesion, 2= light mycelial growth, 3=moderate mycelial growth, 4= severe mycelial growth).

Overall, fruits corresponding to 3 breeding lines (22-T1A-02, 22-T1A-11, and 22-T1A-12) show a reduction of disease incidence (%) over time. A 30-60% incidence of fruit rot was observed when fruits corresponding to 21dpp were inoculated with *P. capsici*, however fruit rot was not observed for fruits corresponding to 28dpp from the 3 experimental lines indicated above. These results indicate age-related

resistance for these 3 lines as previously observed for resistant control lines 22-T1A-14 ('Autumn Cup') and 22-T1A-16 ('Dickinson').

	disease incidence (%)*		P of fruit rot incidence **	
Line 22-ID-ARR	**21dpp**	**28dpp**	**21dpp**	**28dpp**
22-T1A-01	90.0 [z]	70.0	0.96	0.78
22-T1A-02	33.3 [y]	0.0 [y]	0.93	0.02
22-T1A-03	0.0	0.0	0.03	0.04
22-T1A-04	10.0	20.0	1.00	0.97
22-T1A-05	NF	0.0 [x]	1.00	0.02
22-T1A-06	0.0	50.0	0.03	0.41
22-T1A-07	10.0	10.0	1.00	0.98
22-T1A-08	0.0	10.0	0.03	0.98
22-T1A-09	44.4	NF	0.95	1.00
22-T1A-10	50.0	50.0	0.93	0.63
22-T1A-11	60.0	0.0	0.85	0.03
22-T1A-12	30.0	0.0	0.98	0.03
22-T1A-13 (Golden Delicious)	50.0	30.0	0.93	0.92
22-T1A-14 (Autumn Cup F1)	40.0	0.0	0.96	0.04
22-T1A-15 (Thunder F1)	40.0	33.3	0.96	0.88
22-T1A-16 (Dickinson)	0.0	0.0	0.03	0.04

** Based on logistic model; value is the probability for each fruit to have an incidence of "1" at a P-value of 0.05

To evaluate squash breeding lines for Phytophthora crown and root rot resistance at the seedling stage, a greenhouse trial at Michigan State University Plant Science Greenhouses in East Lansing, MI was established. The trial consisted of a total of 8 interspecific breeding lines (BC2F1) and 3 parental lines provided by Dr. Mazourek (Cornell Univ.). On 26-May, 18 seeds per line were directly seeded onto 3x3in plastic containers containing SureMix soilless medium. On 29-June, when seedlings were at 3-4 leaf stages, all seedlings were inoculated with a zoospore suspension ($2x10^4$/ml) of *Phytophthora capsici* (M. Hausbeck *P. capsici* collection, strains SP98 and 12889). Lines were distributed in a complete randomized block design with three replications. Disease ratings based on a 0-5 categorical scale (0= healthy, 1=small lesion at crown, 2= water-soaked lesions beyond cotyledons, 3=wilt and plant partially collapsing, 4= severe wilt and pant completely collapsing, and 5=plant death) were conducted once a week after inoculation on 7-, 13-, and 20-July. A preliminary analysis of the results indicated differences in resistance among the lines tested; 2 lines were more susceptible than the susceptible parental line (cv. 'Golden Delicious') and 6 lines were more susceptible than the resistant parental lines (cv. 'Dickinson' and F1:21-2253x2262). Furthermore, differences in response among the individuals (i.e. each seedling) within each line indicated that resistance is still segregating.

	Average disease rating (0-5)*		
Breeding Line Evaluated	**6-Jul**	**13-Jul**	**20-Jul**
18-A181 (Dickinson) (R)	0.0 b	0.1 b	0.9 cd
21-2253x2262 (F1)	0.0 b	0.0 b	0.4 d
22-2293-01	0.0 b	0.4 b	2.6 a-d
22-2293-02	0.4 b	1.7 ab	4.6 a
22-2293-03	0.3 b	0.4 b	2.4 a-d
22-2293-04	0.3 b	0.8 b	3.0 a-c
22-2293-06	0.0 b	0.7 b	2.1 b-d
22-2293-07	0.2 b	0.8 b	2.2 b-d
22-2293-09	0.2 b	1.4 b	3.7 ab
22-2293-10	0.0 b	1.0 b	3.9 ab
GWAS-197-1 (Golden Delicious) (S)	1.4 a	2.9 a	2.9 b

* Disease rating based on a 0-5 categorical scale (0= healthy, 1=small lesion at crown, 2= water-soaked lesions beyond cotyledons, 3=wilt and plant partially collapsing, 4= severe wilt and plant completely collapsing, and 5=plant death).
**Values are the means of back transformed data using "rank minus 1".

Smart (2022): Trial 1: Greenhouse trial of 14 squash breeding lines with potential resistance to *Phytophthora capsici* from Geoffrey Meru performed in a RCBD and inoculated with a NY isolate of *Phytophthora capsici*. Breeding line SS69-72 had a significantly lower AUDPC relative to all other lines except SSS337-7. Line SSS337-7 had a significantly lower AUDPC than 358-174, 358-195, and Early Prolific. All other AUDPC values between breeding lines were not significantly different from each other.

Trial 2: Field trial with 16 *C. maxima* accessions for fruit processing quality, from Michael Mazourek's program. This included 12 entries and 4 controls. Representative fruit from each treatment were canned in fall 2022 to assess canning yield, dry matter, water separation, and color.

Hausbeck 2023 To Evaluate efficacy of conventional and biorational products to control Phytophthora crown rot of cucurbits using a partially resistant hard squash (*Cucurbita maxima)* cultivar. This study was conducted at the Michigan State University Southwest Research and Extension Center located near Benton Harbor, MI on sandy soil previously planted to squash. The field was plowed, disced, and preplant fertilizer (potassium 180 lb/A, sulfur 25 lb/A, and boron 2.0 lb/A) was applied on 19 May. On 26 May, 6-in. raised plant beds covered with black polyethylene plastic were laid spaced 16 ft apart. A single drip tape (0.65 gpm/100 ft) was installed under the plastic mulch for plot irrigation. On 2 Jun, 3-week-old 'Thunder' winter squash plants were transplanted 18 in. apart. Fertilizer (urea ammonium nitrate 28% N) was applied weekly at a rate of 1 gal/A/day through the drip tape. For each treatment, a replicate consisted of a single 20-ft row with a 5-ft buffer within the row to separate treatments. Treatments were arranged in a randomized complete block design with four replicates. On 23 Jun, plants were inoculated with *P. capsici*-infested millet (100 g sterilized millet, 72 ml distilled water, 0.08 g asparagine, and 7 7-mm plugs of *P. capsici*). *P. capsici* isolates 12889 (A1 mating type, sensitive to mefenoxam, isolated from cucumber) and SP98 (A2 mating type, sensitive to mefenoxam, isolated from pumpkin) were used to infest the millet and were mixed 1:1 prior to inoculation. Holes were made 1 cm from the plant crown and 2 g of millet was inserted. Fungicides were applied using a CO_2 backpack sprayer for soil drench

applications (100 ml/plant) using a single-nozzle boom with one 8006EVS nozzle calibrated at 35 psi to deliver 100 gal/A. Foliar applications were applied using a CO_2 backpack sprayer with three XR8003 flat-fan nozzles spaced 18 in apart calibrated at 35 psi to deliver 50 gal/A. Fungicides were applied on 20, 27, 30 Jun and 7, 11, 18 Jul for the 7-day interval treatments. Fungicide treatments were applied on 20, 30 Jun and 11, 25 Jul for 14-day interval treatments. Dead plants were counted on 18, 25, 31 Jul and 7 Aug and the percentage of dead plants was calculated by dividing the number of dead plants by the total number of plants in a plot (10) and multiplying by 100. The area under the disease progress curve (AUDPC) was calculated using the percentage of dead plants. Data were analyzed using an analysis of variance using (ANOVA) SAS PROC GLIMMIX procedure of the SAS software version 9.4 (SAS Institute, Cary, NC), with mean separation performed using Fisher's protected least significant difference (LSD) at $P<0.05$.

Disease pressure was relatively low (<28% of plat death in all treatments) at the initial rating date on 18 Jul, and no statistical differences (P=0.1438) were observed among the treatments. On the following evaluation date, 25 Jul, the untreated control reached 77.5% plant death. All the treatments expressed significantly (P=0.0144) lower (<35.0 %) percent of plant death than the untreated control. Presidio 4 fl oz expressed the lowest percent of plant death of the treatments evaluated. However, the rest of the treatments were statistically (P=0.0144) similar to Presidio 4 fl oz except Funibiol Gold 32 fl oz and Theia 1.5 lb. + Howler EVO 2.5 lb. Funibiol Gold 32 fl oz and Theia 1.5 lb + Howler EVO 2.5 lb were significantly better than the untreated control and similar to each other at the second rating date. On the final rating date, the untreated control developed 97.5 % of plant death. All treatments statistically (P=0.0249) reduced plant death compared to the untreated control on the final rating date, except Theia 1.5 lb. Theia 1.5 lb. was similar to the untreated control and all other fungicide treatments; all the treatments were similar for plant death on the final rating date. According to the area under the disease progress curve (AUDPC), all treatments had significantly (P=0.0008) less disease than the untreated control. Presidio 4 fl oz had the lowest AUDPC but was similar to all other fungicide treatments except Theia 1.5 lb. and Theia 1.5 lb. plus Howler EVO 2.5 lb. When combined with host resistance, Biorational could efficiently manage Phytophthora crown rot.

4.2.2 Evaluation of integrated disease management in cucurbits combining host resistance and chemical control (Year 3-4).

Watermelon Fusarium wilt (Lead: Schultheis, Secondary Site: Keinath):

Schultheis, Keinath (2023): Triploid watermelon cultivars 'Fascination' and 'Eleanor' were evaluated for disease incidence and yield when treated with one of three fungicide programs (prothioconazole (Proline), prothioconazole + fluopyram (Propulse), Proline and Propulse) while growing in fields infested with *Fusarium oxysporum* f. sp. *niveum*. 'Fascination', 'Eleanor', and 'Shoreline' also were evaluated for disease incidence and yield in grafted (G) and un-grafted (UG) (control) treatments. The study was conducted at two locations at research stations in Clinton, NC, and Charleston, SC, and used a randomized complete block design with four replications. Transplants were established on 3 May 2023 in NC and on 5 April 2023 in SC. Ten triploid plants made up each plot in NC and 12 plants in SC. Four pollenizers, SP-7 and G SP-7, were used per plot. Disease incidence was rated for eight weeks starting on 24 May 2023 in NC and on 25 April 2023 in SC. Proline at 5.7 oz/ac and Propulse at 13.6 oz/ac was applied to the corresponding plots via drip irrigation the day after transplanting. An additional drip application of Proline was applied to the plots receiving the Proline and Propulse treatment 15 days after transplanting. Yield data were collected over 4 harvests in NC from 20 July to 11 August 2023. In SC, yield data were collected over 8 harvests from 19 June to 7 August 2023. G 'Fascination', 'Eleanor', and 'Shoreline' had the lowest disease incidence at both locations with ≤0.6% incidence. Un-grafted

‘Shoreline’ had the highest disease incidence 11 weeks after transplanting at both locations with ≥95.0% incidence. The three G treatments had the highest yields at both locations and produced ≥34,317 lb/ac in NC and ≥33,832 lb/a in SC. In NC the three G treatments yielded higher than all other treatments. . Un-grafted ‘Shoreline’ had the lowest yields at both locations with 0 lb/ac in NC and 916 lb/ac in SC. In this study, grafting was a more effective management option than fungicides against Fusarium wilt of seedless watermelon.

Cucumber downy mildew (Lead: Hausbeck, Secondary Site: Quesada):

Quesada (2023): The experiment was performed at the Horticultural Crops Research Station in Clinton, NC. Experimental plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. Susceptible pickling cucumber ‘Vlasik’ and two tolerant pickling cucumber cultivars, ‘Citadel’ and ‘Chaperon’, were directly seeded on 2 Aug (2-ft in-row spacing, 2 seed/hill) and thinned to one plant per hill after emergence (7 plants/plot). Regular cultural practices like irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Three chemical program treatments and non-treated control were tested in a factorial design with each cultivar in a randomized complete block design with four repetitions. Fungicide treatments were applied using a CO2-pressurized backpack sprayer equipped with a single-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 35 psi on Aug 30, Sep 6, Sep 15, Sep 20, Sep 27 and Oct 4. Disease severity per plot was assessed every week. Yields were assessed every week (3 data points) as marketable and non-marketable (summarized as total marketable and total non-marketable). Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher’s protected least significant differences (LSD) test to separate means.

Downy mildew was first detected on 30 Aug at approximately 2% disease severity in the field. No phytotoxicity was observed for any treatments in the experiment. Only 6 foliar applications were able to be applied because disease at the end of the trial reached 100%, and the trial was concluded. Disease severity in the non-treated ‘Vlaspik’ and ‘Citadel’ plots were significantly different from ‘Chaperon’ non-treated plots. Area under disease progress curve values were significantly different across each variety and varied by fungicide program for each variety. Marketable yields were variable across treatment, with ‘Chaperon’ Howler Evo+Dyne-Amic+Kocide+Dyne-Amic+Citadel producing 21.20 lbs/treatment and ‘Vlaspik’ Howler Evo+Dyne-Amic+Kocide+Dyne-Amic+Citadel producing 10.28 lbs/treatment.

	Treatments	Rate	Application Code[z]	Disease Severity[y] (%) 20 Sep	AUDPC[x]	Marketable Yields (lbs./treatment)
1	Vlaspik	–	–	38.8 a[w]	1918.25 ab	11.98 cd
2	Orondis Opti	2.5 pt/a	AE	36.3 ab	1993.25 a	12.40 cd
	Kocide	1.25 lb ai/a	AE			
	Ranman	2.75 fl oz/a	BF			
	Howler Evo	1.25 lb ai/a	BF			
	Dyne-Amic	0.375 % v/v	BF			
	Previcur Flex	1.2 pt/a	C			
	Kocide	1.25 lb ai/a	C			
	Dyne-Amic	0.375 % v/v	C			
	Zampro	14 fl oz/a	D			
	Howler Evo	1.25 lb ai/a	D			
	Dyne-Amic	0.375 % v/v	D			
	Vlaspik	–	–			
3	Howler Evo	1.25 lb ai/a	ACE	41.3 a	1974.25 a	10.28 d
	Dyne-Amic	0.375 % v/v	ACE			
	Kocide	1.25 lb ai/a	BDF			
	Dyne-Amic	0.375 % v/v	BDF			
	Vlaspik	–	–			
4	Orondis Opti	2.5 pt/a	AE	30.0 abc	1380.00 cd	18.98 abc
	Ranman	2.75 fl oz/a	BF			
	Bravo Weather Stik	2 pt/a	BF			
	Previcur Flex	1.2 pt/a	C			
	Bravo Weather Stik	2 pt/a	C			
	Zampro	14 fl oz/a	D			
	Bravo Weather Stik	2 pt/a	D			
	Vlaspik	–	–			
5	Citadel	–	–	33.5 ab	1513.57 c	17.96 abc
6	Orondis Opti	2.5 pt/a	AE	25.0 bcd	1533.00 bc	16.23 a-d
	Kocide	1.25 lb ai/a	AE			
	Ranman	2.75 fl oz/a	BF			
	Howler Evo	1.25 lb ai/a	BF			
	Dyne-Amic	0.375 % v/v	BF			
	Previcur Flex	1.2 pt/a	C			
	Kocide	1.25 lb ai/a	C			
	Dyne-Amic	0.375 % v/v	C			
	Zampro	14 fl oz/a	D			
	Howler Evo	1.25 lb ai/a	D			
	Dyne-Amic	0.375 % v/v	D			
	Citadel	–	–			
7	Howler Evo	1.25 lb ai/a	ACE	16.8 de	977.00 ef	11.90 cd
	Dyne-Amic	0.375 % v/v	ACE			
	Kocide	1.25 lb ai/a	BDF			
	Dyne-Amic	0.375 % v/v	BDF			
	Citadel	–	–			

8	Orondis Opti	2.5 pt/a	AE	21.8 cd	943.63 ef	13.98 bcd
	Ranman	2.75 fl oz/a	BF			
	Bravo Weather Stik	2 pt/a	BF			
	Previcur Flex	1.2 pt/a	C			
	Bravo Weather Stik	2 pt/a	C			
	Zampro	14 fl oz/a	D			
	Bravo Weather Stik	2 pt/a	D			
	Citadel	–	–			
9	Chaperon	–	–	12.5 de	1054.88 def	16.90 a-d
10	Orondis Opti	2.5 pt/a	AE	17.2 de	1150.29 cde	18.54 abc
	Kocide	1.25 lb ai/a	AE			
	Ranman	2.75 fl oz/a	BF			
	Howler Evo	1.25 lb ai/a	BF			
	Dyne-Amic	0.375 % v/v	BF			
	Previcur Flex	1.2 pt/a	C			
	Kocide	1.25 lb ai/a	C			
	Dyne-Amic	0.375 % v/v	C			
	Zampro	14 fl oz/a	D			
	Howler Evo	1.25 lb ai/a	D			
	Dyne-Amic	0.375 % v/v	D			
	Chaperon	–	–			
11	Howler Evo	1.25 lb ai/a	ACE	9.0 e	710.50 f	21.20 a
	Dyne-Amic	0.375 % v/v	ACE			
	Kocide	1.25 lb ai/a	BDF			
	Dyne-Amic	0.375 % v/v	BDF			
	Chaperon	–	–			
12	Orondis Opti	2.5 pt/a	AE	15.8 de	1372.75 cd	20.43 ab
	Ranman	2.75 fl oz/a	BF			
	Bravo Weather Stik	2 pt/a	BF			
	Previcur Flex	1.2 pt/a	C			
	Bravo Weather Stik	2 pt/a	C			
	Zampro	14 fl oz/a	D			
	Bravo Weather Stik	2 pt/a	D			
	Chaperon	–	–			

[z]Application code based on application date: A = 30 Aug, B = 6 Sep, C = 15 Sep, D = 20 Sep, E= 27 Sep, F= 4 Oct

[y]Disease rating based on percent necrotic foliage caused by *P. cubensis*, 7 weeks after planting.

[x]Area under disease progress curve AUDPC =

[w]Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Fisher's Protected LSD).

Hausbeck (2023): The experiment was established at the Michigan State University Southwest Michigan Research and Extension Center near Benton Harbor, MI, in sandy soil previously planted to cucurbits. The field was plowed and disced, and pre-plant fertilizer (180 lb/A potassium, 25 lb/A sulfur, and 2.0 lb/A boron) was applied and incorporated on 19 May. On 26 May, raised beds were formed in the field with black plastic mulch 8 ft apart with drip tape for irrigation and in-season fertilization. Biweekly mechanical cultivation and hand weeding were used for weed control. Planting occurred on 25 Jul from seed. The cultivars used for this experiment were cucurbit downy mildew (CDM) susceptible 'Vlaspik' and CDM intermediately resistant 'Citadel' and 'Chaperon'. Three fungicide programs, including a standard program, a mix of standard and an organic program, and an organic program, were compared to an untreated control in this trial. The treatments were arranged in a split-plot design with the fungicide program nested within the cultivar and four replications. Each replicate row was 20 ft long with a 5 ft buffer between each plot in a row. In-season fertilization occurred with applications of urea ammonium nitrate (1.0 gal/A/day) through the drip tape daily. Fungicide treatments were applied using a CO_2 backpack sprayer and a broadcast boom equipped with three XR8003 flat-fan nozzles spaced 18 in apart, calibrated at 35 psi, and delivering 50 gal/A. Fungicide applications occurred on 7, 14, 23 and 28 Aug; 5, 15 and 19 Sep. The percent of disease severity was visually assessed using a 0-100 scale on 23 and 30 Aug; 8, 11, 21, and 28 Sep. Mature fruits were harvested on 15 and 21 Sep. Data were analyzed using an analysis of variance using (ANOVA) SAS PROC GLIMMIX procedure of the SAS system 9.4 (SAS Institute, Cary, NC). Least-square means comparisons were performed using Tukey's honestly significant difference test (HSD $P<0.05$). According to ANOVA, significance (HSD $P<0.05$) was detected for cultivars, programs, and the interaction of cultivar*program.

According to marginal means within cultivars, the cultivars 'Citadel' and 'Chaperon' developed statistically (HSD $P<0.05$) less disease severity than 'Vlaspik' during the evaluation period as well as the AUDPC, while 'Citadel' and 'Chaperon' were statistically similar (HSD $P<0.05$) for disease severity and AUDPC. According to the program's marginal means, all the programs statistically limited (HSD $P<0.05$) disease severity and AUDPC compared to the untreated control except for the organic program (Howler EVO 2.5 lb plus Dyne-Amic 0.375% V/V alternated with Kocide 1.25 lb plus Dyne-Amic 0.375% V/V). The mixed program (Orondis Opti 2.5 pt + Kocide 1.25 lb, alternating with Ranman + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, alternated with Previcur Flex 1.2 pt + Kocide 1.15 lb + Dyne-Amic 0.375% V/V alternated with Zampro 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V) and the standard program (Orondis Opti 2.5 pt alternated with Ranman 2.75 fl oz + Bravo WeatherStik 2 pt alternated with Previcur Flex 1.2 pt + Bravo WeatherStik 2 pt alternated with Zampro 14 fl oz plus Bravo WeatherStik 2 pt) similarly limited the disease infection. By observing the simple effect of the fungicide programs sliced by cultivars, 'Vlaspik' plants significantly (HSD $P<0.05$) have less disease when sprayed with the mixed and standard program (6.3% and 1.3% of disease severity) than those plants left untreated or sprayed with the organic program (81.3% and 71.3% of disease severity) at final rating date and the AUDPC. The disease pressure was consistently lower in the 'Citadel' plants than in 'Vlaspik' plants regardless of the treatment sprayed. Non-statistical differences were observed when 'Citadel' plants were untreated or treated with the programs at the final rating day (0% to 12.5 % of disease severity). According to the AUDPC, the mixed and standard programs had a lower AUDPC than the untreated control and the organic programs. The organic program had a similar AUDPC than the untreated control. Disease pressure on 'Chaperon' plants was the lowest of all the cultivars tested; all programs tested were similar to the untreated control at the final rating date (0.0 % to 3.8 % of disease severity) and AUDPC. There was no interaction for harvest data, and no differences were observed among treatments for yield. In Michigan, the management of cucurbit downy mildew could be improved by combining genetic resistance and fungicides.

	Foliar Disease Severity (%)[z]							**Yield (lb)**
Cultivars	23 Aug	30 Aug	8 Sep	11 Sep	21 Sep	28 Sep	AUDPC[y]	Total 20-ft plot
Vlaspik	9.1 a[x]	9.2 a	13.8 a	15.1 a	24.3 a	39.9 a	631.5 a	1.7 a
Chaperon	1.2 b	0.7 b	0.4 b	0.8 b	1.3 b	1.2 b	32.0 b	1.2 a
Citadel	3.3 b	2.1 b	2.8 b	4.6 b	5.7 b	6.3 b	145.5 b	1.7 a
P-value	0.0033	<.0001	<.0001	<.0001	0.0004	<.0001	<.0001	0.2059

[z]Based on visual estimation of the percentage of foliage with downy mildew symptoms.
[y]Area under the disease progress curve.
[x]Means with a letter in common or not letter are not significantly different according to Tukey's honestly significant difference test (HSD; P<0.05).

Treatment Program[z], and rate/A, applied at 7-day intervals, *application schedule*	Foliar Disease Severity (%)[y]						AUDPC[x]	**Yield (lb)** Total 20-ft plot
	23 Aug	30 Aug	8 Sep	11 Sep	21 Sep	28 Sep		
Untreated	9.1 a[w]	8.9 a	10.7 a	12.9 a	21.7 a	32.5 a	549.0 a	1.7 a
Orondis Opti SC 40 fl oz, *apps A, E* -alt- Ranman SC 2.75 fl oz + Bravo WS SC 32 fl oz, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Bravo WS SC 32 fl oz, *apps C, G* -alt- Zampro SC 14 fl oz + Bravo WS SC 32 fl oz, *apps D*	0.0 b	0.2 c	0.8 b	1.0 b	0.9 b	0.6 b	22.7 b	1.8 a
Orondis Opti SC 40 fl oz + Kocide 3000-O DF1.25 lb A, *apps A, E* -alt- Ranman SC 2.75 fl oz+ Howler EVO 2.5 lb + Dyne-Amic 0.375% SL V/V, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Kocide 3000-o DF 1.15 lb + Dyne-Amic 0.375% V/V, *apps C, G* -alt- Zampro SC 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps D*	0.8 b	1.2 c	1.5 b	1.9 b	1.4 b	2.1 b	53.0 b	1.6 a
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps A, C, E, G* -alt- Kocide 3000-O DF 1.25 lb + Dyne-Amic 0.375% V/V, *apps B, D, F*	8.1 a	5.8 b	9.5 a	11.4 a	17.7 a	28.1 a	454.0 a	1.6 a
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9623

[z]Bravo WS=Bravo Weatherstik; -alt-=alternate

[y]Based on visual estimation of the percentage of foliage with downy mildew symptoms.

[x]Area under the disease progress curve.

[w]Means with a letter in common or not letter are not significantly different according to Tukey's honestly significant difference test (HSD; P<0.05).

Treatment Program[z], and rate/A, applied at 7-day intervals, *application schedule*	Vlaspik												Yield (lb)	
	Foliar Disease Severity (%)[y]													
	23 Aug		30 Aug		8 Sep		11 Sep		21 Sep		28 Sep		AUDPC[x]		Total 20-ft plot	
Untreated	18.8	a[w]	20.0	a	25.0	a	26.3	a	50.5	a	81.3	a	1259.9	a	1.6	a
Orondis Opti SC 40 fl oz, *apps A, E* -alt- Ranman SC 2.75 fl oz + Bravo WS SC 32 fl oz, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Bravo WS SC 32 fl oz, *apps C, G* -alt- Zampro SC 14 fl oz + Bravo WS SC 32 fl oz, *apps D*	0.0	b	0.0	c	1.5	c	1.5	c	1.8	c	1.0	b	37.1	b	1.7	a
Orondis Opti SC 40 fl oz + Kocide 3000-O DF1.25 lb A, *apps A, E* -alt- Ranman SC 2.75 fl oz+ Howler EVO 2.5 lb + Dyne-Amic 0.375% SL V/V, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Kocide 3000-o DF 1.15 lb + Dyne-Amic 0.375% V/V, *apps C, G* -alt- Zampro SC 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps D*	2.5	b	3.3	c	4.5	b	5.5	b	4.3	c	6.3	b	155.5	b	2.1	a
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps A, C, E, G* -alt- Kocide 3000-O DF 1.25 lb + Dyne-Amic 0.375% V/V, *apps B, D, F*	15.0	a	13.5	b	24.0	a	27.0	a	40.5	b	71.3	a	1073.6	a	1.3	a
P-value	<.0001		<.0001		<.0001		<.0001		<.0001		<.0001		<.0001		0.7900	

Treatment Program[z], and rate/A, applied at 7-day intervals, *application schedule*	Vlaspik														Yield (lb)	
	Foliar Disease Severity (%)[y]															
	23 Aug		30 Aug		8 Sep		11 Sep		21 Sep		28 Sep		AUDPC[x]		Total 20-ft plot	
Untreated	18.8	a[w]	20.0	a	25.0	a	26.3	a	50.5	a	81.3	a	1259.9	a	1.6	a
Orondis Opti SC 40 fl oz, *apps A, E* -alt- Ranman SC 2.75 fl oz + Bravo WS SC 32 fl oz, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Bravo WS SC 32 fl oz, *apps C, G* -alt- Zampro SC 14 fl oz + Bravo WS SC 32 fl oz, *apps D*	0.0	b	0.0	c	1.5	c	1.5	c	1.8	c	1.0	b	37.1	b	1.7	a
Orondis Opti SC 40 fl oz + Kocide 3000-O DF1.25 lb A, *apps A, E* -alt- Ranman SC 2.75 fl oz+ Howler EVO 2.5 lb + Dyne-Amic 0.375% SL V/V, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Kocide 3000-o DF 1.15 lb + Dyne-Amic 0.375% V/V, *apps C, G* -alt- Zampro SC 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps D*	2.5	b	3.3	c	4.5	b	5.5	b	4.3	c	6.3	b	155.5	b	2.1	a
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps A, C, E, G* -alt- Kocide 3000-O DF 1.25 lb + Dyne-Amic 0.375% V/V, *apps B, D, F*	15.0	a	13.5	b	24.0	a	27.0	a	40.5	b	71.3	a	1073.6	a	1.3	a
P-value	<.0001		<.0001		<.0001		<.0001		<.0001		<.0001		<.0001		0.7900	

[z]Bravo WS=Bravo Weatherstik; -alt-=alternate

[y]Based on visual estimation of the percentage of foliage with downy mildew symptoms.

[x]Area under the disease progress curve.

[w]Means with a letter in common or not letter are not significantly different according to Tukey's honestly significant difference test (HSD; P<0.05).

Treatment Program[z], and rate/A, applied at 7-day intervals, *application schedule*	Citadel												Yield (lb)	
	Foliar Disease Severity (%)[y]													
	23 Aug		30 Aug		8 Sep		11 Sep		21 Sep		28 Sep		AUDPC[x]		Total 20-ft plot	
Untreated	6.3	a[w]	5.0	a	6.0	a	10.0	a	10.8	a	12.5	a	298.0	a	1.2	a
Orondis Opti SC 40 fl oz + Kocide 3000-O DF1.25 lb A, *apps A, E* -alt- Ranman SC 2.75 fl oz+ Howler EVO 2.5 lb + Dyne-Amic 0.375% SL V/V, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Kocide 3000-o DF 1.15 lb + Dyne-Amic 0.375% V/V, *apps C, G* -alt- Zampro SC 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps D*	0.0	b	0.3	a	0.0	c	0.3	b	0.0	b	0.0	a	3.6	b	1.4	a
Orondis Opti SC 40 fl oz, *apps A, E* -alt- Ranman SC 2.75 fl oz + Bravo WS SC 32 fl oz, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Bravo WS SC 32 fl oz, *apps C, G* -alt- Zampro SC 14 fl oz + Bravo WS SC 32 fl oz, *apps D*	0.0	b	0.5	a	1.0	bc	1.5	b	1.0	b	0.8	a	30.9	b	0.4	a
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps A, C, E, G* -alt- Kocide 3000-O DF 1.25 lb + Dyne-Amic 0.375% V/V, *apps B, D, F*	6.8	a	2.8	a	4.0	ab	6.5	a	11.3	a	12.0	a	249.5	a	1.3	a
P-value	<.0001		0.0617		0.0013		0.0003		0.0343		0.0776		0.0067		0.6818	

[z]Bravo WS=Bravo Weatherstik; -alt-=alternate

[y]Based on visual estimation of the percentage of foliage with downy mildew symptoms.

[x]Area under the disease progress curve.

[w]Means with a letter in common or not letter are not significantly different according to Tukey's honestly significant difference test (HSD; P<0.05).

Treatment Program[z], and rate/A, applied at 7-day intervals, *application schedule*	Chaperon							Yield (lb)
	Foliar Disease Severity (%)[y]							Total
	23 Aug	30 Aug	8 Sep	11 Sep	21 Sep	28 Sep	AUDPC[x]	20-ft plot
Untreated	2.3 ab[w]	1.8 a	1.0 a	2.5 a	3.8 a	3.8 a	89.1 a	2.0 a
Orondis Opti SC 40 fl oz + Kocide 3000-O DF1.25 lb A, *apps A, E* -alt- Ranman SC 2.75 fl oz+ Howler EVO 2.5 lb + Dyne-Amic 0.375% SL V/V, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Kocide 3000-o DF 1.15 lb + Dyne-Amic 0.375% V/V, *apps C, G* -alt- Zampro SC 14 fl oz + Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps D*	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	1.3 a
Orondis Opti SC 40 fl oz, *apps A, E* -alt- Ranman SC 2.75 fl oz + Bravo WS SC 32 fl oz, *apps B, F* -alt- Previcur Flex SL 19.2 fl oz + Bravo WS SC 32 fl oz, *apps C, G* -alt- Zampro SC 14 fl oz + Bravo WS SC 32 fl oz, *apps D*	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	3.2 a
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V, *apps A, C, E, G* -alt- Kocide 3000-O DF 1.25 lb + Dyne-Amic 0.375% V/V, *apps B, D, F*	2.5 a	1.0 a	0.5 a	0.8 a	1.3 a	1.0 a	38.8 a	2.2 a
P-value	0.0116	0.7511	0.7616	0.5083	0.8381	0.9163	0.7539	0.4389

[z]Bravo WS=Bravo Weatherstik; -alt-=alternate

[y]Based on visual estimation of the percentage of foliage with downy mildew symptoms.

[x]Area under the disease progress curve.

[w]Means with a letter in common or not letter are not significantly different according to Tukey's honestly significant difference test (HSD; P<0.05).

Squash powdery mildew (Lead: Smart, Secondary Site: Hausbeck):

Smart (2021, 2022): **Year 1:** Smart conducted a field trial evaluating 9 biofungicides to control powdery mildew. Kocide 3000-O (Certis) was the most effective at reducing powdery mildew severity followed by Theia (AgBiome), compared to the untreated control. **Year 2:** Field trial evaluating 11 biorational fungicides (two copper products and ten biologicals) to control powdery mildew. Kocide 3000-O was again the most effective at reducing disease severity, followed by Theia, Dyne-Amic (Helena Agri), and Curezin (VM Agritech), compared to the untreated control.

Hausbeck (2022): A trial was established at the Michigan State University Plant Pathology Farm in East Lansing, MI, in a field of Capac loam soil previously planted to pumpkin. The field was plowed on 20 May and disced 1-June. Preplant fertilizer (80 lb. per acre N and 105 lb. per acre of K) was applied and incorporated on 1-June. On 2-June, raised beds were formed in the field with black plastic mulch 12 ft apart, and drip tape (0.65 gpm/100 ft) for irrigation and in-season fertilization. Biweekly mechanical cultivation and hand weeding was used for weed control. Planting occurred 13-June via transplanting. The cultivar used for this experiment was 'Tiptop' which has intermediate resistance to powdery mildew. The treatments were arranged in a completely randomized block design with four replications. Each replication was 20 ft with a 5 ft buffer between each plot in a row. Each week during the growing season the trial was fertilized with 28% N liquid fertilizer at 1 gal per acre through the drip tape. Presidio (4 fl oz/A) was applied on 21-July. For control of Phytophthora crown rot, Admire Pro was applied through the drip lines on 20-June. for insect control. Spray treatments were applied on 29-July and 5-, 12-, 19-, 26-Aug. using a CO_2 backpack sprayer and a broadcast boom equipped with four XR8003 flat-fan nozzles spaced 18 in. apart, calibrated at 35 psi and delivering 50 gal/A. Foliage was evaluated for infection (%) (both upper and lower side) on 18-, 24-, 29-Aug. and for necrosis on 5-Sep. Area under the disease progress curve was calculated using foliar infection for the upper side of the leaf and using foliar infection for the lower side of the leaf. Area under the disease progress curve was calculated using foliar infection for the upper side of the leaf and using foliar infection for the lower side of the leaf.

Disease on the lower side of the leaf progressed from 3.8% (18-Aug.) to 51.3% (29-Aug.) in the untreated control. A significant increase in disease occurred for all treatments between 18- and 24-August. On 24-August, all treatments had significantly less disease than the untreated control. On the final rating date (29-Aug.), all treatments differed from the untreated control except MBI-121 and Theia + Activator 90 alternated with Microthiol Disperss. According to the area under the disease progress curve (AUDPC), all treatments differed from the untreated control but not each other. Disease on the upper side of the leaf progressed from 2% (18-Aug.) to 45% (29-Aug.) over the course of the trial. According to disease severity on the final rating date (29-Aug.) and AUDPC only treatments that included Microthiol Disperss differed from the untreated control. According to foliar necrosis on 5-Sep., all treatments differed from the untreated control except Trillium and treatments containing Microthiol Disperss had less necrosis overall. No phytotoxicity was observed. In general, programs with either Microthiol Disperss alone or in a program were the only treatments that consistently limited powdery mildew disease progress in our study.

Treatment[z] and rate/A, *application schedule,* applied at 7-day intervals	**Foliar infection on lower leaf surface (%)[y]**							
	18-Aug	**24-Aug**		**29-Aug**		**AUDPC[x]**	
Untreated control	3.8[w]	31.3	a	51.3	a	311.3	a
Microthiol Disperss WP 5 lb., *A-E*	0.3	8.8	b	21.3	d	102.0	b
MBI-121 EC 3 pt., *apps ACE -alt-* Microthiol Disperss WP 5 lb., *BD*	1.3	12.5	b	25.0	b-d	135.0	b
Theia WP 3 lb. + Activator 90 0.125% V/V, *A-E*	0.8	13.8	b	21.3	cd	131.0	b
Theia WP 3 lb. + Activator 90 0.125% V/V, *ACE -alt-* Microthiol Disperss WP 5 lb., *BD*	0.5	15.3	b	40.0	ab	185.4	b
MBI-121 EC 3 qt, *A-E*	2.8	16.3	b	38.8	a-c	194.5	b
Trillium EC 1% V/V, *A-E*	1.3	16.3	b	26.3	b-d	158.8	b
P-value	0.3259	0.0152		0.0112		0.0057	

[z]-alt- = alternate.
[y] Based on visual estimation.
[x]AUDPC = Area under the disease process curve.
[w]Column means with no letter or a letter in common are not significantly different (LSD t-Test; P=0.05).

Treatment[z] and rate/A, *application schedule,* applied at 7-day intervals	**Foliar infection on upper leaf surface (%)[y]**							**Foliar necrosis (%)[y]**	
	18-Aug	**24-Aug**	**29-Aug**		**AUDPC[x]**			**5-Sep**	
Untreated control	2.0[w]	22.8	45.0	a	243.6	a		57.5	a
Microthiol Disperss WP 5 lb., *A-E*	0.3	2.0	6.3	c	27.4	c		27.5	d
MBI-121 EC 3 pt., *ACE -alt-* Microthiol Disperss WP 5 lb., *BD*	0.3	5.3	10.0	bc	54.6	bc		30.0	cd
Theia WP 3 lb. + Activator 90 0.125% V/V, *A-E*	1.0	10.0	17.5	ab	101.8	ab		38.8	b-d
Theia WP 3 lb. + Activator 90 0.125% V/V, *ACE -alt-* Microthiol Disperss WP 5 lb., *BD*	1.5	10.0	15.0	bc	97.0	bc		42.5	bc
MBI-121 EC 3 qt, *A-E*	2.5	11.3	21.3	ab	122.5	ab		42.5	bc
Trillium EC 1% V/V, *A-E*	0.8	8.0	28.8	ab	118.1	ab		45.0	ab
P-value	0.3692	0.1097	0.0168		0.0218			0.003	

[z]-alt- = alternate.
[y] Based on visual estimation.
[x]AUDPC = Area under the disease process curve.
[w]Column means with no letter or a letter in common are not significantly different (LSD t-Test; P=0.05).

Hausbeck (2023): The trial was established at the Michigan State University Plant Pathology Farm in Lansing, MI, in a field of Capac loam soil previously planted to squash. The field was plowed on 9 May and disced 15 May. Preplant fertilizer (130 lb/A urea and 130 lb/A potash) was applied and incorporated on 16 May. On 17 May, raised beds were formed in the field with black plastic mulch 12-ft apart with

drip tape for irrigation and in-season fertilization. Biweekly mechanical cultivation and hand weeding was used for weed control. Planting occurred 31 May via transplanting Unsing three weeks olds seedlings. The cultivar used for this experiment was intermediately resistant 'Tiptop'. The treatments were arranged in a randomized complete block design with four replications. Each replication was 20 ft long with a 5-ft buffer between each plot in a row. Each week during the growing season the trial was fertilized with urea ammonium nitrate (28% N) at 1 gal/A through the drip tape. Admire Pro SC (10.5 fl oz/A) was applied through the drip tape on 5 and 24 Jul and Warrior CS (1.92 fl oz/A) was sprayed on 28 Jul for insect control. Spray treatments were applied on 19 and 27 Jul; 2, 9, 16, 24 and 31 Aug; and 7 Sep using a CO_2 backpack sprayer and a broadcast boom equipped with four XR8003 flat-fan nozzles spaced 18 in. apart, calibrated at 35 psi, and delivering 50 gal/A. Foliage was evaluated for disease severity (both upper and lower surface of the leaf) on 3, 14, 24 and 30 Aug; 7 and 14 Sep and for necrosis on 24 and 30 Aug; 7 and 14 Sep. Evaluations were conducted using a 0 to 100 % scale, with 0% = 0% foliar disease/necrosis and 100% = 100% foliar disease/necrosis. Area under the disease progress curve was calculated for the upper and lower leave surface and for necrosis using disease severity data. Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).

According to disease severity on the final rating date, there were no differences among treatments for either the upper or lower leaf surfaces. On the upper leaf surface, all treatments limited the area under the disease progress curve (AUDPC) compared to the untreated control except for Zonix L 500 ppm plus Kinetic L 6 oz/100 gal. No differences among treatments were observed for AUDPC on the lower leaf surface. According to the foliar disease severity on the final rating date, only treatments that included Sil-Matrix 3 qt./ 100 gal limited necrosis compared to the untreated control. According to AUDPC, only Zonix L 500 ppm plus Sil-Matrix SC 3 qt./ 100 gal had less disease than the untreated control.

Treatment and rate/A, applied at 7-day intervals	Foliar Disease Severity (%) – Upper Leaf Surface[z]										AUDPC[y]	
	14 Aug		24 Aug		30 Aug		7 Sep		14 Sep			
Untreated	4.3	a[x]	14.5	a	23.0	a	9.8	a	9.3	a	403.8	a
Sil-Matrix SC 3 qt./100 gal	1.5	a	6.3	b	12.0	d	6.3	a	7.8	a	215.5	c
Zonix L 500 ppm + Sil-Matrix SC 3 qt./100 gal	2.8	a	6.8	b	15.0	cd	9.8	a	9.8	a	280.0	bc
Zonix L 500 ppm	2.3	a	7.8	b	17.5	bc	8.8	a	7.3	a	286.8	bc
Zonix L 300 ppm	3.0	a	8.3	b	18.3	a-c	9.8	a	8.0	a	309.9	b
Zonix L 500 ppm + Kinetic L 6 oz/100 gal	5.3	a	8.8	b	20.8	ab	11.0	a	5.3	a	342.4	ab

[z]Based on visual estimation of the percentage of the upper leaf surface covered with powdery mildew colonies.

[y]Area under the disease progress curve.

[x]Column means with a letter in common or not letter are not significantly different (LSD t test; P=0.05).

Treatment and rate/A, applied at 7-day intervals	Foliar Disease Severity (%) – Lower Leaf Surface[z]					AUDPC[y]
	14 Aug	24 Aug	30 Aug	7 Sep	14 Sep	
Untreated	1.8[x]	7.0	10.5	8.8	6.8	227.5
Zonix L 500 ppm + Sil-Matrix SC 3 qt./100 gal	0.5	4.3	8.3	6.0	6.5	162.0
Zonix L 500 ppm	1.0	4.8	9.5	7.8	6.5	190.4
Sil-Matrix SC 3 qt./100 gal	1.8	4.0	8.8	8.8	7.8	194.8
Zonix L 300 ppm	2.3	4.0	9.5	9.0	6.5	200.0
Zonix L 500 ppm + Kinetic L 6 oz/100 gal	1.3	4.8	11.3	9.8	4.8	212.8

[z]Based on visual estimation of the percentage of the lower leaf surface covered with powdery mildew colonies.
[y]Area under the disease progress curve.
[x]Column means with a letter in common or not letter are not significantly different (LSD t test; P=0.05).

Treatment and rate/A, applied at 7-day intervals	Foliar Disease Severity (%) – Necrosis[z]								AUDPC[y]	
	24 Aug		30 Aug		7 Sep		14 Sep			
Untreated	1.3	a[x]	3.0	a	18.0	a	48.5	ab	329.5	ab
Zonix L 500 ppm + Sil-Matrix SC 3 qt./100 gal	0.5	a	1.8	a	9.8	a	35.5	c	211.1	c
Sil-Matrix SC 3 qt./100 gal	0.8	a	2.3	a	15.5	a	35.8	c	259.4	bc
Zonix L 500 ppm	0.8	a	2.0	a	14.8	a	47.0	ab	291.4	a-c
Zonix L 300 ppm	0.8	a	2.3	a	17.5	a	43.8	bc	302.4	ab
Zonix L 500 ppm + Kinetic L 6 oz/100 gal	1.0	a	2.0	a	20.8	a	54.8	a	364.3	a

[z]Based on visual estimation.
[y]Area under the disease progress curve.
[x]Column means with a letter in common or not letter are not significantly different (LSD t test; P=0.05).

Smart (2023): One trial evaluating the effectiveness of integrating breeding lines with biorational fungicides to manage powdery mildew (PM): There were 2 lines and 1 susceptible control, and 3 fungicides with 1 untreated control. Fungicides included Curezin (copper zinc), Kocide 3000-O (copper hydroxide), and Theia (Bacillus subtilis). One breeding line, TR2-03, consistently had the least disease, regardless of fungicide treatment. Within breeding lines, we found no impact of PM severity on yield. The susceptible control yielded significantly more fruit weight per plot than the other lines. However, the susceptible control was no different than TR2-06 in fruit number per plot. TR2-03 yielded significantly less than both the susceptible control and TR2-06 in both fruit number and weight per plot. We conclude that, of the products evaluated, host resistance played a larger role than fungicides in reducing PM.
One trial evaluating the canning quality of processing pumpkin breeding lines: There were 2 lines, plus both parents, 'Dickinson' and 'Bugle'. 'Bugle' is a powdery mildew-resistant butternut squash, and 'Dickinson' is a standard processing cultivar. Fruit from each line fruit were puréed, canned, and compared to both parents, with assessments including moisture content (%), ˚Brix, pH, consistency, and color. Purées from the two breeding lines were more similar to 'Dickinson' than 'Bugle'.

Squash Phytophthora blight (Lead: Hausbeck, Secondary Site: Smart):

Hausbeck (2022): A study was conducted at the Michigan State University Southwest Research and Extension Center located near Benton Harbor, MI on sandy soil previously planted to squash. Preplant fertilizer (nitrogen 100 lb./A, potassium 180 lb./A, sulfur 25 lb./A, and boron 2.0 lb./A) was applied on 31 May. On 7-June 3-week-old 'Enterprise' summer squash plants were transplanted 18 in. apart into 6-in. raised plant beds covered with black polyethylene plastic and spaced 8 ft apart. A single drip tape (0.65 gpm/100 ft) was installed under the plastic mulch for plot irrigation. Fertilizer (nitrogen 28%) was applied weekly at a rate of 1 gal/A through the drip irrigation. For each treatment, a replicate consisted of a single 20-ft row with a 5-ft buffer within the row to separate treatments. Treatments were arranged in a randomized block design with four replicates. On 5-July, plants were inoculated with *P. capsici*-infested millet (100 g sterilized millet, 72 ml distilled water, 0.08 g asparagine, and seven 7-mm plugs of *P. capsici*). *P. capsici* isolates 12889 (A1 mating type, sensitive to mefenoxam, isolated from cucumber) and SP98 (A2 mating type, sensitive to mefenoxam, isolated from pumpkin) were used to infest the millet and were mixed 1:1 prior to inoculation. Holes were made 1 cm from the plant crown and 1 g of millet was inserted. Fungicides were applied with a CO_2 backpack sprayer as a soil drench (100 ml/plant) using a hand-wand without nozzle at 20 psi. Fungicide treatments were applied on 1-, 8-, 15-, and 22-July. Dead plants were counted on 19-, 22-, 26-, 29-Jul., and 2 August. Data were analyzed using an analysis of variance (ANOVA), with mean separation performed using Fisher's Protected Least Significant Difference (LSD).

The percentage of dead plants progressed over the course of the study from 30.8% to 75% for the untreated control plots from 19 July to 2 August. Differences among treatments were not observed on 19-July. Presidio SL was the most effective treatment with <10% dead plants on the last rating date (2-Aug), significantly less than Double Nickel LC treatment but not significantly different from the remaining treatments. Mega 128, Rootshield Plus WP, MGCI Phytalix, and Double Nickel LC did not differ significantly from the untreated control ($p \leq 0.05$). The AUDPC data indicated that Presidio SL was the only effective product and that Mega 128, Rootshield Plus WP, MGCI Phytalix, and Double Nickel LC were the least effective products for controlling Phytophthora crown rot in 2022.

Treatment and rate/A, *application schedule*[z], applied at 7-day intervals	**Plant death (%)**										
	19-Jul	**22-Jul**		**26 -Jul**		**29-Jul**		**2-Aug**		**AUDPC[x]**	
Untreated control	30.8[y]	51.9	a	63.5	a	73.1	a	75.0	a	855.8	a
Presidio SL, 4 fl oz, *apps A,B*	1.9	1.9	b	3.8	b	3.8	b	9.6	b	55.8	b
Mega 128 fl oz, *apps A-D*	7.7	32.7	ab	34.6	ab	36.5	ab	38.5	ab	451.9	ab
Rootshield Plus WP 32 oz, *apps A,C*	13.5	32.7	ab	36.5	b	38.5	ab	32.7	ab	462.5	ab
MGCI Phytalix 10 fl oz, *apps A-D*	17.3	34.6	ab	50.0	a	48.1	ab	51.9	ab	594.2	a
Double Nickel LC 64 fl oz, *apps A-D*	13.5	42.3	a	50.0	a	67.3	a	69.2	a	717.3	a

[z] *apps* = applications. A=1 Jul, B=8 Jul, C=15 Jul, D=22 Jul.

[y] Column means with a letter in common or no letter are not significantly different (LSD t-Test; *P*=0.05).

[x] Area Under the Disease Progress Curve.

Smart (2022): The identical trial to that of Hausbeck in MI was conducted in NY. We tested product efficacy against *Phytophthora capsici* on squash. The experiment included 4 biofungicides, 1 conventional fungicide (Ridomil) and an untreated control (exactly as described above by Hausbeck). Plants were inoculated on August 4, 2022 using *Phytophthora capsici* growing in vermiculite. One gram (about 1 teaspoon) of inoculum was buried 1 centimeter from the crown of each plant. To determine the zoospore concentration of the inoculum, we placed one gram in 100 ml of water (repeated three times) and used a hemacytometer to determine the number of zoospores per one gram of inoculum. This was 2 x 10^6 zoospores per one gram of inoculum. Plants were rated 6 times. Only Ridomil was effective in reducing disease in the NY trial. Plants in all treatments other than Ridomil died within two weeks of inoculation.

Hausbeck (2023):
This study was conducted at the Michigan State University Southwest Research and Extension Center located near Benton Harbor, MI on sandy soil previously planted to squash. The field was plowed, disced, and preplant fertilizer (potassium 180 lb/A, sulfur 25 lb/A, and boron 2.0 lb/A) was applied on 19 May. On 26 May, 6-in. raised plant beds covered with black polyethylene plastic were laid spaced 16 ft apart. A single drip tape (0.65 gpm/100 ft) was installed under the plastic mulch for plot irrigation. On 2 Jun, 3-week-old 'Thunder' winter squash plants were transplanted 18 in. apart. Fertilizer (urea ammonium nitrate 28% N) was applied weekly at a rate of 1 gal/A/day through the drip tape. For each treatment, a replicate consisted of a single 20-ft row with a 5-ft buffer within the row to separate treatments. Treatments were arranged in a randomized complete block design with four replicates. On 23 Jun, plants were inoculated with *P. capsici*-infested millet (100 g sterilized millet, 72 ml distilled water, 0.08 g asparagine, and 7 7-mm plugs of *P. capsici*). *P. capsici* isolates 12889 (A1 mating type, sensitive to mefenoxam, isolated from cucumber) and SP98 (A2 mating type, sensitive to mefenoxam, isolated from pumpkin) were used to infest the millet and were mixed 1:1 prior to inoculation. Holes were made 1 cm from the plant crown and 2 g of millet was inserted. Fungicides were applied using a CO_2 backpack sprayer for soil drench applications (100 ml/plant) using a single-nozzle boom with one 8006EVS nozzle calibrated at 35 psi to deliver 100 gal/A. Foliar applications were applied using a CO_2 backpack sprayer with three XR8003 flat-fan nozzles spaced 18 in apart calibrated at 35 psi to deliver 50 gal/A. Fungicides were applied on 20, 27, 30 Jun and 7, 11, 18 Jul for the 7-day interval treatments. Fungicide treatments were applied on 20, 30 Jun and 11, 25 Jul for 14-day interval treatments. Dead plants were counted on 18, 25, 31 Jul and 7 Aug and the percentage of dead plants was calculated by dividing the number of dead plants by the total number of plants in a plot (10) and multiplying by 100. The area under the disease progress curve (AUDPC) was calculated using the percentage of dead plants. Data were analyzed using an analysis of variance using (ANOVA) SAS PROC GLIMMIX procedure of the SAS software version 9.4 (SAS Institute, Cary, NC), with mean separation performed using Fisher's protected least significant difference (LSD) at $P<0.05$.

Disease pressure was relatively low (<28% of plat death in all treatments) at the initial rating date on 18 Jul, and no statistical differences ($P=0.1438$) were observed among the treatments. On the following evaluation date, 25 Jul, the untreated control reached 77.5% plant death. All the treatments expressed significantly ($P=0.0144$) lower (<35.0 %) percent of plant death than the untreated control. Presidio 4 fl oz expressed the lowest percent of plant death of the treatments evaluated. However, the rest of the treatments were statistically ($P=0.0144$) similar to Presidio 4 fl oz except Funibiol Gold 32 fl oz and Theia 1.5 lb. + Howler EVO 2.5 lb. Funibiol Gold 32 fl oz and Theia 1.5 lb + Howler EVO 2.5 lb were significantly better than the untreated control and similar to each other at the second rating date. On the final rating date, the untreated control developed 97.5 % of plant death. All treatments statistically ($P=0.0249$) reduced plant death compared to the untreated control on the final rating date, except Theia 1.5 lb. Theia 1.5 lb. was similar to the untreated control and all other fungicide treatments; all the treatments were similar for plant death on the final rating date. According to the area under the disease progress curve (AUDPC), all treatments had significantly ($P=0.0008$) less disease than the untreated

control. Presidio 4 fl oz had the lowest AUDPC but was similar to all other fungicide treatments except Theia 1.5 lb. and Theia 1.5 lb. plus Howler EVO 2.5 lb. When combined with host resistance, Biorational could efficiently manage Phytophthora crown rot.

Treatment and rate[z]/A, applied at 7- or 14-days intervals, *applications*	Plant Death (%)[y]								AUDPC[x]	
	18 Jul		25 Jul		31 Jul		7 Aug			
Untreated	27.5	a[w]	77.5	a	95.0	a	97.5	a	1558.8	a
Presidio 4 fl oz, *apps AB*	0.0	a	2.5	c	15.0	c	22.5	b	192.5	c
Theia 1.5 lb., *apps BDEF -alt-* Presidio 4 fl oz, *apps AC*	7.5	a	7.5	bc	17.5	bc	22.5	b	267.5	bc
Theia 3 lb., apps *A-F*	7.5	a	20.0	bc	42.5	bc	45.0	b	590.0	bc
RootShield Plus 32 fl oz, *apps AB*	10.0	a	20.0	bc	42.5	bc	47.5	b	607.5	bc
MGCI 8 fl oz, *apps A-D*	12.5	a	20.0	bc	37.5	bc	57.5	b	618.8	bc
Funibiol Gold 32 fl oz, *apps A-D*	20.0	a	27.5	b	35.0	bc	47.5	b	642.5	bc
Theia 1.5 lb., *apps A-F*	10.0	a	22.5	bc	47.5	b	60.0	ab	700.0	b
Theia 1.5 lb. + Howler EVO 2.5 lb., *apps A-F*	15.0	a	32.5	b	45.0	bc	57.5	b	757.5	b
P-value	0.1438		0.0144		0.0017		0.0249		0.0008	

[z]*-alt-* = alternate. Presidio 4 fl oz: sprench applications A and B at 7 day intervals; Theia 1.5 lb. *-alt-* Presidio 4 fl oz, Theia 3 lb., Theia 1.5 lb., and Theia 1.5 lb. + Howler EVO 2.5 lb. sprench applications A to F at 7-day intervals; Funibiol Gold 32 oz: sprench applications A-D at 14 day intervals; RootShield Plus 32 oz: sprench applications A and B at 14 day intervals; MGCI 8 fl oz: foliar applications A-D at 14 day intervals.

[y]Based on the number of dead plants in a plot divided by the total number of plants in a plot (10) multiplied by 100.

[x]AUDPC = Area under the disease progress curve.

[w]Column means with a letter in common are not significantly different (LSD t test; P=0.05).

4.2.3 Analysis of pathogen populations to inform breeding and disease management (Year 2-3).

<u>*Phytophthora capsici*</u> (<u>Smart</u>): We now have a panel of about 120 isolates of *P. capsici* for which we have genotype data, using genotyping-by-sequencing, to identify a SNP panel of over 64,000 SNPs. For this same panel, we have phenotypic data including pathogenicity on cucurbits and pepper as well as sensitivity to the fungicide mefenoxam, and mating type for each of these isolates. Using a genome-wide association study, we were able to map the gene that is likely responsible for resistance to the fungicide mefenoxam, and identified a potential effector that is recognized by a subset of host plants. During the 2022 field season, we collected 121 additional isolates from western New York and 69 isolates from California. We are currently completing single zoospore isolation from each of these isolates, and genotyping will begin in the near future. During the 2023 field season we plan to get additional isolates from states including North Carolina, South Carolina, and potentially Michigan, Georgia or Tennessee. These isolates will be included in our genotyping and phenotyping studies and used in GWAS to map traits of interest.

Collection of *Phytophthora capsici* isolates for population study:
26 isolates were collected in New York. In 2024, a total of 248 isolates collected between 2022-2023 will be genotyped. These isolates comprise populations from California, New York, and North Carolina, and

were collected from *Cucurbita moschata*, *C. pepo*, and pepper. Additionally, fungicide insensitivity (mefenoxam) was observed in approximately 40% of the 248 isolates.
The above work is a follow-up to the work a previous student, Greg Vogel, had done as part of CucCAP2 looking at differences in virulence, and presumably effector composition, of 118 isolates. This work was presented to the CucCAP2 team in March 2023.

Pseudoperonospora cubensis (Quesada): Since its reemergence in 2004, *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew (CDM), has experienced significant changes in fungicide sensitivity. Presently, frequent fungicide applications are required to control the disease in cucumber due to the loss of host resistance. Carboxylic acid amides (CAA) and quinone outside inhibitors (QoI) are two fungicide groups used to control foliar diseases in cucurbits, including CDM. Resistance to these fungicides is associated with single nucleotide polymorphism (SNP) mutations. In this study, we used population analyses to determine the occurrence of fungicide resistance mutations to CAA and QoI fungicides in host-adapted clade 1 and clade 2 *P. cubensis* isolates. Our results revealed that CAA-resistant genotypes occurred more prominently in clade 2 isolates, with more sensitive genotypes observed in clade 1 isolates, while QoI resistance was widespread across isolates from both clades. We also determined that wild cucurbits can serve as reservoirs for *P. cubensis* isolates containing fungicide resistance alleles. Finally, we report that the G1105W substitution associated with CAA resistance was more prominent within clade 2 *P. cubensis* isolates while the G1105V resistance substitution and sensitivity genotypes were more prominent in clade 1 isolates. Our findings of clade-specific occurrence of fungicide resistance mutations highlight the importance of understanding the population dynamics of *P. cubensis* clades by crop and region to design effective fungicide programs and establish accurate baseline sensitivity to active ingredients in *P. cubensis* populations.

4.3. Economic impacts of disease and gains from control tools and valuation of crop attributes (Year 1-4). (Tregeagle)

4.3.1. Determine economic impacts of disease and control tools (Year 1-4).

Cucumber production costs were collected from participants at the SE Vegetable & Fruit Expo

4.3.2 Estimate industry valuation of improvement in crop attributes (Year 1-4).

Draft evaluation surveys were prepared for cucumbers, watermelon, summer squash, and winter squash. Drafts were shared with members of the CucCAP2 team and the stakeholder advisory board. Comments were received and incorporated.
Cucumber survey was launched online and data collection is ongoing.

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS

REFEREED PUBLICATIONS, BOOK CHAPTERS, CONFERENCE PROCEEDINGS
(items in bold added for 2024 report)

Refereed Publications

1. Acharya B, Mackasmiel L, Taheri A, Ondzighi-Assoume CA, Weng Y, Dumenyo CK (2021) Identification of bacterial wilt (*Erwinia tracheiphila*) resistances in USDA melon collection. P**lants** 10: 1972
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Book Chapters

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EXTENSION PUBLICATIONS and OUTREACH RESOURCES

1. Adams M. L., Batts T. M. , Collins H., Quesada-Ocampo L. M. (2021) Evaluation of fungicides for control of downy mildew on cucumber, Saratoga, NC 2020. Plant Disease Management Reports: 15: V106.
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117. Smart, C.D. 2021. Managing Phytophthora in pumpkins & melons – the New York experience. Proceedings of the 2021 Minnesota Fruit and Vegetable Convention.

118. Smart, C.D. 2021. Phytophthora of cucurbit crops Disease fact sheet https://www.vegetables.cornell.edu/pest-management/disease-factsheets/phytophthora-blight/

119. Smart, C.D. 2021. Cucurbit Downy Mildew Disease fact sheet https://www.vegetables.cornell.edu/crops/cucurbits/downy-mildew/

120. Tekip, A. 2020. Monitoring and managing cucumber downy mildew. Interview, Michigan State University AgBioResearch, Futures: Summer 2020.

121. Uebbing MR, Hausbeck MK. 2022. Evaluation of single product treatments for control of downy mildew on pickling cucumbers, 2021. Plant Disease Management Reports 16: V137.

122. Uebbing MR, Hausbeck MK. 2022. Evaluation of disease forecasters for control of downy mildew on pickling cucumbers, 2021. Plant Disease Management Reports 16: V136.

123. Uebbing M, Hausbeck MK. 2022. Downy Mildew Update in Pickling Cucumber. Great Lakes Farm, Fruit and Vegetable Expo. Grand Rapids, MI. 6 Dec. https://glexpo.com/2022-session-handouts

124. Uebbing, M.R. and Hausbeck, M.K. 2023. Evaluating pickling cucumber cultivars for resistance to downy mildew under natural infection conditions, 2022. Plant Disease Management Reports 17:V157.

125. Uebbing, M.R. and Hausbeck, M.K. 2023. Efficacy of organic products for control of powdery mildew on moderately resistant acorn squash, 2022. Plant Disease Management Reports 17:V158.
126. Uebbing, M.R. and Hausbeck, M.K. 2023. Evaluation of biological and conventional products to control downy mildew on pickling cucumbers, 2022. Plant Disease Management Reports 17:V156.
127. Walker K. 2001. Downy mildew in cucumbers this season. FarmWorld. Interview, 7/30.
128. Wintermantel, W.M. 2022. Cucurbit chlorotic yellows virus. ecucurbitviruses.org (Emerging Viruses in Cucurbits Working Group website); https://ecucurbitviruses.org/resources/facts-sheets-videos/cucurbit-chlorotic-yellows-virus/
129. Wintermantel, W.M. 2023. Cucurbit yellow stunting disorder virus. ecucurbitviruses.org (Emerging Viruses in Cucurbits Working Group website)

SCIENTIFIC CONFERENCE and UNIVERSITY PRESENTATIONS

1. Adams, L., Josiah, S., Legendre, R., & McGregor, C. (2022). The Effect of Three Genetic Loci on Rind Thickness in Watermelon. HortScience, S291
2. **Alzohairy, S.A., Moore, B.M., Hammerschmidt, R., Shiu, S., and Hausbeck, M.K. 2022. Lignin biosynthesis gene expression is associated with age-related resistance of winter squash to *Phytophthora capsici*. Cucurbitaceae 2022 Abstract Book. Naples, FL, 30 Oct-2 Nov.**
3. Andreason, S. and Kousik, C.S. 2022. Detection of cucurbit leaf crumple virus infectious clones from the virus vector *Bemisia tabaci*. American Phytopathological Society Annual Meeting, Pittsburg, PA. August 2022.
4. Agudelo P., Corbin J., Desaeger J., Gorny A., Grabau Z., Guan Z., Hajihassani A., Mueller J., Quesada-Ocampo L. M., Rutter W., and Wadl P. 2022. Multi-state effort to contain and manage *Meloidogyne enterolobii* on vegetable crops. Cucurbitaceae, Naples, FL, November 2022.
5. **Bhuiyan, M. Z. R., D'Arcangelo, K. N. and Quesada-Ocampo L. M. Populations of *Pseudoperonospora cubensis* Causing Downy Mildew in Squash and Cucumbers are Structured by Host Genotype. American Phytopathological Society Annual Meeting, Plant Health 2023, Denver, CO, August 2023.**
6. **Biswas, A., V. Ganaparthi, S. Kousik, P. Wechter, A. Levi, and S. Branham. 2023. Genomic Selection (GS) Approach to incorporate Fusarium wilt race 2-resistance into Watermelon Cultivars. National Association of Plant Breeders, Greenville, SC July16-20.**
7. **Branham SE. 2023. Genomics-assisted vegetable breeding to develop new varieties for South Carolina. Clemson University, AGSC 4100/6100 Newman Seminar and Lecture Series**
8. Branham SE. 2022. Marker-assisted vegetable breeding for production in the Southeastern US. Cornell University, School of Integrative Plant Science Spring Seminar Series.
9. Branham SE, Ganaparthi V, Kousik S, Wechter WP, Park YH, Wehner T, Davis A, Tetteh A, Hammar S, Grumet R, Levi A. 2023. XP-GWAS and marker development for resistance to powdery mildew race 2W in watermelon (*Citrullus lanatus*). Plant and Animal Genome.
10. Branham SE, *Ganaparthi V, Kousik S, Wechter WP, Park YH, Wehner T, Davis A, Tetteh A, Hammar S, Grumet R, Levi A. 2022. XP-GWAS and marker development for resistance to powdery mildew race 2W in watermelon (*Citrullus lanatus*). Cucurbitaceae.

11. Branham, S.E., Wechter, W.P., Ling, K., Katuuramu, D.N., Levi, A. 2021. QTL mapping and pyramiding resistance to *Fusarium oxysporum* f. sp. *niveum* (races 1 and 2) and potyviruses in watermelon. Eucarpia Cucurbitaceae Symposium Proceedings.
12. **Bhuiyan, M. Z. R., D'Arcangelo, K. N. and Quesada-Ocampo L. M. 2023. Populations of *Pseudoperonospora cubensis* causing downy mildew in squash and cucumbers are structured by host genotype. Phytopathology 113: S3.108**.
13. Chanda, B., Ikerd, J.L., Adkins, S., Kousik, C.S. 2021. Understanding the disease resistance mechanism through RNA-Seq analysis of SqVYV-resistant watermelon. Annual Meeting of the American Phytopathological Society.
14. Chanda, B., Shamimuzzaman, M., Gilliard, A., and Ling, K.-S. 2021. Managing the spread of *Tomato brown rugose fruit virus* and *Cucumber green mottle mosaic virus* using chemical disinfectants. Plant Health -2021 annual meeting of American Phytopathological Society, on-line, August 2-6, 2021.
15. Chen FC 2022. Genetic architecture of the downy mildew resistance locus *dm4.1* in PI 330638 (WI7120). Cucurbitaceae 2022, Naples, FL
16. **Cochran, S., Miles, T., Quesada-Ocampo L. M. (2024) Development of a Recombinase Polymerase Amplification (RPA) assay for rapid detection of *Phytophthora capsici*. American Phytopathological Society-Southern Division, Columbia, SC, February 2024.**
17. Condo, I., Prieto-Torres, M., Quesada-Ocampo L. M. Monitoring populations in *Pseudoperonospora cubensis* using biosurveillance and molecular markers. Undergraduate Research and Creativity Symposium. North Carolina State University. Raleigh, North Carolina. July 2022
18. Culp C, Chen YC, Grumet R. 2022. Testing Cucumber Accessions for Phytophthora Fruit Rot Resistance. MSU Undergraduate Research Forum. East Lansing MI
19. D'Arcangelo, K. N. and Quesada-Ocampo L.M. Characterization of the population dynamics of alleles related to Carboxilic Acid Amide and Quinone Outside Inhibitor resistance in the host-adapted clades of *Pseudoperononspora cubensis* to facilitate crop-specific management of cucurbit downy mildew. Department of Entomology and Plant Pathology Seminar. Raleigh, NC, October, 2021.
20. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. Utilizing a population genetics approach to facilitate crop-specific management of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*. Annual Southern Division American Phytopathological Society Meeting, Virtual Meeting. February, 2021. Phytopathology 111:S1.14
21. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. (2020) Leveraging population genetics to develop disease control practices: a study in the crop-specific management of cucurbit downy mildew. Phytopathology 110: S2.203.
22. D'Arcangelo, K. N., Rahman, A., Miles, T. D., and Quesada-Ocampo, L. M. 2021. Distribution of alleles related to carboxylic acid amide and quinone outside inhibitor resistance in host-adapted clades of *Pseudoperonospora cubensis*. American Phytopathological Society Annual Meeting, Plant Health 2021, Memphis, TN, August 2021. Phytopathology 111:S2.114
23. Deaton T, Rosado-Rivera Y, Quesada-Ocampo L. M. Crop varieties assessment for susceptibility to downy mildew in North Carolina. Undergraduate Research and Creativity Symposium. North Carolina State University. Raleigh, North Carolina. July 2022
24. De Figueiredo Silva, F., Keinath, A. P., Kunkel, D. Economic impact of the foliar disease downy mildew under fungicide applications in cucumber production: a preliminary analysis. Southern Agricultural Economics Association Annual Meeting. February 2021. https://saea2021.org/schedule/

25. **Fei Z. 2024. Cucurbit Genomics Database (CuGenDB) v2: an updated database for cucurbit genomics. Plant & Animal Genome Conference. San Diego, CA. January 2024**
26. **Fei Z. 2023. Genomic basis of watermelon origin, domestication, and breeding. School of Plant Integrative Science, Cornell University. February 2023**
27. **Fei Z. 2023. Genomic basis of watermelon origin and domestication. Nanjing Forestry University. November 2023**
28. **Fei Z. 2023. Pan-genomes of fruit crops. 10th International Horticulture Research Conference. Guanzhou, China. November 2023**
29. **Fei Z.(2023. Genomic insights into watermelon origin and domestication. Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences. November 2023**
30. **Fei Z. 2023. Genomic basis of watermelon origin and domestication. Hunan Agricultural University. November 2023**
31. **Fei Z. 2023. Genomic insights into watermelon origin and domestication. Fruit Quality Biology/ Food Quality and Safety International Conference 2023. Hangzhou, China. November 2023**
32. **Fei Z. 2023. Genomic basis of watermelon origin and domestication. Shanghai Normal University. November 2023**
33. **Fei Z. 2023. Pangenomes of wild and dessert watermelons. VII International Symposium on Cucurbits. Zhengzhou, China**
34. **Fei Z (2023) Pan-genomes of horticultural crops. Nature Genetics & Plant Editorial Communities**
35. **Fei Z (2023) Genomic and pan-genomic basis of watermelon origin, domestication and breeding. Molecular Horticulture**
36. **Fei Z (2023) Genomic and pan-genomic basis of watermelon origin, domestication and breeding. National Engineering Research Center for Vegetables. Beijing, China**
37. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. The 9th International Horticulture Research Conference. Wuhan, China. November 2022
38. Fei Z. 2022. A super-pangenome of cultivated and wild watermelon species. Cucurbitaceae 2022. Naples, FL. October 2022
39. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. University of North Carolina. October 2022
40. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. Shandong Academy of Agricultural Sciences. October 2022
41. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. The 2022 International Symposium of Horticulture and Plant Biology. Wuhan, China. August 2022
42. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. BTI PGRP Summer Intern. July 2022
43. Fei Z (2021) Genomic analyses shed light on watermelon origin and the genetic history of domestication and agronomic traits. Zhejiang University
44. Fei Z (2021) Genomic analyses shed light on watermelon origin and the genetic history of domestication and agronomic traits. CSHL Plant Genomes, Systems Biology & Engineer
45. Fei Z. Genomic analyses shed light on the genetic history of watermelon domestication and agronomic traits. The 2021 Cold Spring Harbor meeting on Plant Genomes, Systems Biology and Engineering. December 2021
46. Fei Z. The origin, history and future of watermelon. BTI Breaking Ground series. November 2021

47. Fei Z. Genomic analyses provide insights into the genetic history of watermelon domestication and agronomic traits. The 7th Asia-Pacific Agrobiological Genome Symposium, Korea. November 2021
48. Frank A, Lin YC, Grumet R. 2022. Measurement and Analysis of Cucumber Fruit Curvature. MSU Undergraduate Research Forum. East Lansing MI
49. Gaines DD, Panicker GK, Henry K, Leonard K, Reddy UK. 2020. Evaluation of Grafted Watermelons (*Citrullus lanatus*) grown on a Heavy Soil with Organic Treatments for Their Biomass, Quality, Yield, and Adaptability. ASHS Annual Conference, 2020
50. **Ganaparthi VR, Wechter WP, Levi A, Branham SE. 2024. *Citrullus amarus* as a source of disease resistance and introgression of Fusarium wilt resistance into cultivated watermelon. Cucurbit Genomics, Plant and Animal Genome**.
51. Ganaparthi V, Wechter WP, Rennberger G, Levi A, Branham SE. 2022. QTL mapping and marker development for resistance to *Fusarium oxysporum* f. sp. *niveum* race 2 in an interspecific *Citrullus amarus/lanatus* population. Cucurbitaceae.
52. Ganaparthi V, Branham S, Levi A, Robinson S, Katawczik M, Wechter P. 2022. Mapping and Validation of QTLs Imparting Fon Race 2 Resistance in Watermelon. Cucurbitaceae, 2022, Naples, FL.
53. Grumet R. 2022. Grumet R. 2022. Leveraging applied genomics to increase disease resistance in cucurbit crops. Corteva Symposium, Cornell University. March 2022
54. Grumet R. 2022. Grumet R. 2022. Cucurbit germplasm - genomic tools and disease resistance. National Association of Plant Breeders. Ames Iowa. August 2022
55. Grumet R, Fei Z, Branham S, Levi A, Wechter WP, Weng Y, Wang Y, McCreight J, Mazourek M, Mansfeld BM, Lin Y-C (2021) Application of genomic tools for mapping and analysis of disease resistance traits in cucurbits: The CucCAP experience. XII Eucarpia Meeting on Cucurbit Genetics and Breeding. Virtual.
56. Grumet, R., Z. Fei, A. Levi, M. Maxourek, J.D. McCreight. J. Schultheis, Y. Weng, M. Hausbeck, S. Kousik, K.S. Ling, A. Linares-Ramirez, C. McGregor, L. Quesada-Ocampo, U. Reddy, C. Smart, P. Wechter, T. Wehner, L. Wessel-Beaver, and W.M. Wintermantel. (2020). The CucCAP project: Leveraging applied genomics to improve disease resistance in cucurbit crops. Acta Horticulturae 1294:101-114.
57. **Hausbeck, M.K. 2023. Grower-Driven Research Leads to Integrated Disease Management. Departmental Seminar, Michigan State University, East Lansing, MI, 27 Apr.**
58. Hausbeck, M.K., Harlan, B.R., Bello, J.C., and Kenny, G. 2021. Downy Mildew Management in Pickling Cucumbers. Agriculture Agri-Food Canada, Ontario, Canada. Virtual, Apr.
59. Heagy, K., T. Birdsell, and J. Schultheis. 2022. Effects of pumpkin spacing on fruit weight, quantity, and size. HortScience 57(9) Supplement (Part 2). S224 (Abstr.)
60. Heagy, K., T. Birdsell, and J. Schultheis. 2022. Effects of pumpkin spacing on fruit weight, quantity, and size. Watermelon Research and Development group, ASHS - Southern Region, New Orleans, LA. Feb. 11.
61. Indermaur, E. J., Day, C. T. C., and Smart, C. D. Biofungicides for organic management of powdery mildew in winter squash (poster). Cucurbitaceae. Naples, FL, October 2022.
62. Katuuramu DN, Branham SE, Levi A, Wechter WP. 2021. Genome-wide association analysis of downy mildew resistance in a pre-breeding watermelon (*Citrullus amarus*) collection. Eucarpia Cucurbitaceae Symposium Proceedings.
63. Kelly B, Salcedo A, Rahman A, Wallace EC, Crouch JA, Quesada-Ocampo LM. 2022. Does a *Pseudoperonospora cubensis* cryptospecies population caused a cucurbit downy mildew epidemic in the U.S.? Undergraduate Research and Creativity Symposium. North Carolina State University. Raleigh, North Carolina. July 2022

64. **Keinath, A. P. 2023. In-Field Food Crop Agent Training on Vegetable Diseases. In-service Training, Clemson Extension. September 26, 2023**
65. Keinath, A. P. and Silva, F. D. Economic impacts of downy mildew and reduced fungicide efficacy on slicing cucumber. Canadian Reduced-Risk Strategy for Cucumber Downy Mildew Annual Meeting 2021.
66. Keinath, A. P., and Silva, F. D. Economic impacts of downy mildew and reduced fungicide efficacy on slicing cucumber. Plant Health 2021, American Phytopathological Society (virtual). https://events.rdmobile.com/Lists/Details/1179331
67. Kenny, G. 2020 Cucumber field data. Departmental seminar, Department of Plant Pathology, Michigan State University, East Lansing, MI. Virtual.
68. Kikway, I., Keinath, A. P., and Ojiambo, P. S. Field occurrence and overwintering of oospores of *Pseudoperonospora cubensis* in the eastern United States. Plant Health 2021, American Phytopathological Society (virtual). https://events.rdmobile.com/Lists/Details/1179538
69. Kilduff, A. and Tregeagle, D. Eureka! Or Sassy? Producer Valuations of Cucumber Traits. Department of Agricultural and Resource Economics, NC State University, Raleigh, NC, March 2023.
70. Kousik, C.S. 2020. Breeding for resistance to whitefly transmitted viruses in watermelon. Presented at the Whitefly Symposium. Entomological Society of America Annual Meeting, November 2020.
71. Kousik, C.S., Chanda, B., Mandal, M., Ikerd, J., Sudarshana, M., Turechek, W.W., Adkin, S. 2022. Identifying and Confirming Resistance to Whitefly-Transmitted Cucurbit Leaf Crumple Virus in Watermelon Using Infectious Clones. Cucurbitaceae 2022. Naples FL. Keynote talk.
72. Kousik, C.S., Chanda, B., Mandal, M., Ikerd, J., Sudarshana, M., Turechek, W.W., Adkin, S. 2022. Phenotyping resistance to whitefly-transmitted *Cucurbit leaf crumple virus* in watermelon using infectious clones and confirming resistance using dPCR. American Phytopathological Society Annual Meeting, Pittsburg, PA. August 2022. (Poster Presentation).
73. Kousik, C.S., Chanda, B., Mandal, M., Ikerd, J., Sudarshana, M., Turechek, W.W., Adkin, S. 2022. Developing resources for breeding watermelon varieties for resistance to whitefly- transmitted viruses. Southern Division American Phytopathological Society Annual Meeting, March 2022. Chattanooga, TN. (Virtual talk)
74. Kousik, C.S., Chanda, B., Suren, H., M., Ikerd, J., Turechek, W.W., Adkin, S. 2021. Advances in breeding for resistance to whitefly transmitted viruses in watermelon. Entomological Society of America Annual Meeting. Invited Virtual Talk. November 2022
75. **Kumar R, Chanda, B., Adkins, S. and Kousik, C.S. 2024. Transcriptomic analysis of Watermelon-Squash Vein Yellowing Virus Interactions Reveals Elevated Expression of Callose and RNA Silencing genes in Resistant Genotype. Presented at the Plant and Animal Genome Conference, January 2024, SanDiego, CA.**
76. Landrón A, Linares AM. 2022. Screening for powdery mildew resistance in *Cucurbita moschata* in Lajas, Puerto Rico. Proceedings Cucurbitaceae 2022, November, 2022, Naples. Florida p.37
77. Landron A. and A. Linares Ramirez. 2022. Identification of the Powdery Mildew Causal Agent in *Cucurbita moschata* Duch. for Breeding Purposes in Lajas, Puerto Rico. American Society of Horticultural Sciences. Chicago, IL.
78. Levi A. 2022. Challenges and progress in genetic research and in enhancing disease resistance in watermelon. South Korean Society of Plant Breeders and Geneticists.
79. Lin YC, Grumet R (2021) QTL-seq of Young Fruit Resistance to *Phytophthora capsici* in Cucumber. 2020 American Society for Horticultural Science Annual Meeting; August 9th, 2020; Virtual
80. Lin YC, Grumet R. 2022. QTL Mapping of Young Fruit Resistance to *Phytophthora capsici* in Cucumber. Cucurbitaceae 2022. Naples FL

81. Lin YC, Grumet R. 2023. QTL Mapping of Young Fruit Resistance to *Phytophthora capsici* in Cucumber. Plant Animal Genome Conference, San Diego CA
82. Lin YC, Rett-Cadman S, Grumet R. 2022. Phenotypic and Genetic Analysis of Fruit Morphological Traits for the USDA Cucumber Core Collection. American Society of Horticultural Sciences. Chicago, IL
83. **Lin YC, Tang X, Weng Y, Fei Z, Grumet R. 2024. Identification of QTL Associated with Young Fruit Resistance to Phytophthora Fruit Rot in Cucumber. Plant Animal Genome Conf., San Diego CA**
84. Lin YC, Weng Y, Fei Z, Grumet R. (2021) Phenotypic analysis of the U.S. cucumber PI core collection for fruit morphological diversity. National Association of Plant Breeders Annual Meeting; August 18th, 2021; Virtual.
85. **Lin YC, Weng Y, Fei Z, Grumet R. 2024. Genetic Analysis of Morphological Diversity for Fruit Quality Traits in the Cucumber Core Collection. Plant Animal Genome Conf., San Diego CA**
86. Luckew, A., Bag, S., Srinivasan, R., Dutta, B., Da Silva, A.L.B.R., Meru, G., and C.E. McGregor. 2022. Genome wide association study of *C. pepo* evaluated for whiteflies and their transmitted viruses. Cucurbitaceae, Naples, FL.
87. Luckew, A. and C.E. McGregor. 2022. Identifying resistance to whitefly transmitted viruses in Cucurbita. Cucurbitaceae, Naples, FL.
88. Luckew, A. and C.E. McGregor. 2022. Evaluation of Cucurbita germplasm for resistance to whitefly transmitted viruses. Southern Region American Society for Horticultural Science, New Orleans, LA.
89. Luckew, A. and C.E. McGregor. 2022. Evaluation of Cucurbita germplasm for resistance to whitefly transmitted viruses. Joint Southeastern Branch Entomological Society of America and American Phytopathological Society- Caribbean Division, San Juan, PR.
90. Mandal, M.K., Chanda, B., and Kousik, C.S. 2021. Identification of Powdery Mildew Resistant Marker in Watermelon by Metabolomics and Genomics Approach. Annual Meeting of the American Phytopathological Society.
91. Mandal M K, Thompson D, Harris R, CS. 2021. Bacterial Biocontrols in Sustainable Management of Phytophthora Crown and Fruit Rot in Pepper and Watermelon. Annual Meeting of the American Phytopathological Society.
92. **Mazourek M. 2023. National Association of Plant Breeders Conference. "Culinary Driven Plant Breeding" July 17, 2023**
93. Mazourek M. 2022. Combining Resistance with Quality in Squash. Asia Pacific Seed Association Cucurbinar. Sept 29, 2022.
94. Mazourek M, Frost E 2022. Combining Cucurbits for Downy Mildew Resistance and More. OSSI Webinar Series. May 11, 2022.
95. **Mcgregor, C. (2023). Citrullus germplasm with resistance to whiteflies and whitefly transmitted viruses. In American Society of Horticultural Science 2023 Annual Conference**
96. McGregor CE (2022) Where the Wild Things Are: Using Crop Wild Relatives for Watermelon Improvement. Invited seminar in Department of Horticulture, Michigan State University (virtual)
97. McGregor CE, Rijal S. and S Josiah (2022) The Use of Citrullus Crop Wild Relatives in Watermelon Breeding. American Society for Horticultural Science, Chicago, IL
98. McGregor*, C., Luckew, A., Wang, E., Mathews, P., Carvalho, R., da Silva, A., . . . & R. Srinivasan (2020). Evaluation of Cucurbita germplasm for resistance to whiteflies and whitefly-transmitted viruses. Entomological Society of America national meeting (Virtual).
99. Meru, G. 2023. Squash Breeding and Genetics: Building Blocks for Success in a Genomics Era. Horticultural Sciences Department Seminar, Michigan State University, East Lansing, MI.

100. Meru, G., Fu, Y., Shrestha, S., Sabharwal, P., Thakur, S., Michael, V. 2023. Development and application of genomic tools for squash breeding and genetics. Annual meeting of the American Society of Horticultural Sciences, Orlando, Florida. August 2023

101. Meru, G. 2022. Advancing the cucurbit industry through a genomics-enabled breeding and extension program. Horticultural Sciences Department Seminar, University of Florida, Gainesville, FL.

102. Meru G. 2021. Squash breeding and genetics: building blocks for success in a genomics era. Presented at the University of Georgia, Department of Horticulture, Spring 2021 semester seminar series, held virtually February 24, 2021.

103. Meru, G., Fu, Y., Michael, N. and Shrestha, S. 2022. Genomics-enabled breeding in squash: progress towards high -throughput application. 135th annual meeting of Florida State Horticultural Society, Sarasota, FL, June 5-7, 2022.

104. Meru, G., Michael, N., Acharya, S., Fu, Y., Shrestha, S. and Sabharwal, P. 2022. RNA-SEQ reveals potential defense mechanisms against *Phytophthora capsici* in squash. Cucurbitaceae 2022. Naples FL, Oct. 30- Nov. 2, 2022.

105. **Michel, S.*, J.R. Schultheis, A.P. Keinath, and L. Quesada-Ocampo. 2023. 2023 Results for Managing Fusarium Wilt of Watermelon with Cultivar, Fungicide, and Grafting in North and South Carolina. American Society of Horticultural Science Southern Region Annual Conference, Atlanta, GA. Feb 3**.

106. Michel, S.*, J.R. Schultheis, B. Parker, and B. Stickley. 2023. 2023 Mini Watermelon Cultigen Yield and Quality Results for North Carolina. American Society of Horticultural Science Southern Region Annual Conference, Atlanta, GA. Feb 3.

107. Michel S., Schultheis J., Keinath A., and Quesada-Ocampo L. M. 2022. Incidence and yield response of seedless watermelon cultivars affected with Fusarium wilt. Cucurbitaceae, Naples, Florida, November 2022.

108. Michael, V.N.; Fu, Y.; Shrestha, S.; Meru, G. 2020. QTL mapping of Phytophthora crown rot resistance in squash. Presented at the 133rd Annual Meeting for the Florida State Horticultural Society, held virtually October 18 - October 20, 2020.

109. Mondal S, Wintermantel WM, McCreight J. 2022. Development of CYSDV-resistant lines using marker-assisted selection. Cucurbitaceae 2022 Naples, FL

110. Mondal S, Chen C, Jenkins-Hladky LL, Wintermantel WM. 2022. Spatio-temporal accumulation of two closely related criniviruses in melon plants during co-infection. Annual Meeting of the American Phytopathological Society, Pittsburgh, PA, August 6-10, 2022

111. Mondal S, Wintermantel W, McCreight J. 2022. Advancement of CYSDV-resistant Melon using Marker-Assisted Selection. Annual Meeting of the American Phytopathological Society, Pittsburgh, PA, August 6-10, 2022

112. Mondal, S., Ando, K., Tamang, P., Fashing, P., Chen, C., Wintermantel, W.M., and McCreight, J.D. 2023. Advancement of CYSDV-resistant melon lines using marker-assisted selection. American Phytopathological Society, Annual meeting, August 12 – 16, Denver, Colorado.

113. Natarajan P, Nimmakayala P, Abburi V, Lopez-Ortiz C, Levi A, Wehner T, Reddy UK. (2022) Whole-Genome Scanning Using QTL-Seq and GWAS for Gummy StemBlight Resistance in Watermelon. Cucurbitaceae 2022. October 30-November 2, 2022. Naples, FL, USA.

114. Natarajan P, Nimmakayala P, Lopez-Ortiz C, Rathnagiri A, Rivera-Burgos LA, Sari N, Wehner TC, Levi A, Tomason Y, Reddy UK. (2023) Whole-Genome Scanning Using QTL-Seq and GWAS for Gummy Stem Blight Resistance in Watermelon. Plant & Animal Genome Conference: PAG 30. January 13-18, 2023. San Diego, CA, USA.

115. Pandey, S., Luckew, A., McAvoy, T., Meru, G., Simmons, A., & Mcgregor, C. (2023). Evaluation of Citrullus Genotypes for Resistance to Whitefly Transmitted Viruses. In Southern Region— American Society for Horticultural Science

116. Pandey, S., Luckew, A., McAvoy, T., Meru, G., and C.E. McGregor 2022. Evaluation of *Citrullus* genotypes for resistance to whitefly transmitted viruses. Southern Region ASHS annual meeting Oklahoma City, OK.

117. Parada Rojas C. H. and Quesada-Ocampo L.M. Improving Knowledge of Host Resistance Against Soilborne Vegetable Pathogens. Department of Entomology and Plant Pathology Seminar. Raleigh, NC, February, 2023.

118. Parada-Rojas C. H. and Quesada-Ocampo L. M. *Phytophthora capsici* populations structure by host, geography, and fluopicolide sensitivity. Cucurbitaceae, Naples, Florida, November 2022.

119. Parada-Rojas C. H. and Quesada-Ocampo L. M. (2022) populations structure by host, geography, and fluopicolide sensitivity. Phytopathology 112: S3.102.

120. Perkins-Veazie, P., M. Trandel, J. Schultheis and T. Birdsell. 2020. Pumpkin Postharvest: Stem Retention and Moisture with Storage. *HortScience*, 55(9S): S410-S411(abstr.).

121. Perla, D. Evaluate strategies for management of Phytophthora blight *Phytophthora capsici* in Michigan processing squash. Departmental seminar, Department of Plant Pathology, Michigan State University, East Lansing, MI. Virtual

122. Perla D., and Hausbeck M.K. Vegetable Disease Management using host resistance and fungicides. American Phytopathological Society North Central Division Meeting, Lincoln, NE, June 2022.2020-5132139

123. Perla, D.E, Hayden, Z.D., and Hausbeck, M. K. Commercial hard squash cultivars exhibit differences in resistance to Phytophthora fruit and crown rot. Cucurbitaceae 2022. Naples, FL, November 2022.

124. Perla, D.E., Hayden, Z.D., and Hausbeck, M.K. 2022. Assessment of hard squash cultivars for resistance to crown rot caused by *Phytophthora capsici* and sugar content. American Phytopathological Society North Central Division Meeting. Lincoln, NE, 21-23 Jul.

125. **Perla, D., Medina-Mora, C.M., Engfehr, C., and Hausbeck, M.K. 2023. Evaluating hard squash cultivars for susceptibility to powdery mildew and fruit rot. 12th International Congress on Plant Pathology, The International Society for Plant Pathology and the French Phytopathological Society. Lyon, France. 20 Aug-25 Aug**.

126. Peterson, A.M., Bello, J.C., Kenny, G., Perla, D., Uebbing, M., Hausbeck, M.K. 2022. Burkard spore traps for detection of *Pseudoperonospora cubensis* sporangia in cucurbit production. American Phytopathological Society Annual Meeting, Pittsburg, PA. August 2022

127. **Prieto Torres, M., and Quesada-Ocampo L. M. 2023. Monitoreo y biovigilancia en poblaciones de mildeo velloso en cucurbitáceas (*Pseudoperonospora cubensis),* en Carolina del Norte. I Simposio Internacional de Fitopatología y Microbiología Agrícola, Universidad Nacional Mayor de San Marcos, Peru, November 2023**.

128. Prieto-Torres, M. and Quesada-Ocampo L. M. (2023) Monitoring oxathiapiprolin fungicide resistance mutations in *Pseudoperonospora cubensis* populations in North Carolina. Phytopathology 113: S3.18.

129. Prieto-Torres, M. and Quesada-Ocampo L. M. (2023) Monitoring for mutations related to oxathiapiprolin fungicide resistance in *Pseudoperonospora cubensis* populations. Phytopathology 113: S2.31

130. Prieto-Torres, M. and Quesada-Ocampo L. M. Monitoring for mutations related to oxathiapiprolin fungicide resistance in *Pseudoperonospora cubensis* populations. American Phytopathological Society-Southern Division, Durham, NC. February 2023.

131. Prieto-Torres, M. and Quesada-Ocampo L. M. Monitoring populations and fungicide resistance in *Pseudoperonospora cubensis* using biosurveillance and molecular markers. Cucurbitaceae, Naples, Florida, November 2022.
132. Purayannur S., Cano L.M., Bowman M.J, Childs K.L., Quesada-Ocampo L.M. Effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Annual American Phytopathological Society Meeting, Virtual Meeting. August, 2021. Phytopathology 111:S2.7
133. Purayannur S., Cano L.M., Bowman M.J, Childs K.L., Quesada-Ocampo L.M. Differential expression of effector-encoding genes in two clades of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. International Society for Molecular Plant-Microbe Interactions Congress eSymposia series. September 2021.
134. Purayannur, S., Cano, L. M., Bowman, M. J., Childs, K. L., and Quesada-Ocampo, L. M. (2020) Clade-specific RXLR effectorome of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Phytopathology 110: S2.6.
135. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Pathology, Gainesville, FL, November 2023.
136. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Botany and Plant Pathology, West Lafayette, IN, November 2023.
137. Quesada-Ocampo L. M., Xiang, L., Brown H., and Vijapurapu R. Evaluation of commercially available spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits. CALS Tailgate Chancellor Suite, Raleigh, NC, October 2023.
138. Quesada-Ocampo L. M.2023. Translational research for detection and management of diseases of vegetable crops. NC Plant Sciences Initiative Senator Visit, Raleigh, NC, June 2023.
139. Quesada-Ocampo L. M. 2023. Translational research for detection and management of diseases of vegetable crops. NC Plant Sciences Initiative Partners Event, Raleigh, NC, May 2023.
140. Quesada-Ocampo L. M. 2023. Applied genomics for disease management in vegetable crops. Department of Biochemistry and Molecular Biology Seminar, Reno, NV, September 2023.
141. Quesada-Ocampo L. M. 2023. Applied genomics for disease management in vegetable crops. Department of Plant Pathology Seminar, University of Georgia, Athens, GA, March 2023.
142. Quesada-Ocampo L. M. 2023. Next generation technologies for plant pathogen detection. NC Plant Sciences Initiative State of the Union. North Carolina Plant Sciences Initiative. North Carolina State University, Raleigh, NC, February 2023.
143. Quesada-Ocampo L. M. 2022. Translational research for detection and management of diseases of vegetable crops. Universidad Nacional Mayor de San Marcos. Lima, Peru, November 2022.
144. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Genetics and Genomics Academy Seminar, North Carolina State University, Raleigh, NC, November 2022.
145. Quesada-Ocampo L. M., Next generation technologies for plant pathogen detection. Commercializing Academic Research Showcase & Innovation Expo. North Carolina Plant Sciences Initiative. North Carolina State University, Raleigh, NC, November 2022.
146. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Biology Seminar, University of Massachusetts, September 2022.
147. Quesada-Ocampo L. M. Disease management in vegetable crops. AgBiome seminar, Durham, NC, July 2022.

148. Quesada-Ocampo L. M. Translational strategies to improve management of re-emerging pathogens of vegetable crops. Australasian Plant Pathology Society, Australia, November 2021.
149. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve disease management in vegetable crops. Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, September 2021.
150. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve management of cucurbit downy mildew. Agriculture Agri-Food Canada, Ontario, Canada, April 2021.
151. Quesada-Ocampo L. M. Leveraging population genetics, epidemiology, and genomics to improve management of re-emerging pathogens of vegetable crops. Department of Plant Pathology, Kansas State University, Manhattan, KS, November 2020.
152. Quesada-Ocampo L.M. Population genetics and epidemiology approaches for management of re-emerging pathogens of vegetable crops. Department of Plant Pathology, University of Minnesota, St. Paul, MN, October 2020.
153. Quesada-Ocampo L.M. From the field to the lab and back: translational strategies to improve disease control in vegetable crops. Department of Plant Pathology, Washington State University, Pullman, WA, September 2020.
154. Quesada-Ocampo L. M., Xiang, L., Brown H., and Vijapurapu R. 2023. Evaluation of commercially available spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits. NC State Chancellor Innovation Fund Meeting, Raleigh, NC, May 2023.
155. Rett-Cadman S, Hammar S, Grumet R. 2023. Biochemical and Genetic Analyses of Cucumber Fruit Peel Lipid Droplets. Plant Animal Genome Conference. San Diego CA
156. Rett-Cadman S, Grumet R. 2024. Genomide Wide Association Study of Cuticle and Lipid Droplet Properties of Cucumber Fruit. Plant and Animal Genome Conference, San Diego CA
157. Rett-Cadman S, Hammar S, Grumet R. 2022. Isolation and Characterization of Lipid Droplets in Cucumber Fruit. Cucurbitaceae 2022, Naples FL
158. Rijal, S. And C.E. McGregor. 2022. Marker-Assisted Breeding for Gummy Stem Blight Resistance in Watermelon. Cucurbitaceae, Naples, Florida.
159. Rijal S, McGregor CE. 2022.Watermelon improvement for gummy stem blight (GSB) resistance through marker-assisted introgression of resistance quantitative trait loci (QTL) from the wild relatives. Southern Region American Society for Horticultural Science, New Orleans, LA.
160. Rijal S, McGregor CE. 2022. Introgression of gummy stem blight (GSB) resistance QTL into elite watermelon cultivars. Joint Southeastern Branch Entomological Society of America and American Phytopathological Society- Caribbean Division, San Juan, PR. HortScience, S233
161. Rijal, S. 2022. Watermelon's Wild Friends: Introgressing Important Traits in a Favorite Fruit. IPBGG Research Seminar, UGA.
162. Rosado-Rivera Y. I., Adams M. L., D'Arcangelo K. N. and Quesada-Ocampo L. M. Downy mildew disease management of cucumber and squash in North Carolina. Cucurbitaceae, Naples, Florida, November 2022.
163. Sabharwal, P., Smart, C., Indermaur, L., Day, C.T., Mazourek, M., Inzinna, G., Hausbeck, M., Medina-Mora, C., and Meru, G. 2023. Evaluation of *Cucurbita pepo* germplasm for resistance to powdery mildew. Annual Conference, American Society for Horticultural Science. Orlando, FL. 31 Jul-4 Aug.
164. Salcedo, A., Parada-Rojas C. H., Purayannur S.,Quesada-Ocampo L. M. Accelerating Resistance Breeding in Cucurbits. CucCAP2 meeting, Virtual Meeting, October 2021

165. Schultheis, J.R.*, S. Michel, B. Parker, and B. Stickley. 2023 Standard Size Watermelon Cultigen Yield and Quality Results for North Carolina. American Society of Horticultural Science Southern Region Annual Conference, Atlanta, GA. Feb 3.

166. Schultheis JR, Keinath A, Quesada-Ocampo L. 2022. Watermelon cultivar symptom plant incidence and yield response in fields in North and South Carolina containing *Fusarium oxysporum* f. sp *niveum*. Watermelon Research and Development group, ASHS - Southern Region, New Orleans, LA, Feb. 11. HortScience 57:S290

167. Schultheis, J.R. and K.D. Starke. 2022 Triploid watermelon standard size cultivar yield and quality results, North Carolina, 2021. Watermelon Research and Development group, ASHS - Southern Region, New Orleans, LA Feb. 11.HortScience 57:S290

168. Schultheis, J. and K. Starke 2021. Melon cultigens and their adaptation in the southeastern United States when grown in North Carolina (abstr.)

169. Shaonpius Mondal, K. Ando, P. Tamang, P. Fashing, W.M. Wintermantel, and J.D. McCreight. 2022. Advancement of CYSDV-resistant melon using marker-assisted selection. Cucurbitaceae 2022, October 30–November 2, 2022.

170. Singh, G., Luckew, A., Mcgregor, C., & Srinivasan, R. (2023). Harnessing host plant resistance: A promising approach to tackle whiteflies and viral diseases in squash. In Entomological Society of America, Annual Meeting. National Harbor, MD

171. Singh, G., Mcgregor, C., & Srinivasan, R. (2023). Screening newly-developed Squash (*Cucurbita* spp.) germplasm lines for resistance against whitefly-transmitted Begomovirus and Crinivirus mixed infection. In Entomological Society of America, Annual SEB meeting. Little Rock, Arkansas

172. Smart, CD. American Phytopathological Society. August 15, 2023. Phytopathologist of Distinction talk. A passion for plant pathology: Pairing discovery with public engagement.

173. **Smart CD. Colorado State University. March 22, 2023. Our veggies are dying: The intersection of climate, consumers and pathogens**.

174. Smart CD. Cornell University Plant Pathology & Plant-Microbe Biology Section, School of Integrative Plant Science. September 13, 2023 Water-limited Agriculture; My Colorado Experience.

175. Sun H. 2024. Graph-based pangenome of the *Citrullus* genus provides insights into watermelon evolution and domestication. Plant & Animal Genome Conference. San Diego, CA. January 2024

176. Sun H. The Citrullus genus super-pangenome. BTI Monday Morning Seminar Series. March 2023.

177. Tan JY, Weng Y. 'Sequence variation in sigma factor binding protein1 (CsSIB1) contributes to downy mildew resistance in cucumber.' A presentation at 2nd Intl Symposium for Hort Plant Biol and Biotech (Nov 17-18, 2022, Beijing, China)

178. Toporek, S.M., and Keinath, A. P. Clade and mating type distribution and population structure of *Pseudoperonsopra cubensis* on *Cucumis melo* in the eastern United States. Plant Health 2021, American Phytopathological Society (virtual). https://events.rdmobile.com/Lists/Details/1179180

179. Trandel, M.A., S. Johanningsmeier, C. Gunter, J. Schultheis, and P. Perkins-Veazie, P. 2020. Cell wall architecture in grafted and non-grafted 'Liberty' watermelon with hollow heart. HortScience, 55(9S):S129 (abstr.).

180. Trandel, M.A., P. Perkins-Veazie, S. Johanningsmeier, J. Schultheis, and C. Gunter. 2020. The Backbone of Fruit: Cell Wall Polysaccharides in Grafted and Non-grafted 'Liberty' Watermelon at Varying Levels of Hollow Heart. HortScience, 55(9S): S423-S424 (abstr.)

181. Trandel, M.A. P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. (2020). Grafting watermelon onto interspecific hybrid squash reduces hollow heart. Acta Horticulturae II International Symposium of Vegetable Grafting. 1302:225-232.
182. Turechek, W.W. Adkins, S., Kousik, C.S., Smith, H. 2020. Towards Areawide Pest Management of Whitefly-Transmitted Viruses in Florida Vegetable Production Systems. Presented at the Whitefly Symposium. Entomological Society of America Annual Meeting, November 2020.
183. Uebbing M.R., Hausbeck M.K. Managing Cucurbit Downy Mildew in Pickling cucumber using disease forecasters and fungicides. Department of Plant Soil and Microbial Sciences, Michigan State University, March 2022.
184. Uebbing M.R., and Hausbeck M.K. Using weather conditions to time fungicide application intervals for control of downy mildew on cucumber. American Phytopathological Society North Central Division Meeting, Lincoln, NE, June 2022. 2020-51181-3219
185. Weng Y (2023) Marker-assisted QTL pyramiding expedites development of cucumbers with multiple disease resistances. Plant and Animal International Conference 30 (Jan 15, 2023, San Diego, CA)
186. Weng Y. 2023. 'Development of 3K SNP panel for targeted genotyping in cucumber.' An oral presentation at ISHS Cucurbits Symposium (online, June 12, 2023, Zhengzhou, China)
187. Weng Y. 2023. 'The USDA-ARS, UW-Madison Cucumber Breeding Program.' A seminar presentation at Tennessee State University, Nashville (October 23, 2023; Nashville, TN).
188. Weng Y (2022) Genetic basis of downy mildew resistance in cucumber. An invited talk on Cucurbinars 2022 organized by APSA (Asian and Pacific Seed Association) (Sept 30, 2022)
189. Weng Y . 2022. Cucumber, genetics, genomics and breeding research: my journey. 'Lecture Series for Post Graduate Students' organized by the Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India (Virtual, February 24, 2022).
190. Weng Y. 2022. Cucumber Breeding: All Things Considered. 'Global Connect Series Lectures' organized by the Dr. Y.S.R. Horticultural University, India (Virtual, April 20, 2022).
191. Weng, Y. 2022. The Gy14v2.0 pickling cucumber genome. Cucurbitaceae 2022 international meeting (Naples, FL, November 2, 2022)
192. Weng Y (2021). Disease resistances in cucumber. SIPS seminar. Cornell University, Ithaca, NY. Virtual.
193. Weng Y, Xu XW, Dymerski R, Wang YH, Copetti D, Luo MC, Fei ZJ, Sun HH, Qu SJ, Jiang N, Bostan H, Iorizzo M (2022) The US processing cucumber genome assembly Gy14v2.0. Cucurbitaceae 2022 (November 1, 2022, Naples, FL)
194. Wilds E., Purayannur S., Quesada-Ocampo L. M. 2022. Differential expression of two effector-encoding genes in Clade 1 and Clade 2 of the cucurbit downy mildew pathogen (*Pseudoperonospora cubensis*) Undergraduate Research and Creativity Symposium. North Carolina State University. Raleigh, North Carolina. July 2022.
195. Wintermantel WM. 2022. Whitefly-transmitted virus infection patterns in mixed infections vary among three cucurbit production regions in the United States. International Symposium on Plant Virus Epidemiology. Madrid, Spain, June 2022
196. Wintermantel WM. 2022. Seasonal prevalence and spread of whitefly-transmitted viruses in California production regions. Cucurbitaceae 2022. Naples FL.
197. Wintermantel WM. 2022. Whitefly populations in the Central Valley of California Lead to introduction and establishment of whitefly-transmitted viruses in melon. Entomological Society of America.
198. Wintermantel WM. 2022. Emerging viruses threatening cucurbit crops. Annual Meeting of the American Phytopathological Society, Pittsburgh, PA, August 6-10, 2022

199. Wintermantel, W.M. 2023. A historical perspective of cucurbit virus emergence and impact in the United States. American Phytopathological Society, Annual meeting, August 12 – 16, Denver, Colorado.

200. Wintermantel, W.M., and Mondal, S. 2023. Competitiveness of whitefly-transmitted yellowing viruses can influence virus dominance in cucurbit crops. Southern Division American Phytopathological Society Annual Meeting, February 13-16, 2023, Durham, NC.

201. Wintermantel, W.M., and Mondal, S. 2023. Factors influencing epidemiology and spread of whitefly-transmitted cucurbit viruses in the US vary among production regions. International Congress of Plant Pathology, August 20-25, 2023, Lyon, France.

202. Wu S. (2023) Super-pangenome of wild and cultivated watermelons. Plant & Animal Genome Conference. San Diego, CA. January 2023.

203. Wu S (2022) Pan-genome of wild and cultivated watermelons. University of Georgia

204. **Zia, B., Levi, A., Simmons, A. and Ling, K.-S.2023. Identification of SNPs associated with cucumber green mottle mosaic virus resistance in watermelon. Plant Health 2023, Denver, CO, August 12-16, 2023**.

205. Zia B, Weng Y, Chen F, Levi A, Cutulle MA, Ling K-S. 2022. Identification and characterization of genetic resistance in cucumber and watermelon to *Cucumber green mottle mosaic virus*. Cucurbitaceae 2022, Naples, FL, USA, October 30 - November 2, 2022

206. Zia B, Weng Y, Cutulle MA, Ling, KS (2022) Identification of genetic sources of resistance to the emerging *Cucumber green mottle mosaic virus* in cucumber lines (APS meeting 2022, Pittsburgh, PA)

EXTENSION/OUTREACH PRESENTATIONS

1. Adams ML, Quesada-Ocampo LM. 2021. Cucurbit Disease Identification and IPM. Piedmont Research Station Horticulture and Specialty Crops Field Day. Salisbury, NC, August 2021.
2. Baugher, N. and J.R. Schultheis. 2022. NC pollinizer research report. NC Watermelon Production meeting. Virtual, Feb. 7, https://gates.ces.ncsu.edu/2022/03/2022-watermelon-production-meeting-recording/
3. Birdsell T, Heagy K, Schultheis J. 2021. Pumpkin Cultivars to Consider Growing in North Carolina; Pumpkin Spacing Considerations: Effects on Yield, Size and Fruit Uniformity. North Carolina Vegetable Growers Association Ag Expo, Raleigh, Dec. 1Grumet R. 2021. The CucCAP2 project. BASF. January 2021.
4. **Branham SE. 2024. Breeding Fon race 2 resistant watermelon using crop wild relatives. Giant Watermelon Growers Association Meeting**.
5. Branham S. Clemson University Coastal Research and Education Center 2022 Field Day. Exhibited melon lines in the field, as well as pathogenicity assays in the greenhouse and growth chamber to 100+ individuals.
6. **Collins, H., and Quesada-Ocampo L. M. Management of Phytophthora capsici on cucurbit crops. 36th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, November 2023**

7. **Grumet R. 2023. Genetic mapping of resistance to Phytophthora fruit rot in cucumber. Pickling Cucumber Research Committee**.
8. Grumet R., Weng Y. 2021. Breeding for disease resistance in pickling cucumber. Great Lakes Fruit, Vegetable, Farm Market Expo. December, 2021
9. Grumet R, Lin YC. 2020. Resistance of cucumber fruit to *Phytophthora capsici*. Pickle Packers International. Virtual conference, October 2020.
10. **Hausbeck, M.K. 2023. Phytophthora control: lessons learned in Michigan. Ontario Fruit and Vegetable Convention. Niagara Falls, ON, Canada, 22 Feb.**
11. **Hausbeck, M.K. 2023. Phytophthora management: What really works? The Ohio State University 76th Annual Celeryville Muck Crops School. Willard, OH, 4 Jan. 25 attendees.**
12. **Hausbeck, M.K. 2023. Southwest Hort Days: Phytophthora crown rot session. Benton Harbor, MI. 1 Feb. 20 attendees**
13. Hausbeck, M.K. Developments in downy mildew and *Phytophthora capsici* control in pickling cucumber. Annual Meeting of the Pickle Packers International. Las Vegas, NV, October, 2022.
14. Hausbeck, M.K. 2022. Phytophthora Crown Rot and Fruit Rot on Cucurbits and Peppers. Syngenta Canada Fruit and Vegetable Webinar. Ontario, Canada. Virtual, 2 Feb. 70 attendees.
15. Hausbeck, M.K. 2022. Putting together a Phytophthora program that works. MSU Extension meeting. Oceana County, MI, Mar. 25 attendees.
16. Hausbeck, M.K. 2022. Developing Tools to Limit Phytophthora on hard squash. Michigan IMP Alliance EPA Crop Tour, Phytophthora stop. Oceana County, MI, 23 Aug. 20 attendees.
17. Hausbeck, M.K. and Uebbing, M. 2021. Downy Mildew: New Insights on Control. Great Lakes Farm, Fruit and Vegetable Expo. Grand Rapids, MI, 8 Dec 2021. 2020-51181-32139
18. Hausbeck, M.K. and Uebbing, M.R. 2021. Pickles in a pickle: Trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Annual Meeting of the Pickle Packers International. Virtual, 19 Oct. 78 attendees.
19. Hausbeck, M.K. 2021. A Partnership to protect Michigan's Cucumber Industry. Farm Lane Society meeting, Virtual, 5 Mar.
20. Hausbeck, M.K. 2020. Management of Phytophthora Blight in Processing Squash. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 10 Dec. 167 attendees.
21. Hausbeck, M.K. 2020. Downy Mildew Management in Pickling Cucumbers. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 8 Dec. 174 attendees.
22. Hausbeck, M.K. 2020. Vegetable and Root Crop Field Day: Disease control of Vegetables. Sept, Virtual, 39 attendees. https://www.canr.msu.edu/events/oceana-research-tour-virtual-field-day
23. Hausbeck, M.K. 2020. 2021 Spray Program. Southeast Vegetable Meeting. Virtual, 4 Nov. 90 attendees.
24. Hausbeck, M.K. and Higgins, D.S. 2020. The Grounder, the Line Drive, and the Pop Fly: Fielding Three Very Different Vine Crop Diseases. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 9 Dec. 178 attendees.
25. Hausbeck, M.K. and Kenny, G. 2020. From the Field to the Lab and Back: Monitoring Fungicide Resistance in Cucurbit Downy Mildew. Pickle Packers International Annual Meeting. Virtual, 19 Oct. 78 attendees.
26. Hausbeck, M.K. and Kenny, G. 2020. From the Field to the Lab and Back: Monitoring Fungicide Resistance in Cucurbit Downy Mildew. Pickle Packers International Annual Meeting. Virtual, 19 Oct. 78 attendees.
27. Heagy, K. and Schultheis, J. R. UAV images and objective detection software: estimating pumpkin yield and fruit size. North Carolina Vegetable Growers Association Ag Expo. Raleigh, NC, November 29, 2022.

28. Higgins D, Hausbeck MK. 2021.Phytophthora Crown Rot and Fruit Rot, and Powdery Mildew for Fresh Market Growers. Great Lakes Farm, Fruit, and Vegetable Expo. Grand Rapids, 9 Dec. 115 attendees.
29. **Hurry, N. and J. Schultheis. 2023. Pollinizer Options for Seedless Watermelon Production. 36th Annual Southeast Vegetable & Fruit EXPO, Watermelon Section. Myrtle Beach, SC., Nov. 27**
30. Indermaur, E. J., Day, C. T. C., and Smart, C. D. Tools for managing powdery mildew in winter squash organically: cultivars & biofungicides. Empire State Producers Expo. Syracuse, NY, February, 2023.
31. Indermaur, E. J., Day, C. T. C., and Smart, C. D. Winter squash cultivar evaluations for resistance to powdery mildew. Empire State Producers Expo. Syracuse, NY, February, 2022.
32. **Indermar L, Smart CD. 2023. Specialty crop tour. Geneva NY September 16, 2023. Current research on diseases of specialty crops at Cornell AgriTech.**
33. Katuuramu D. N. 2022. GWAS for Resistance to Cucurbit Downy Mildew in Watermelon. 2022 Watermelon Research & Development Group Annual Meeting.in South Carolina. Edisto REC Watermelon Field Day, Blackville, SC. July 14, 2022.
34. **Keinath, A. P. 2023. 2023 Vegetable Disease Update: Downy Mildew on Cucumbers and Anthracnose on Peppers. Pee Dee Vegetable Meeting, Florence, SC. 2/28/2023**
35. **Keinath, A. P. 2023. 2023 Midlands Vegetable Disease Update: Downy Mildew, Black Rot, Black Spot, and Anthracnose. Midlands Spring Vegetable Meeting, Pelion, SC. 3/7/23**
36. **Keinath, A. P. 2023. Integrated management of Fusarium wilt on seedless watermelon. Clemson Coastal REC Field Day. Presented twice on 6/7/23. 94 attendees, 96% reported learning something new.**
37. **Keinath, A. P. 2023. The SC Grower Exchange: June 30, 2023 - with Dr. Anthony Keinath. Podcast. 6/30/23. Major topic was cucurbit downy mildew. https://open.spotify.com/episode/1RGY5V6c3mukTVZbj9thIM**
38. **Keinath, A. P. 2023. Fusarium Biology and Disease Management. Clemson Edisto REC Watermelon Field Day. 7/13/23**
39. Keinath, A. P. 2023. How Much Does It Cost to Not Spray Watermelon? And Other Economic Impacts of Vegetable Disease Management. Virginia Tech Eastern Shore Agricultural Research and Extension Center Lunch and Learn. March 24, 2023.
40. Keinath, A. P. 2023. Tebuconazole Resistance in the Gummy Stem Blight Fungus in South Carolina. 35th Southeast Vegetable & Fruit Expo. November 29, 2022.
41. Keinath, A. P. 2023. 2023 Vegetable Disease Update: Downy Mildew on Cucumbers and Anthracnose on Peppers. Pee Dee Vegetable Meeting, Florence, SC. February 23, 2023.
42. Keinath, A. P. 2023. 2023 Midlands Vegetable Disease Update: Downy Mildew, Black Rot, Black Spot, and Anthracnose. Midlands Spring Vegetable Meeting, Pelion, SC. March 7, 2023.
43. Keinath, A. P. 2022. Management and Economics of Three Common Foliar Diseases on Cucumber and Leafy Greens. Long Island Ag Forum (virtual). January 18, 2022.
44. Keinath, A. P. 2022. All You Need to Know about Downy Mildew on Cucumbers for the 2022 Growing Season. Clemson Extension Cucurbit Pre-Plant Meeting (virtual). February 8, 2022.
45. Keinath, A. P. 2022. Reduced Sensitivity to Tebuconazole is Common in South Carolina Isolates of *Stagonosporopsis*, Causal Agent of Gummy Stem Blight. Watermelon Research and Development Group Annual Meeting (virtual). February 11, 2022.
46. Keinath, A. P. 2022. Cucurbit Disease Update with 2021 Clemson Trial Results. Clemson Extension Cucurbit Grower Meeting (virtual). February 17, 2022.
47. Keinath, A. P. 2022. Tebuconazole Resistance is Widespread in the Gummy Stem Blight Fungus Keinath, A.P. 2021. In-service training for Commercial Horticulture Agents on cucurbit downy mildew, 9/30/2021 and 10/26/2021, 4 agents.

48. Keinath, A.P. 2021. Advanced Master Gardener Training: Identifying Downy Mildew in the Field on Cucurbits and Basil, Completed, Demonstration, Attendees: 11, (October 5 and 13, 2021)
49. Keinath, A.P. 2021. "Management and Economics of Three Common Foliar Diseases on Cucumber and Leafy Greens," Long Island (NY) Ag Forum (virtual), January 18, 2022. Estimated Attendees: 95
50. Keinath, A.P. 2021. CU Cucurbit Grower Meeting (virtual). Cucurbit Disease Update with 2021 Clemson Trial Results, Presented, Clientele Groups, Estimated Attendees: 8, (February 17, 2022)
51. Keinath, A.P. 2021. CU Cucurbit Pre-Plant Meeting (virtual). All You Need to Know about Downy Mildew on Cucumbers for the 2022 Growing Season, Presented, Clientele Groups, Estimated Attendees: 20, (February 8, 2022)
52. Keinath, A.P. 2020 Watermelon Fungicide Trial Results: Foliar and Fruit Anthracnose and Foliar Gummy Stem Blight. 2021 Watermelon Research and Development Group Annual Meeting. Virtual Meeting, February 2021.
53. Keinath, A.P. Understanding Root Diseases of Vine Crops. 2021 Mid-Atlantic Fruit & Vegetable Conference. Virtual Meeting, February 2021. https://amr.swoogo.com/mafvc2021/sessions
54. Keinath, A.P. New seedless watermelon cultivars and Fusarium wilt. CREC Field Day in-field presentation, June 2021. https://news.clemson.edu/clemsons-coastal-rec-research-helps-improve-vegetable-prduction-in-south-carolina/
55. Keinath, A.P. Seedling date and fungicides to manage cucumber downy mildew. CREC Field Day in-field presentation, June 2021. https://news.clemson.edu/clemsons-coastal-rec-research-helps-improve-vegetable-production-in-south-carolina/
56. Keinath, A.P. Managing anthracnose and gummy stem blight on watermelon with fungicides in 2021. Southwest Indiana Melon Growers. Virtual Meeting, March 2021. https://ag.purdue.edu/arge/swpap/Documents/Tony.pdf
57. Keinath, A.P. and Silva, F.D. Economic Impact of Downy Mildew and Fungicide Resistance on Cucumbers. Clemson Hort Team Virtual Cucurbit Meeting, February 2021. https://ensemble.clemson.edu/hapi/v1/contents/permalinks/Nj39MtRo/view
58. **Kumar R, Branham S., Wechter W., Kousik C.S. 2024. KASP Marker Development for Powdery Mildew Resistance in Watermelon (Citrullus lanatus) using Bulk Segregant Analysis (BSAseq) and RNAseq. Presented at the Watermelon Research and development Group meeting held in conjunction with Southern Region American Society for Horticultural Science. February 2024, Atlanta, GA.**
59. Levi A. 2023. History, Genomic Tools and Enhancing Disease Resistance in Watermelon. Giant Watermelon and Pumpkin Grower Group. March 25th, 2023, Elkin, NC.
60. Levi A. 2023. Genetic research in watermelon. Tour of State agricultural leaders, Senators, Representatives, and Minister of Agriculture from Canada. The U.S. Vegetable Laboratory (USVL), Charleston SC.
61. Levi A. 2022. Presentation to Minister of Agriculture from Qatar. May 11th, 2022.
62. Ling, K.-S. 2022. An integrated approach to prevent emerging viral disease outbreak in greenhouse vegetable production. Canadian Greenhouse Conference, Niagara Fall, Canada, October 5-6, 2022)
63. **Ling, K.-S. 2023. Managing the Emerging Cucumber Green Mottle Mosaic Virus on Cucurbit Crops. Emerging viruses in Cucurbits working group meeting, Durham, NC**.
64. **Mauch KE, Wintermantel WM. 2024. Updates on resources to combat emerging virus threats. the California Melon Research Board Symposium in San Diego, CA (January 11, 2024)**
65. **Mazourek M, Inzinna G, Fabrizio J, Fenn M. 2023. Peppers and Cucurbits. VBI Field days. Freeville and Ithaca, NY. August 28-29, 2023**.

66. Mazourek M, Haga, ER, Jenny P, Mueller, K. 2023. Selecting High-quality Breeding Material. Part of Webinar Series: Practical Training for On-Farm and Collaborative Plant Breeding. Jan 17, 2023.
67. Mazourek M. 2022. Vegetable Breeding Institute Field Days. August 29-20, 2022. Ithaca and Freeville, NY.
68. Mazourek M. 2021. Winter Squash Background, Diversity and Breeding. Winter Squash Sagra. Culinary Breeding Network. January 25, 2021
69. McGregor, C. & G. Boyhan (2020) Breeding better Cucurbits. Vegetable & Specialty Crop News, September 2020: 16-17
70. Meru G. 2021. Progress towards breeding resilient cultivars for Florida. Presented at the Southeast Florida Extension Meeting, held virtually April 8, 2021.
71. Meru G. 2021. Progress towards breeding resilient cultivars for Florida. Presented at the Extension Field Day for Vegetable Growers in Miami-Dade County, held virtually February 18, 2021.
72. **Michel, S., Quesada-Ocampo, L., Schultheis, J., and Keinath, A. 2023. Triploid Watermelon Cultigen Responses in a Field Infested with *Fusarium oxysporum* f. sp. *niveum*. 35th Southeast Vegetable & Fruit Expo, Raleigh, NC. 11/29/23**
73. **Michel, S., L. Quesada-Ocampo, J. Schultheis, and T. Keinath. 2023. Management Results of Fusarium Wilt in Watermelon Using Cultivar, Fungicide, and Grafting. 36th Annual Southeast Vegetable & Fruit EXPO, Watermelon Section. Myrtle Beach, SC., Nov. 27**
74. **Michel, S.*, J.R. Schultheis, A.P. Keinath, and L. Quesada-Ocampo. Management Results of Fusarium Wilt of Watermelon Using Cultivar, Fungicide, and Grafting. Tri-County North Carolina Vegetable Production Meeting, Online, February 2024.**
75. **Michel, S.*, J.R. Schultheis, A.P. Keinath, A. P., and L. Quesada-Ocampo. Management Results of Fusarium Wilt of Watermelon Using Cultivar, Fungicide, and Grafting. North Carolina Watermelon Production Meeting, Online, January 2024.**
76. Michel, S., J. Schultheis, A. Keinath, and L. Quesada-Ocampo. Triploid watermelon cultigen responses in a field infested with *Fusarium oxysporum* f sp. *niveum*. North Carolina Vegetable Growers Association Ag Expo. Raleigh, NC, November 29, 2022.
77. Michel S., Quesada-Ocampo, L. M., Schultheis J., and Keinath T. Triploid watermelon cultigen responses in a field infested with *Fusarium oxysporum* f.sp. *niveum*. 35th Annual Southeast Vegetable and Fruit Expo. Durham, NC, December 2022.
78. **Nelson E, Smart CD. 2023. Rain and Hail Insurance Company Tour. Geneva NY July 19, 2023. Phytophthora blight of vegetable crops.**
79. **Parker, B., S. Michel, B. Stickley, A. Pfefferkorn, and J. Schultheis. 2023 Standard Size and Mini Watermelon Cultivar Study Results 2023. 36th Annual Southeast Vegetable & Fruit EXPO, Watermelon Section. Myrtle Beach, SC., Nov. 27**
80. **Quesada-Ocampo L. M., Xiang L., Prieto M., Rosado Y., Collins H., Lorscheider M. New technologies for disease detection in cucurbit crops. NC Legislature field day. Clayton, NC, June 2023**
81. **Quesada-Ocampo L. M. 2023. Field monitoring of the cucurbit downy mildew pathogen: the next frontier. Pickle Packers International Spring Meeting. Raleigh NC, April 2023.**
82. Quesada-Ocampo L. M. Management of watermelon diseases. North Carolina Watermelon Production Meeting. Virtual, February 2023.
83. Quesada-Ocampo L. M., Rosado-Rivera Y.I., and Prieto M. Management of downy mildew in cucurbit crops. 35th Annual Southeast Vegetable and Fruit Expo. Durham, NC, December 2022.
84. Quesada-Ocampo, L. M. Pickles in a pickle: trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. North Carolina Vegetable Growers Association Ag Expo.

Wilmington, NC, November 2021.

85. Quesada-Ocampo L. M. and Hausbeck M. K. Pickles in a pickle: trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Pickle Packers International Spring Meeting. Austin, TX, October 2021.
86. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve management of cucurbit downy mildew. Agriculture Agri-Food Canada, Ontario, Canada, April 2021.
87. Quesada-Ocampo L.M. Management of Fusarium wilt and anthracnose in watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
88. Quesada-Ocampo L.M. Cultural and chemical control options for Phytophthora fruit rot of watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
89. Quesada-Ocampo L.M. Never a dill moment when managing cucumber downy mildew. 2020 Eastern NC Certified Crop Adviser Training. Virtual Meeting, December 2020.
90. Quesada-Ocampo L.M. From the field to the lab and back: monitoring fungicide resistance in cucurbit downy mildew. Pickle Packers International Annual Meeting. Virtual Meeting, October 2020.
91. **Schultheis, J.*, A. Pfefferkorn*, S. Michel, B. Stickley, and B. Parker. 2023. New Zucchini Cultivar Options; What About Ideal Melons?; Canary Melons, a Potential New Crop for North Carolina. 36th Annual Southeast Vegetable & Fruit EXPO, Myrtle Beach, SC., Nov. 27. (50 attenders)**
92. **Schultheis, J.*, K.Heagy, and M. Knuth. 2023. Pumpkin Economic Considerations Based on Plant Spacing; Considerations for Standardization of Pumpkin Categories. 36th Annual Southeast Vegetable & Fruit EXPO, Myrtle Beach, SC., Nov. 27. (30 attenders)**
93. **Schulthies J. 2023. Watermelon variety trials. Watermelon Production Meeting (virtual).Feb. 17, 2023 (~ 65 in attendance)**
94. **Schulthies J. 2023. Watermelon cultivar update. Regional Watermelon Grower meeting, Turkey, NC Fe. 13 (~25 in attendance)**
95. Schultheis, J., A. Pfefferkorn, S. Michel, B. Stickley, and B. Parker. 2023. New Zucchini Cultivar Options; What About Ideal Melons?; Canary Melons, a Potential New Crop for North Carolina. 36th Annual Southeast Vegetable & Fruit EXPO, 2023 Yearbook. Cucurbit Section. Myrtle Beach, SC., Nov. 27. (abstr.)
96. Schultheis, J., K.Heagy, and M. Knuth. 2023. Pumpkin Economic Considerations Based on Plant Spacing; Considerations for Standardization of Pumpkin Categories. 36th Annual Southeast Vegetable & Fruit EXPO, 2023 Yearbook. Cucurbit Section. Myrtle Beach, SC., Nov. 27. (abstr.)
97. Schultheis, J. and A. Keinath. Watermelon cultivar incidence and yield response in fields in North and South Carolina containing *Fusarium oxysporum* f. sp. *niveum*. Mar-Del Watermelon Association meeting. Cambridge, MD, February 3, 2023.
98. Schultheis, J. and S. Michel. Watermelon cultigen yield and quality results, North Carolina, 2022. Mar-Del Watermelon Association meeting. Cambridge, MD, February 3, 2023.
99. Schultheis, J. and S. Michel. Watermelon cultigen yield and quality results, North Carolina, 2022. North Carolina Vegetable Growers Association Ag Expo. Raleigh, NC, November 29, 2022.
100. Schultheis JR, Quesada-Ocampo L. 2022. Watermelon Cultivar Evaluations in Fields with Minimal or High Levels of Fusarium Wilt; Potential Fusarium Wilt Management Strategies, NC Watermelon Production meeting, Virtual, Feb. 7, https://gates.ces.ncsu.edu/2022/03/2022-watermelon-production-meeting-recording/
101. Schultheis J, Quesada-Ocampo LM, Keinath T. 2021. Watermelon cultivar evaluations with and without Fusarium wilt. North Carolina Vegetable Growers Association Ag Expo. Wilmington, NC, November 2021.

102. Schultheis, J.R. 2021. Hollow heart considerations and pollenizer cultivar comparisons. North Carolina Watermelon Growers Association. Virtual meeting, January, 2021. 65 attendees
103. Schultheis, J. R. and L. Quesada-Ocampo. 2021 Watermelon cultivar evaluations in fields with minimal or high levels of Fusarium wilt: Potential Fusarium wilt management strategies. North Carolina Vegetable Growers Association Ag Expo. Raleigh, NC, December 1 2021.
104. Smart C. Winter Squash Cultivar Evaluations for Resistance to Powdery Mildew. 2022. NY State producers expo.
105. Smart, C.D., Western NY vegetable twilight meeting. August 3, 2021. *Cucurbit diseases of 2021.* 2 x 1 hour meetings with 30 growers and educators in each.
106. Smart, CD. 2023. Western NY vegetable disease discussions – primarily focused on *Phytophthora capsici*. September 20, 2023.
107. Smart, CD. 2023. Monroe County NY vegetable disease discussions – primarily focused on *Phytophthora capsici* September 27, 2023
108. Smart CD. 2023. Master Gardener plant disease talk and discussion. October 4, 2023. 1.5 hour talk to 40 people.
109. Smart CD. 2023. Cornell AgriTech Legislative Tour. October 13, 2023. 30 minute talk and 1 hour tour of greenhouses. 44 people
110. Smart, CD. 2023. NY Capital District vegetable disease discussions – primarily focused on *Phytophthora capsici* October 17, 2023
111. Smart CD. 2023. Hudson Valley vegetable, apple and hemp disease grower discussions. October 19, 2023. 3x1 hour visits to farms, 5 people per farm.
112. Smart CD. 2023. Western NY winter vegetable meeting. Eden, NY. Fungicide resistance, two case studies. 60 minute talk to 46 people.
113. Smart CD. Hudson Valley vegetable, apple and hemp disease grower discussions. October 19, 2023. 3x1 hour visits to farms, 5 people per farm.
114. Smart CD. 2024. Long Island Agricultural Forum. Riverhead NY, January 10, 2024. Vegetable disease management updates. 30 minute talk to 50 growers.
115. Smart CD. 2024. New York State Vegetable Growers Expo. Syracuse NY, January 23, 2024. Strategies to manage Phytophthora in cucurbits. 30 minute talk to 30 growers.
116. Tregeagle D. 2023. Economic Outlook for Pickles and Overview of Cucumber Seed Trait Valuation Research. Pickle Packers International Annual Meeting. Raleigh, NC. Apr 20, 2023.
117. Tregeagle D. 2023. Developing a Pickling Cucumber Budget. Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. November 27, 2023.
118. Toporek, S. M., and Keinath, A. P. Grafting to manage downy mildew on cantaloupe. CREC Field Day in-field presentation, June 2021. https://news.clemson.edu/clemsons-coastal-rec-research-helps-improve-vegetable-production-in-south-carolina/
119. Uebbing M.R. and Hausbeck M.K. Downy Mildew Update in Pickling Cucumber. Great Lakes Farm, Fruit and Vegetable Expo. Grand Rapids, MI, December 2022.
120. Uebbing M.R. and Hausbeck M. Using disease forecasters to time fungicide applications to control downy mildew in pickling cucumbers. MSU Pickle & Pepper Research Committee meeting. Grand Rapids, MI, December 2022.
121. Uebbing M.R. and Hausbeck M.K. Practical and effective strategies to keep downy mildew in check. Pickle Packers International 2022 Annual Meeting. Las Vegas, NV, October 2022.
122. Wechter W, Branham S. 2023. Clemson University Coastal Research and Education Center 2023 Field Day. Exhibited melon lines in the field.

123. Weng Y. 2024. 'Accelerate development of disease resistant varieties with molecular breeding in cucumber.' An outreach talk with cucumber industry stakeholders (February 22, 2024, Madison, WI)

124. Weng Y. 2023. 'QTL mapping of downy mildew resistances in cucumber – a update.' An outreach talk with cucumber industry stakeholders (December 20, 2023, online).

125. Weng Y. 2023. 'The USDA-ARS, UW-Madison Cucumber Breeding Program.' A seminar presentation at Tennessee State University, Nashville (October 23, 2023; Nashville, TN).

126. Weng Y. 2023. 'The USDA-ARS and UW Madison Cucumber Improvement Program.' Field talk at Hancock Agriculture Research Station (Hancock, Wisconsin, 9/1/2023).

127. Weng, Y. 2022. Genetic basis of downy mildew resistances in cucumber. Asia Pacific Seed Association. September 2022

128. Weng Y (2022) Development of pickling cucumber inbreds with multiple disease resistances conferred by a novel mutant. Pickle Packers international (PPI) annual meeting (October 19, 2022 Las Vegas, NV)

129. Weng Y. 2022. A cucumber leaf color mutation associated with biotic and abiotic stress tolerance. Pickle Packer International (PPI) annual meeting (Las Vegas, NV, October 19, 2022)

130. Weng Y (2022) Marker-assisted QTL pyramiding for multiple disease resistances in cucumber. Midwest Pickle Association annual meeting (December 6, 2022, Grand Rapids, MI)

131. Weng Y (2021) The Gy14v2.0 cucumber draft genome. Chinese Cucumber Breeders Association 2021 Annual Meeting and Variety Show. April 2021. Virtual.

132. Y, Chen FF, Tan JY (2020) Marker-assisted QTL pyramiding for downy mildew (DM) and phytophthora fruit rot (PFR) resistances in pickling cucumber. Pickle Packers International. Virtual conference, October 2020.

133. Wintermantel W. 2022. California Melon research Board, January, 2022. Online presentation to ca. 70 Board members growers and seed company personnel.

134. Wintermantel W. 2022. University of California Extension, Melon and Tomato Crops Meeting, WSREC, Five Points, CA, October 12, 2022. Symptoms and biology of potentially invasive melon viruses.

135. Wintermantel WM. 2024. Monitoring the incidence of whitefly-transmitted viruses in melon fields, weeds and alternate crops in the Central Valley and Low Desert production regions. California Melon Research Board Symposium in San Diego, CA (January 11, 2024)

136. Wintermantel WM. 2024. The Emerging Viruses in Cucurbits Working Group and new viruses of concern for U.S. cucurbit production. American Seed Trade Association's 63rd Annual Vegetable & Flower Seed Conference in Monterey, CA (January 26-30, 2024)