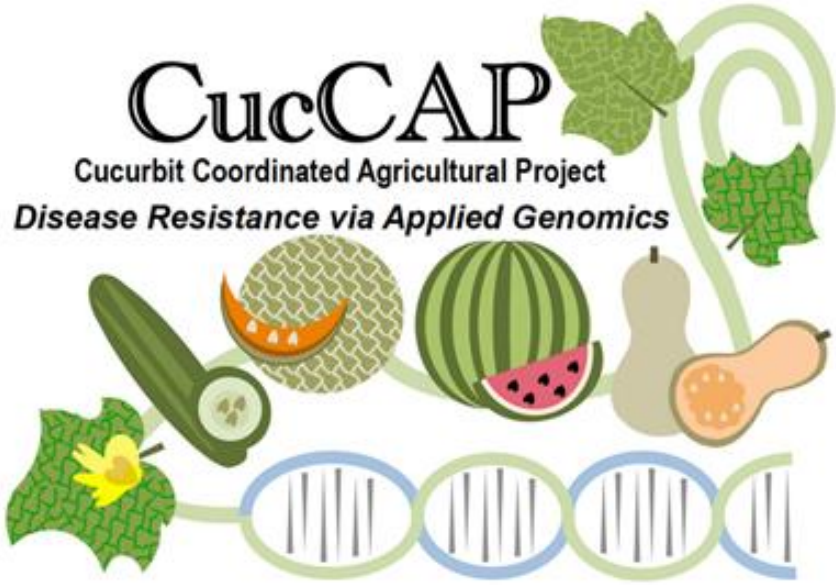
CucCAP2

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



CucCAP2 Team Meeting

April 13-14, 2023

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AGENDA

CucCAP2 team meeting – April 13-14, 2023

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

THURSDAY, APRIL 13

8:00-8:15 Arrival, welcome, Introduction of participants
8:15-8:30 Introduction -
- Project objectives
- Plans for the meeting, logistics

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

8:30-9:15 Overview of progress: bioinformatics platforms, databases, marker sets, pan-genomic analyses (Fei, Wu)
9:15-9:35 Discussion and feedback
Priorities, data access and distribution, phenotype data being collected
9:35-9:45 Status of core panels (seed stocks; resequencing; seed handling and distribution) (Grumet)
9:45-10:15 Discussion of strategy for seed handling and distribution

10:15-10:30 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

10:30-11:40 **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)
11:40-12:00 Feedback/priorities from industry

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

1:00-1:50 **Melon**: Status for each disease
powdery mildew, CMV, CYSDV, Fusarium
(McCreight, Branham, Kousik, Wechter, Wintermantel)
1:50-2:00 Feedback/priorities from industry

2:00-2:35 **Cucumber**: Status for each disease
downy mildew, Phytophthora, CGMMV
(Weng, Grumet, Keinath, Ling)
2:35-2:50 Feedback/priorities from industry

2:50-3:05 ***Break***

3:00-4:00 **Squash**: Status for each disease
C. moschata – powdery mildew, *Phytophthora*, *C. maxima – Phytophthora*,
C. pepo – powdery mildew, *Phytophthora*
(Mazourek, Hausbeck, Kousik, Meru, Ramirez, Smart)
4:00-4:15 Feedback/priorities from industry

Planning Session 2
4:15-5:00 Integrated Disease Management
Genomic Tools

6:00 ***CucCAP Networking Dinner***
Host: Mary Hausbeck, 306 Norris Road, Dewitt, MI

FRIDAY, APRIL 14

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)
CucCAP3? Industry priorities? Research priorities? Leadership? Timeline?

11:00-11:30 Wrap up discussions, feedback from external reviewers

CucCAP Team

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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	Eben Ogundiwin	R&D Technical Lead Cucurbits, BASF Vegetable Seeds
Bayer Crop Science	Nischit Shetty	NAM Cucurbit Breeding Lead, Seminis Vegetable Seeds
East-West Seeds	Caleb Orchard Marilyn Hindo	
Enza Zaden	Bart Kay Walter Verweij	Research Molecular Biology
Hollar Seed Company	Bruce Carle	Plant Breeder
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	

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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
	Shaker Kousik	(SK)	ARS-SC
	Pat Wechter	(PW)	ARS-SC
	Bill Wintermantel	(BW)	ARS-CA
Cucumber	***Yiqun Weng- Team Leader***	(YW)	ARS-WI
	Rebecca Grumet	(RG)	MSU
	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

Table 3. TIMELINE CHART					
Objective	**Personnel/Institution**	**Year**			
	(initials and institution abbreviations as ***p. 11***)	1	2	3	4
Obj. 1. Develop genomic, bioinformatic, mapping approaches and tools for cucurbits					
1.1. Develop genomic and bioinformatics platforms					
1.1.1. High resolution genotyping	ZF, SW (BTI)	X	X		
1.1.2. Pan-genome platforms	ZF, SW (BTI)	X	X	X	X
1.1.3. De novo genome assembly, pan-genome construction	ZF, SW (BTI)	X	X	X	X
1.1.4 Web-based database for phenotypic, genotypic, QTL information	ZF (BTI), members of crop teams	X	X	X	X
1.1.5. Genomic, bioinformatics workshops	ZF, SW (BTI), members of crop teams	X	X	X	X
1.2. Provide community resource for genome wide association studies (GWAS)					
1.2.1. Seed multiplication of core populations - watermelon - melon - cucumber - squash	 AL (ARS-SC) JM (ARS-CA) YW (ARS-WI), MM (CU)	 X X X X	 X X X X	 X	
1.2.2. Deep sequencing cores (sets of 100)	ZF, SW (BTI)	X	X		
1.2.3 Population genetics and phenotype-genotype associations	Crops teams	X	X	X	X
Obj. 2. Map and develop markers for disease resistance					
2.1 QTL mapping of resistances *(QTL, QTL-seq, GWAS)*	Developing populations **(P)**, phenotyping (**Ph)**, QTL mapping **(Q),** Refining/Fine mapping (**F**)				
2.1.1. Watermelon - CGMMV - Fusarium race 2 - gummy stem blight - Phytophthora - powdery mildew - downy mildew	 KSL, AL (ARS-SC) AL, PW (ARS-SC), SB (CLU) PW,AL (ARS-SC), SB,AK(CLU) CM (UGA), UR (WVSU) SK (ARS-SC) SK (ARS-SC) PW, AL (ARS-SC), SB (CL)	 P Ph Ph P Ph Ph Ph	 Ph Ph Ph Ph Ph Q Ph	 Q Q Q Q Q Q	 F F Q
2.1.2. Melon - powdery mildew - downy mildew - CYSDV	 SK,PW (ARS-SC), JM(ARS-CA) PW (ARS-SC) JM, WW (ARS-CA)	 PhQ Ph	 Q Ph PhQ	 Q Q	

2.1 QTL map resistances (continued) *(QTL, QTL-seq, GWAS)*	Developing populations **(P)**, phenotyping (**Ph)**, QTL mapping **(Q),** Refining/Fine mapping (**F**)				
		Y1	Y2	Y3	Y4
2.1.3. Cucumber					
- downy mildew	YW (ARS-WI), AK (CLU)	P,Ph	Q,F	Q,F	F
- Phytophthora	RG (MSU)	P,Ph	Q	F	
- CGMMV	KL (ARS-SC), YW (ARS-WI)	Ph,P	P,Q	Q	Q
2.1.4 Squash					
- Powdery – *C. pepo*	GM (UF), MH (MSU), CS(CU)	Ph	Ph	Q	F
C. moschata	MM, CS (CU), ALR (UPR), MH (MSU)	P	P,Ph	Ph,Q	F
- Phytophthora – *C. pepo*	GF (UF)	P	Ph	Q	F
C. maxima	MM, CS (CU), MH (MSU)	P	P,Ph	P, Ph	Ph
C. moschata	SK (ARS-SC), MM (CU)	P	P	Ph	Q
2.2 Marker development and verification	Develop marker **(M)**, verify **(V)**				
2.2.1. Watermelon					
- Fusarium race 1	AL, PW (ARS-SC), SB (CLU)		V	V	
race 2	AL, PW (ARS-SC), SB (CLU)	M	V	V	
- gummy stem blight	PW,AL (ARS-SC), SB,AK(CLU)			M	V
	CM (UGA),	MV	MV	V	V
	UR (WVSU)			M	V
- Phytophthora	SK (ARS-SC)			M	V
- powdery mildew	SK (ARS-SC)		M	V	
-downy mildew	PW, AL (ARS-SC), SB (CLU)			M	V
- PRSV-W	AL, KSL (ARS-SC), SB (CLU)	M	V		
-ZYMV	AL, KSL (ARS-SC), SB (CLU)	M	V		
-CGMMV	AL, KSL (ARS-SC)			M	V
2.2.2. Melon					
- powdery mildew	SK,PW(ARS-SC), JM (ARS-CA)	M	V		
- Fusarium	PW (ARS-SC)	M	V		
- CYSDV	WW, JM (ARS-CA)			M	V
2.2.3. Cucumber					
- downy mildew	YW (ARS-WI), TK (CLU)	M	MV	V	V
- Phytophthora	RG (MSU)		M	MV	V
2.2.4 Squash					
- Powdery – *C. pepo*	GM (UF)			M	V
C. moschata	MM (CU)			M	V
- Phytophthora – *C. pepo*	GM (UF)			M	V

Obj. 3. Introgress, pyramid/stack resistances into advanced breeding lines	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
		Y1	Y2	Y3	Y4
3.1. Watermelon					
- Fusarium wilt (FW) race 1	AL, PW (ARS-SC), SB (CLU)	I	I	A	AR
race 2		I	I	A	AR
- gummy stem blight	PW, AL (ARS-SC)				B
	CM (UGA),	B	I	I	A
	UR (WVSU)			B	B
- Phytophthora	SK (ARS-SC)	I	A	A	AR
- powdery mildew (PM)	SK (ARS-SC)	I	A	A	AR
- PRSV-W	AL, KSL (ARS-SC), SB (CLU)	B	I	A	AR
-ZYMV	AL, KSL (ARS-SC), SB (CLU)	B	I	A	AR
Pyramiding FW races 1and 2 +PM +PRSV +ZYMV into advanced breeding lines.	AL, PW, SK, KL (ARS-SC), SB (CLU)	I	I	A	AR
3.2. Melon					
- powdery mildew	SK (ARS-SC), JM (ARS-CA)	B	I	I	IA
- Fusarium	PW (ARS-SC)	B	B	I	IA
- CYSDV	JM, WW (ARS-CA)	I	I	IA	IAR
3.3. Cucumber					
- downy mildew	YW (ARS-WI), AK (CLU)	B,I	I,A	I,A	R
- Phytophthora	RG (MSU)	B	B,I	I,A	A,R
- Pyramid downy mildew + Phytophthora	YW (ARS-WI), RG (MSU), AK (CLU)	B	B,I	I,A	R
3.4 Squash (*C. pepo, C. moschata*)					
- powdery mildew	MM (CU), GM (CU)	I	I	A	R
- Phytophthora	MM (CU), CS (CU), MH (MSU)	I	I	A	AR
- Pyramid powdery mildew + Phytophthora	MM (CU), SK (ARS-SC)			B	B
Obj. 4. Economic impact analyses, disease control information					
4.1 Provide readily accessible disease management information and recommendations					
- Maintain centralized website	Integrated disease management team	X	X	X	X
- Provide up-to-date disease alerts, diagnostic resources, disease control recommendations		X	X	X	X
- Field days and demonstration plots		X	X	X	X

		Y1	Y2	Y3	Y4
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 - watermelon/powdery mildew - watermelon/fusarium - cucumber/downy mildew - squash/powdery mildew - squash/Phytophthora	 SK (ARS-SC), LQ (NCSU) JS (NCSU), AK (CLU) LQ (NCSU), MH (MSU), AK (CLU) CS (CU), MH (MSU) CS (CU), MH (MSU)	 X X X X X	 X X X X X		
4.2.2. Evaluation of integrated disease management in cucurbits combining host resistance and chemical control - watermelon/fusarium - cucumber/downy - squash/Phytophthora - squash/powdery	 JS (NCSU), AK (CLU) LQ (NCSU), MH (MSU) MH (MSU), CS (CU) MH (MSU), CS (CU)			 X X X X	 X X X X
4.2.3 Analysis of pathogen populations to inform breeding and disease management. *P. capsici* *P. cubensis*	 CS (CU) LQ (NCSU)		 X X	 X X	
4.3. Determine economic impacts of disease and control tools and valuation of crop attributes					
4.3.1. Crop budgets MI – cucumber squash NC – cucumber watermelon NY - squash	 DT (NCSU) DT (NCSU) DT (NCSU) DT (NCSU) DT (NCSU)	 X X X X X	 X X X X X		
4.3.1. Partial budgeting MI – cucumber/downy mildew squash/phytophthora NC – cucumber/downy mildew watermelon/fusarium NY – squash/phytophthora SC – watermelon/fusarium	 DT (NCSU), MH (MSU) DT, LQ (NCSU) DT, JS (NCSU) DT (NCSU), CS (CU) DT (NCSU), AK (CLU)			 X X X X X X	 X X X X X X
4.3.2. Valuation of crop attributes - develop set of measurable variety attributes - develop and administer choice survey at curcurbit meetings - Data analysis	 DT (NCSU), MH (MSU), LQ (NCSU), JS (NCSU), CS (CU), AK (CLU) DT (NCSU)	 X	 X	 X X	 X X

TEAM REPORTS

Genomics and Bioinformatics Team

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Michael Mazourek (Cornell University)
Jim McCreight (USDA, ARS)
Rebecca Grumet (Michigan State University)

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 core collection (comprising 388 accessions) and the squash core collection (207 *Cucurbita pepo* accessions) has been completed (except one in the squash core). The average sequencing depths of cucumber and squash cores are 44.7× and 53.3×, respectively. These data have been processed for SNP and small indel calling using the Gy14 genome (v2.1) as the reference for the cucumber core and the MU-CU-16 genome (v4.1) as the reference for the squash core. Statistics of called variant are summarized in **Table 1**. Biallelic variants with MAF>0.01 of cucumber and squash core collections are available for mining at CuGenDBv2 (http://cucurbitgenomics.org/v2/genotype).

For melon core collection (384 accessions), genome sequencing of 308 accessions has been completed. SNPs and small indels have been called for these 308 samples, and will be updated once the sequencing of the remaining accessions in the core are generated. Sample collection and DNA preparation of the remaining 76 accessions and all accessions in the watermelon core are currently underway (**Table 1**). In addition, we have also completed genome resequencing for 26 *C. maxima* and seven *C. moschata* accessions.

Table 1 Summary of genome sequencing of cucurbit core collections

	cucumber	**squash**	**melon**	**watermelon**
No. accessions	388	207	384	366
No. DNA prepared	388	207	314	313
No. sequenced	388	206	308	0
Average sequencing depth	44.7	53.3	47.3	-
No raw SNPs	5,332,225	5,007,376	21,022,493	-
No. biallelic SNPs with MAF>0.01	2,513,882	4,104,452	10,294,239	-
No raw indels	1,385,149	2,008,251	4,481,966	-
No. biallelic indels with MAF>0.01	490,882	1,264,224	1,540,406	-

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.

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*, 13 *C. amarus*, nine *C. mucosospermus*, four *C. lanatus* var. *cordophanus*, seven landraces, and 88 cultivars and five interspecific hybrids. HiFi sequences have been generated for 124 accessions, and DNA of the remaining 11 accessions (four *cordophanus*, two amarus, two *colocynthis*, two *C. ecirrhosus* and one *C. rehmii*) have been prepared and sent to the sequencing facility for HiFi read generation.

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.

For melon, a total of 16 representative accessions have been selected for HiFi sequencing, including eight *C. melo* ssp. *melo* and eight *C. melo* ssp. *agrestis* accessions, among which eight from India/Pakistan, two from Turkey, two from Americas, and one each from Africa, Central/West Asia, East Asia, and Europe. Sample collection, DNA preparation and HiFi sequencing are underway.

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. One accession (WI5551) had an unexpectedly large size of the assembled genome (~610 Mb), possibly due to sample contamination, and was thus discarded. The assembled genome sizes of the remaining 24 accessions range from 272.0 Mb to 318.5 Mb (average: 294.9 Mb) and N50 sizes from 5.07 Mb to 23.28 Mb (average: 13.45 Mb). Protein-coding genes are being predicted in these genomes. Using the assembled 'Poinsett 76' genome as the reference/backbone, large structural variants (SVs) are being called and integrated for the other 23 assembled genomes and an additional of 11 previously published chromosome-scale cucumber genomes (seven cultivated, one Xishuangbanna and three wild *hardwickii*). A graph pan-genome will be constructed using the 'Poinsett 76' genome and the called SVs.

For watermelon, we have finished chromosome-scale genome assemblies and gene predictions of 124 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). The numbers of predicted protein-coding genes range from 21,209 to 23,314 (average: 21,948). Using the newly assembled '97103' genome as the backbone, SVs are being called in the 123 watermelon accessions. Once the HiFi data of the remaining 11 accessions are received, genome assembling, gene predictions and SV calling will be performed. The final SVs and the '97103' reference genome will be used to construct a *Citrullus* graph pan-genome.

For *Cucurbita* species, we have finished genome assemblies and gene predictions of three squash (*C. pepo*) accessions, and genome assemblies of *C. maxima* Rimu and *C. moschata* Rifu (**Table 2**). Annotation of Rimu and Rifu genomes are underway. SVs are being called and a *Cucurbita* graph pan-genome will be constructed.

Table 2 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
N50 (bp)	12,573,384	9,281,623	7,690,470	9,175,707	10,726,264
No. genes	-	-	31,528	30,412	31,327

All the constructed pan-genomes will be used as the reference to genotype SVs in core collections and other populations through mapping the genome resequencing reads.

In addition, we have constructed four species-level watermelon pan-genomes and a *Citrullus* super-pangenome with the 'map-to-pan' strategy using the four genome assemblies (one from each of the four species, *C. lanatus*, *C. mucosospermus*, *C. amarus* and *C. colocynthis*) and genome resequencing data we previously generated. The resequencing data are from 547 accessions, including 349 *C. lanatus* (243 cultivars, 88 landraces and 18 *C. lanatus* subsp. *cordophanus*), 31 *C. mucosospermus*, 131 *C. amarus* and 36 *C. colocynthis*. The species-level pan-genomes contain 2,288, 583, 1,922 and 2,521 novel genes that are not present in reference genomes of *C. lanatus*, *C. mucosospermus*, *C. amarus* and *C. colocynthis*, respectively. Analysis of presence/absence variations (PAVs) of genes in the *Citrullus* super-pangenome identified many genes showing differential presence frequencies between different populations, including 17 genes related to disease resistance that are completely absent or present at very low frequencies in domesticated watermelons while present at very high frequencies in at least one of the three wild species populations.

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 melon 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. These phenotypic data will be used to develop visualization and analysis tools 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 2023, seeds for 106 accessions with more than 1000 seeds per accession have been received. Seed increase for the majority of the 388 accessions is expected to be completed by the end of 2023.

For watermelon, HM.Clause are increasing the seeds for 249 accessions in the core collection. We shipped to HM.Clause 249 seed packs (accessions-PIs) with 50 S2 seeds in each pack. Prior to shipping the seeds to the HM.Clause station in Davis, California, they were tested for presence of Bacterial fruit blotch using an RT-PCR procedure. HM.Clause conducted seed health testing in California and the lots were shipped to the HM.Clause in Thailand. HM.Clause are planning to provide us 3-4 self-pollinated seed lots per accession - enough to reach the 1,000 seed/accession target. S2 seeds of 167 additional PI are being increased at the U.S. Vegetable Laboratory and at University of Georgia, and seed lots per accession - enough to reach the 500 seed/accession target will be increased during 2023-2024.

For melon, we have increased 312 to date and will harvest fruit from another 21 later this month.

For squash, we have finished seed increase for 130 accessions, of which 100 have >1,000 seeds and 30 have 500-1000 seeds. We are in the process of harvesting seeds for an additional of 20 accessions currently growing in the greenhouse, and are waiting for increased seeds from Villa

Plant for another 50 accessions. The remaining seven accessions in the core will be increased this summer.
The status of the core populations is summarized in Table 3.

Table 3. Status of CucCAP Core (CCC) population development

	Cucumber (*Cucumis sativus*)	**Melon (*Cucumis melo*)**	**Watermelon (*Citrullus lanatus*) [*C. amarus, C. colocynthis*]**	**Squash (*Cucurbita pepo*) *[C. moschata, C. maxima]*)**
No. accessions listed in NPGS	1335 (available)	2083 (available)	1870 [1619,77,151,23]	743 [302,614]
No. sequenced by GBS	1234	2083	1365 [1211,52,102]	829 [314,534]
No. chosen for core collection	395	384	377 [306,23,38,10]	229 [7, 26]
Portion of genetic diversity represented in core collection	96%	99%	96%	>99%
No. accessions selfed (Generations of selfing prior to sequencing)	388 2-3 generations	S1: 55 S2: 296	366 2-3 generations	207 [7,26] 2-3 generations
Seed multiplication in progress	BASF, East-West, Enza Zaden, Vilmorin, VoloAgri	Sakata, and tentatively: BASF & United Genetics	H.M. Clause Sakata In-House	MazLab; Villa Plants CR
No. of accessions with seed (≥1000 seed/accession)	106 remainder in progress		249 increased 126 still to be increased	Almost done: 130 >1000 seed; 30, 500-1000 seed; remainder in progress

1.2.2. Population genetics and phenotype-genotype association analysis
Phylogenies of accessions in the cucumber and melon cores have been inferred using the LD-pruned SNPs at four-fold degenerate sites, which are largely consistent with their geographic origins.
Preliminary GWAS analysis of cucumber fruit traits identified several QTL, including several that have been previously identified in the literature (e.g. Wang et al, , 2020, 2021; Sheng et al., 2020) as highlighted in light blue in Fig. 1. Additional analyses are in progress.

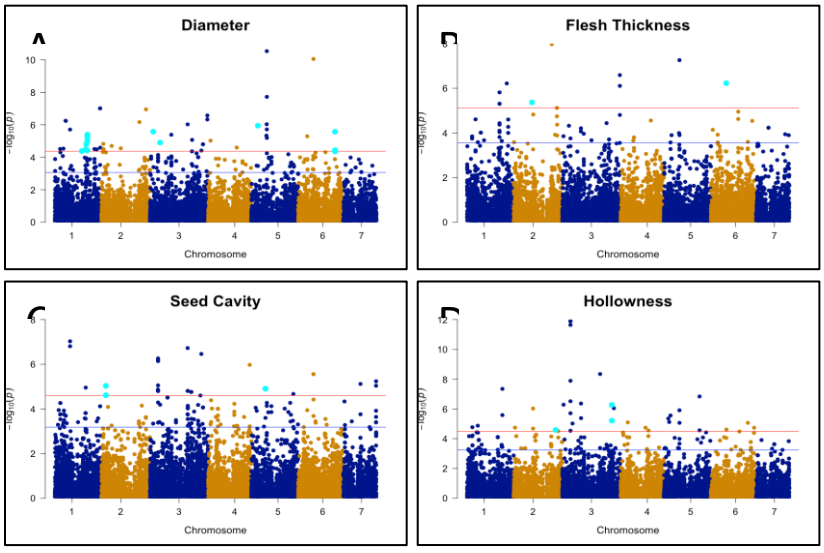

Figure 1. Manhattan plots of GWAS of internal traits. (A) Fruit diameter; (B) Flesh thickness; (C) Seed cavity size; (D) Fruit hollowness. Blue and red lines indicate FDR 0.05 and 0.01, respectively.

Watermelon Team

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1.2.1 Seed multiplication of core collections

Forty-seven accessions were obtained from the USDA germplasm collection for inclusion in the watermelon core collection. These included 39 *C. amarus*, six *C. lanatus* and one *C. mucosospermus* accessions. S_2 seed for 40 accessions were sent to MSU for DNA extractions.

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 additional *Stagonosporopsis* isolates.

We also developed a large (n = 2,000) F_2 population WPop GSB3 from a cross between Crimson Sweet and PI 482379 (resistant). The plant was validated as an F_1 and then vegetatively propagated to make 14 clones in order to obtain a large population from a single F_1 plant. QTL seq 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 marker assisted selection (MAS)

We previously developed and/or validated KASP marker assays for selection of *Qgsb5.1* (syn. Gimode et al., 2020), *Qgsb5.2* (Adams & McGregor, 2022), *Qgsb7.1* (syn. Gimode et al., 2020), *Qgsb8.1* (Ren et al., 2020) and *Qgsb8.2* (syn. *qLL8.1* and *qSB8.1*; Lee et al. (2021). These QTL were currently being introgressed into elite backgrounds (Table 1). After the two generations of a backcross, recombinant markers and background markers for domestication genes were added to the selection process. Background markers were developed to select cultivar-type alleles for the fruit quality-related domestication traits; loss of bitterness (basic helix-loop-helix, bHLH), soluble sugar content (ClAGA2), and red flesh color (LYCB) (Gong et al., 2022; Ren et al., 2018; Wang et al., 2019). Backcross lines will be evaluated in the field in summer 2023.

Table 1. Progress of introgression of Gummy stem blight resistance loci into elite backgrounds.

Line	Generation	QTL introgressed	Source of resistance
5, 73, 80 &140	BC_3	*Qgsb5.2, Qgsb8.1, Qgsb8.2*	PI 189225
9, 35, 62, 177 & 262	BC_4	*Qgsb7.1, Qgsb5.1*	PI 482276
22	BC_3	*Qgsb7.1, Qgsb5.1*	PI 482276

We also evaluated fourteen genotypes obtained from North Carolina State University (Rivera-Burgos et al., 2021a) in the field. These included four released lines, NC-GSB-530W, NC-GSB-531W, NC-GSB-532W and NC-GSB-528W (Rivera-Burgos et al., 2021b). Sugar Baby and Crimson Sweet 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. NCSU-RIL-033, NCSU-RIL-002, NCSU-RIL-027, NCSU-RIL-204 and NCSU-RIL-117 had significantly lower gummy stem blight disease symptoms (AUDPC) than susceptible controls, but symptoms were more severe than resistant controls (Table 2).

Table 2. Results from gummy stem blight field trial, artificially infected with *S. citrulli*.

Genotype	AUDPC		Flesh color	Brix	
Sugar Baby	1516	a	Red	10.0	abc
NC-GSB-532W	1193	b	Red	9.3	abcd
NC-GSB-530W	1182	b	Red	10.7	a
NC-GSB-531W	1165	b	Red	7.3	cdef
NC-GSB-528W	1160	b	Red	6.3	efg
NCSU-RIL-300	1160	b	Red	8.3	abcde
NCSU-RIL-257	1088	b	Red	10.0	abc
NCSU-RIL-042	1083	b	Pink (variable)	4.0	g
NCSU-RIL-158	1079	b	Red	7.0	def
Crimson Sweet	1065	b	Red	10.3	ab
NCSU-RIL-075	999	b	Pink (variable)	6.3	efg
NCSU-RIL-033	678	c	Pink	7.0	def
NCSU-RIL-002	632	c	Yellow	5.3	fg
NCSU-RIL-027	608	c	Pink (variable)	7.7	bcdef
NCSU-RIL-204	523	c	Pink (variable)	6.3	efg
NCSU-RIL-117	493	c	Pink (variable)	4.0	g
UGA1081	20	d	green	not mature	
UGA11	20	d	green	not mature	
UGA81	8	d	green	not mature	

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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, AL/PW/SB, Ph

- 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 in preparation.
- 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. Manuscript under review at *Plant Disease*.

-GSB, PW/AL/SB/AK, Ph

- Completed disease screening (two replicated tests) of the *C. amarus* core collection for GSB resistance and used the phenotypes for GWAS. Manuscript in preparation.

-Downy mildew, PW/AL/SB, Ph

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, AL/PW/SB, M

- 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 reponse to inoculation with Fon race 2 in two replicated tests and the phenotypes used for QTL mapping with the KASP markers. Manuscript under review.

-Powdery mildew race 2w

- 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

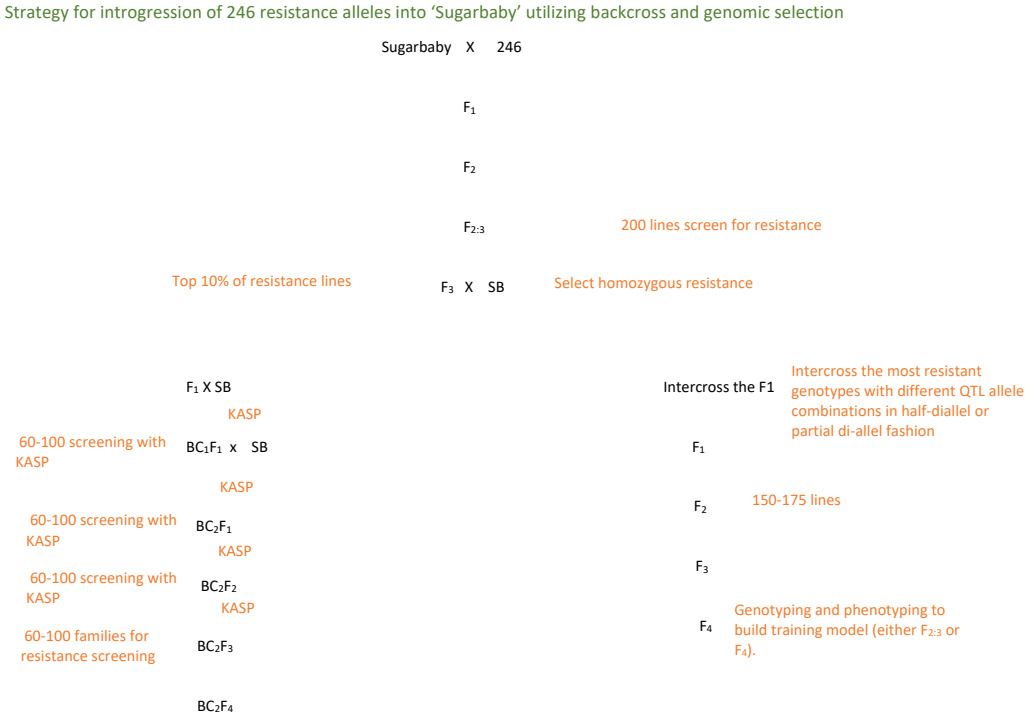
the genome with a significant signal. They were validated in two hundred accessions from the extremes of the distribution. Manuscript in preparation.

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, AL/PW/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 F4 and BC1F4 generations from this cross. We have also crossed USVL246 to 'All Sweet' and 'Crimson Sweet' to begin introgression into a variety of elite backgrounds.



Crop	Current seed being generated	Number of plants (selections)
watermelon	F_3 (Charleston Gray x 246)	6
watermelon	F_4 (Sugarbaby x 246)	8
watermelon	F_1 (Crimson Sweet x 246)	NA
watermelon	F_1 (All Sweet x 246)	NA
watermelon	BC_1F_2 (Charleston Gray x 246) x Charleston Gray	6
watermelon	BC_1F_1 (Sugarbaby x 246) x Sugarbaby	8

Obj. 3B. Using 'genomic selection' approach to incorporate Fusarium wilt race 2 resistance into watermelon cultivars

Constructing and utilizing training populations for 'genomic selection' experiments
USVL246 x USVL114 (RIL), USVL252 x USVL114 (F3)
USVL246 x Sugar Baby (F3), USVL 252 x Sugar Baby (F3; F4; BC1;F2;F3)

Developing small-seeded lines with multiple disease resistance (FW races 1, 2 and Potyviruses)

Advanced (BC5F2) PRSV/ZYMV-Resistant Lines

Brix =10.8 (SD = 0.71)

Dennis Katuuramu, Sandra Branham, Amnon Levi and Patrick Wechter

Genome Wide Association Analysis of Resistance to Downey Mildew in the USDA-ARS Citron Watermelon Germplasm Collection

We screened 122 *Citrullus amarus* accessions for resistance to Cucurbit downy mildew (CDM) over two tests (environments). The accessions were genotyped by whole-genome resequencing to generate 2,126,759 single nucleotide polymorphic (SNP) markers. A genome-wide association study was deployed to uncover marker-trait associations and identify candidate genes underlying resistance to CDM. Our results indicate the presence of wide phenotypic variability (1.1 - 57.8%) for leaf area infection, representing a 50.7-fold variation for CDM resistance across the C. *amarus* germplasm collection (Table 1). Broad-sense heritability estimate was 0.55, implying the presence of moderate genetic effects for resistance to CDM. The peak SNP markers associated with resistance to *P. cubensis* were located on chromosomes Ca03, Ca05, Ca07, and Ca11 (Table 2). The significant SNP markers accounted for up to 30% of the phenotypic variation and were associated with promising candidate genes encoding disease resistance proteins, leucine-rich repeat receptor-like protein kinase, and WRKY transcription factor.

Table 1. Analysis of variance showing mean squares percentage of total variance explained, and *F* and *P* values for the cucurbit downy mildew leaf area infection of the 122 *Citrullus amarus* genotypes evaluated over two screening tests under growth chamber conditions

Source of variation	d_f[a]	Mean square	% of TSS[b]	*F* value	*P* value
Genotype	121	575.9	40.2	2.20	0.0001
Test	1	8,733.7	5.0	33.34	0.0001
Genotype × test	121	254.2	17.7	0.97	0.5689
Rep (test)	2	413.3	0.5	1.58	0.2085
Residual error	242	261.9	36.6	NA[c]	NA

[a] d_f, degrees of freedom.
[b] % of TSS, Percentage of total sum of squares (total variance) explained.
[c] NA, not applicable.

Table 2. Details of the peak SNPs and candidate genes associated with resistance to cucurbit downy mildew across 122 *Citrullus amarus* genotypes evaluated over two screening tests under growth chamber conditions

Chr.	SNP	Position (bp)[a]	Major allele	Minor allele	MAF[b]	*P* value	R^2 (%)	Method[c]	Candidate gene: annotation[d]
Ca01	S1_37497469	37,497,469	A	T	0.05	5.23E-12	26.2	GLM	*CaU01G30010*: LRR–RLK
Ca02	S2_5605535	5,605,535	T	C	0.07	7.44E-12	25.3	GLM	–
Ca03	S3_16504881	16,504,881	A	G	0.05	2.19E-07	28.9	GLM, MLM	–
Ca03	S3_17354658	17,354,658	T	C	0.05	2.19E-07	28.9	GLM, MLM	–
Ca05	S5_732202	732,202	G	A	0.25	9.92E-08	30.2	GLM, MLM	*CaU05G00920*: LRR–RLK
Ca05	S5_1047147	1,047,147	T	G	0.18	2.92E-07	27.9	MLM	*CaU05G01280*: WRKY transcription factor
Ca05	S5_26089384	26,089,384	G	A	0.05	3.58E-07	27.5	MLM	–
Ca06	S6_12840690	12,840,690	A	G	0.06	1.23E-11	24.0	GLM	–
Ca07	S7_22984409	22,984,409	C	T	0.05	2.15E-07	28.6	GLM, MLM	–
Ca07	S7_22985428	22,985,428	T	A	0.05	2.15E-07	28.6	GLM, MLM	–
Ca11	S11_5245781	5,245,781	A	T	0.05	1.72E-07	29.1	MLM	*CaU11G06010*: LRR–RLK

[a] bp, basepair.
[b] MAF, minor allele frequency.
[c] GLM, general linear model; MLM, mixed linear model.
[d] LRR–RLK, leucine-rich repeat receptor-like protein kinase.

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Watermelon resistance to CGMMV

Cucumber green mottle mosaic virus (CGMMV) is an emerging tobamovirus in North America. Following its first detection in 2013 in California, the preventative measures, including quarantine, official control and certified seeds tested negative for CGMMV, have relatively restricted the virus in that state. However, severe CGMMV outbreaks have been reported in Asia, Australia and Europe, which resulted in severe yield losses on various cucurbit crops,

including watermelon. 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. Seedlings from these populations were inoculated with CGMMV and assessed for their resistance using serological assay for virus titer and their phenotypic reaction. 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 was conducted to identify SNPs that are associated with loci that are associated with the resistance to CGMMV infection in watermelon. A segregating F2 population was phenotyped. Resistant and susceptible bulks of F2 individuals were selected for whole genome resequencing. Bulk segregant analysis revealed a total of four tightly associated SNPs to the CGMMV resistance (Figure 1, Table 1). Further analysis revealed several resistance-associated candidate genes in the genome sequence region. The identified markers could be useful to accelerate breeding watermelon with CGMMV resistance through marker-assisted selection.

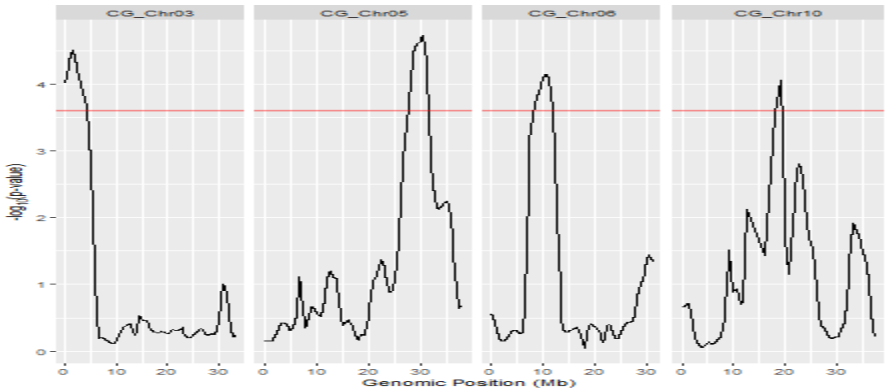

Figure 1. Genomic position of significant SNPs associated with resistance to the CGMMV pathogen in watermelon.

Table 1. Identified SNPs 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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1.1 Plant material and phenotyping

Three hundred MAGIC derivatives of F8 generation have been created using intercrosses involving resistant accessions of Citrullus amarus (PI 482342, PI 189225, PI 526233, PI 482283, PI 482374) and susceptible accessions of Citrullus lanatus (Charleston Grey, Calhoun Gray, Mickylee, Minilee, All sweet, Crimson Sweet, Petite Sweet). Genomic DNA was isolated from 30 GSB susceptible and 30 GSB resistant individuals using the E.Z.N.A. Plant DNA DS Kit (Omega Bio-Tek, USA). The quantity of genomic DNA was determined using a Qubit fluorimeter (Thermo Scientific, USA), and the quality was evaluated using agarose gel electrophoresis. The resistant and susceptible bulks were constructed by combining equimolar DNA from 30 extremely resistant RILs and 30 extremely susceptible RILs. The two bulks were subjected to whole-genome sequencing on the Illumina platform using paired-end sequencing chemistry (2x150 bp), generating more than one billion reads per bulk and attaining deep genome coverage. Subsequently, the reads were mapped to the parental genomes (USVL246 and Charleston Grey), and the mapping percentage was above 99%. By mapping to the USVL 246 genome, we identified 7,163,041 and 7,266,736 variants from the resistant and susceptible bulks, respectively. Furthermore, when mapping to the Charleston Grey genome, we found 2,205,134 and 517,389 variants from the resistant and susceptible bulks, respectively.

The QTLs associated with GSB resis”ance’were Identified using the qtlseqr (Mansfeld and Grumet, 2018) R package with single-nucleotide polymorphism-index (SNP-index) and Gprime methods (Figure 1). We have identified statistically significant variants/loci on chromosomes 1, 2, 3, 5, 7,8, and 10 associated with GSB resistance. QTL-seq analysis identified several key candidate genes for GSB resistance based on their physical location in the important QTL regions on these chromosomes (Table 1). Significant loci associated with GSB resistance were used to develop PACE genotype markers. Allele-specific primers were designed for the QTLseq SNP and INDEL markers. Polymerase chain reaction (PCR) allelic competitive extension (PACE) genotyping chemistry constituting FAM, HEX, and ROX fluorophores was used to analyze the SNPs (3CR Bioscience, Essex, UK). The polymorphic PACE SNP/INDEL markers were used for genotyping the mapping populations used for QTLseq (N = 60) with contrasting phenotypes.

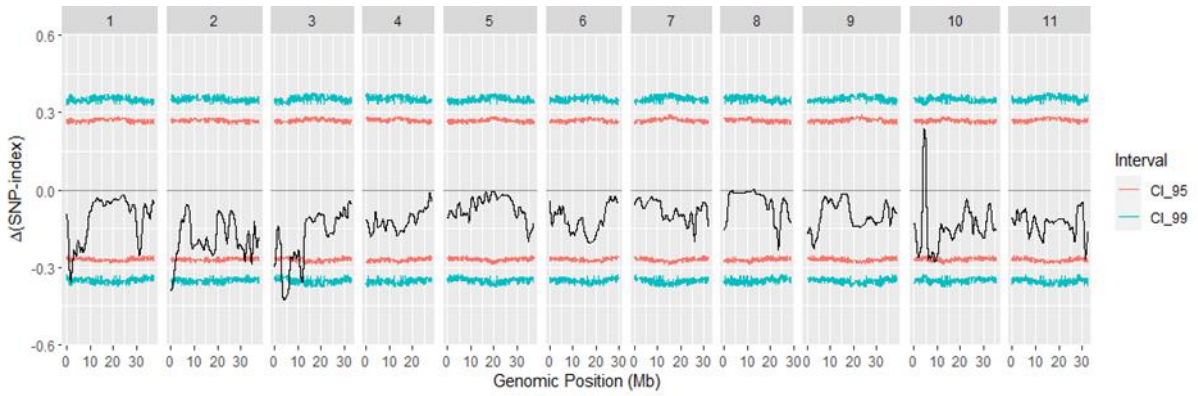

Figure 1. Delta SNP-index plot generated using QTLseqr based on USVL 246 genome mapping. The X-axis represents the genomic position along the chromosomes, and the Y-axis shows the delta SNP-index values. The red line indicates the genome-wide significance threshold (confidence interval CI=95), and the regions above the threshold are considered as putative QTL regions associated with the GSB resistance.

Table 1. Most significant QTLs are associated with GSB resistance based on USVL 246 genome.

CHR	POS	REF	ALT	Res	Res (Depth)		Sus	Sus (Depth)	Gene ID	AnnotationSupport
1	1172937	G	A	G/A	107,97	A	6204	CaU01G01480	RING/U-box superfamily protein	GWAS
1	4120974	C	T	C/T	202,166	T	11412	CaU01G04280	Amine oxidase	
1	31178046	C	T	C/T	73,125	T	160	CaU01G20910	Myosin-1	GWAS
2	4713467	T	C	T/C	35,63	C	277	CaU02G05730	SAUR-like auxin-responsive protein family	
2	34675982	A	T	A/T	141,154	T	26369	CaU02G24910	Exostosin family protein	
3	3600506	AGCCACCT	A	AGCCACCT/A	163,171	A	8327	CaU03G03630	BEL1-like homeodomain 8, putative	GWAS
3	4114411	GGGTGCTTGTTATGGTTTTATATTGTTTTTAGGTGT	G/GGTGCTTGTTATGGTTTTATATTGTTTTTAGGTGT	111,115	GGTGCTTGTTATGGTTTTATATTGTTTTTAGGTGT	12270	CaU03G04260	Ubiquitin-like-specific protease 1D		
3	11822834	TTAAAT/TAAA	70,93	TAAA	132	CaU03G10970	Zinc finger CCCH domain-containing 15	GWAS		
3	27245327	TAT/A	215,140	A	28407	CaU03G15610	Beta-glucosidase	GWAS		
5	3475336	TCTAAGTTATTTGCT	TCTAAGTTATTTGC/T	95,168	T 1194	CaU05G04550	Dihydroxy-acid dehydratase, putative			
5	6388421	AACC	A/ACC	104,111	ACC	20253	CaU05G08720	Coffea canephora DH200=94 genomic scaffold, scaffold_3		
5	9812945	CA	C/A	132,212	A	13365	CaU05G12170	WRKY transcription factor, putative		
5	35131544	T	TGAC	T/TGAC	57,108	TGAC	7145	CaU05G30400	TPX2 (Targeting protein for Xklp2) family protein, putative	
7	25998571	G	A	G/A	35,82	A	18116	CaU07G12990	Avr9/Cf-9 rapidly elicited protein	Gimode et al. 2021
7	31508852	T	TA	T/TA	152,214	TA	39403	CaU07G18420	U3 small nucleolar RNA-associated protein	
8	23446204	G	A	G/A	109,157	A	12325	CaU08G13240	Transcriptional regulatory plant protein, putative	
10	6115210	C	T	C/T	63,86	T	157	CaU10G05530	Transmembrane protein, putative	
10	8882288	T	C	T/C	143,180	C	11368	CaU10G07250	ABC transporter G family member 9	
10	30520112	AATTATGTAATTTTTATCAATGTTTTCAAAACTCAAGTCAG	A/ATTATGTAATTTTTATCAATGTTTTCAAAACTCAAGTCAG	42,44	ATTATGTAATTTTTATCAATGTTTTCAAAACTCAAGTCAG	82	CaU10G19030	Homeobox associated leucine zipper protein		

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.

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Powdery mildew of watermelon.

- Assembled seeds of commercial seedless cultivars for powdery mildew resistance screening. Sent seeds to CucCAP2 collaborator in Raleigh, NC in 2021 and 2022.
- 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.
- Evaluated and collected data on powdery mildew development on 190 RIL lines in the field (Spring 2022). Data analysis is in progress.
- Evaluated and collected data on powdery mildew development on RIL lines in the field (summer 2022). Five plants of each RIL line was planted per plot and each RIL line had two replications. The experiment will be conducted again in summer 2023 and currently the seedlings are being grown in the greenhouse and will be transplanted during the last week of April.
- 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.
- Crossed Advanced RIL lines with red flesh to the cultivar Dixie Lee to generate F_1 seed. F_1 plants were sown in February 2023 to generate F_2 seed for phenotyping for resistance to powdery mildew.
- Backcrossed USVL608-PMR with Dixie Lee to develop powdery mildew resistant germplasm lines with good horticultural traits. Dixie Lee is a watermelon cultivar with good horticultural traits including high brix and uniform red flesh. Backcross populations will be evaluated phenotypically by inoculating with powdery mildew and genotypically with KASP makers in Fall 2023.
- Evaluated multiple disease resistant (MDR) lines (powdery mildew and Phytophthora fruit rot) in the field. (Fall 2022). Of these 36 lines display high levels of resistance to powdery mildew and have been advanced from various PI. Of the 36, 13 are also resistant to Phytophthora fruit rot and can be considered as multiple disease resistant.
- 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.
- Continued development of KASP markers for powdery mildew resistance in watermelon. We utilized the KASP marker developed based on *ClaPMR2* in watermelon Chr02 to identify resistant F_2 plants in a cross of USVL608-PMR X USVL677-PMS. Based QTLseq analysis done in first SCRI CucCAP grant we had identified one region in Chr02 that was tightly linked to powdery mildew resistance in USVL608-PMR. Time course

RNAseq experiments conducted on powdery mildew inoculated plants in a growth chamber (23° ± 1 C) on USVL608-PMR and USVL677-PMS identified the same region in Chr02 with the NBS-LRR gene *ClaPMR2* being highly up regulated compared to USVL677-PMS at three and eight days after inoculation. No visible powdery mildew development was observed on USVL608-PMR eight and 14 days after inoculation. As expected USVL677-PMS displayed severe powdery mildew development. Individual F_2 plants were genotyped using KASP markers in Chr02. A strong correlation between the resistant phenotype and genotype was observed (Figure 1)

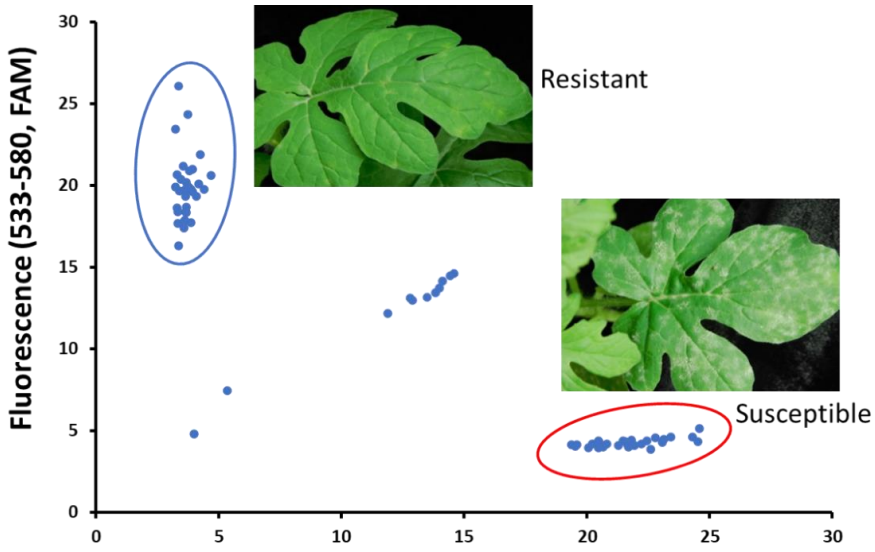

Figure 1. Analysis of F_2 population from a cross of USVL608-PMR (resistant parent) X USVL677-PMS (susceptible parent). KASP marker analysis based on one major QTL in watermelon Chr02. The KASP marker was developed based on the NBS-LRR gene *ClaPMR2* in Chr02 that was observed to be upregulated in RNAseq studies in USVL608-PMR compared to USVL677-PMS.

Phytophthora fruit rot of watermelon

- Completed studies and published manuscript on broad resistance to post-harvest fruit rot in USVL watermelon germplasm lines. U.S. vegetable Laboratory (USVL) developed germplasm lines USVL020-PFR, USVL203-PFR, USVL782-PFR, USVL489-PFR, and USVL531-MDR and two susceptible cultivar Sugar Baby and Mickey Lee were evaluated against 20 isolates of *Phytophthora capsici* collected from different states and crops in the USA. All five resistant germplasm lines were significantly more resistant than the two susceptible checks to all 20 *P. capsici* isolates. Among the five resistant germplasm lines, USVL020-PFR, USVL782-PFR and USVL531-MDR had broad resistance. Some *P. capsici* isolates induced minor lesions and rot on USVL489-PFR compared to the other resistant lines. Variation in virulence and genetic diversity among the 20 *P. capsici* isolates was also observed. The five watermelon germplasm lines will be useful for developing commercial watermelon cultivars with broad resistance to *P. capsici*. We have made crosses of the germplasm lines with susceptible cultivars and have developed breeding populations (F_1, F_2 and back cross populations). NIFA SCRI CucCAP grants were acknowledged in this publication.

- Crossed Advanced RIL lines (F_{10}-F_{11}) that displayed Phytophthora fruit rot resistance and with red flesh to the cultivar Dixie Lee to generate F_1 seed (Fall 2022). Resistance in each of the advanced RIL plants was confirmed prior to making the cross to generate the F_1 seed. F_1 plants were sown in February 2023 to generate F_2 seed for phenotyping for resistance. The F_2 population will be grown in a hoop house in September 2023, self-pollinated and the fruit from each individual F_2 plant will be evaluated for resistance to Phytophthora fruit rot in a walk-ingrowth chamber. Leaf samples will be collected from all the F_2 plants for use in QTLseq and marker analysis.

- Collected leaf samples for extraction of DNA from 190 advanced RIL lines.

- Completed phenotyping 190 RIL lines for resistance to Phytophthora fruit rot in summer 2022. We will phenotype the RIL lines again in 2023. Data analysis from 2022 is in progress.

- 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.

- Advanced F_{11} RIL lines with res flesh and fruit rot resistance and increased seeds. Another round of seed increase is in progress (March-June 2023) in the hoop house in Charleston, SC.

- We are currently evaluating six advanced lines (F_{11}) with Phytophthora fruit rot resistance for horticultural traits and fruit rot resistance at the U.S. Horticultural Research Laboratory FL and in Charleston, SC. One to three of the advanced lines that display good horticultural traits (uniform red flesh and decent brix) will be evaluated again in the fall in Fort Pierce, FL and Charleston prior to public release.

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 Dennis Katuuramu

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.

Accession	*Citrullus* **Sp.**	FW R1	FW R2	GSB	PM	Phyto-phtora	PRSV	ZYMV	SqVYV	BFB	Root-knot Nemato de	**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

R-resistant (tolerant), MR-moderate resistance (tolerance) S-susceptible

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

Melon Team

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Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances
-Powdery 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 two major QTL and validated them in a set of unrelated cultivars population.
-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.
-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. Manuscript accepted in *Theoretical and Applied Genetics*.

- Powdery mildew: 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.
- downy mildew

- 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).

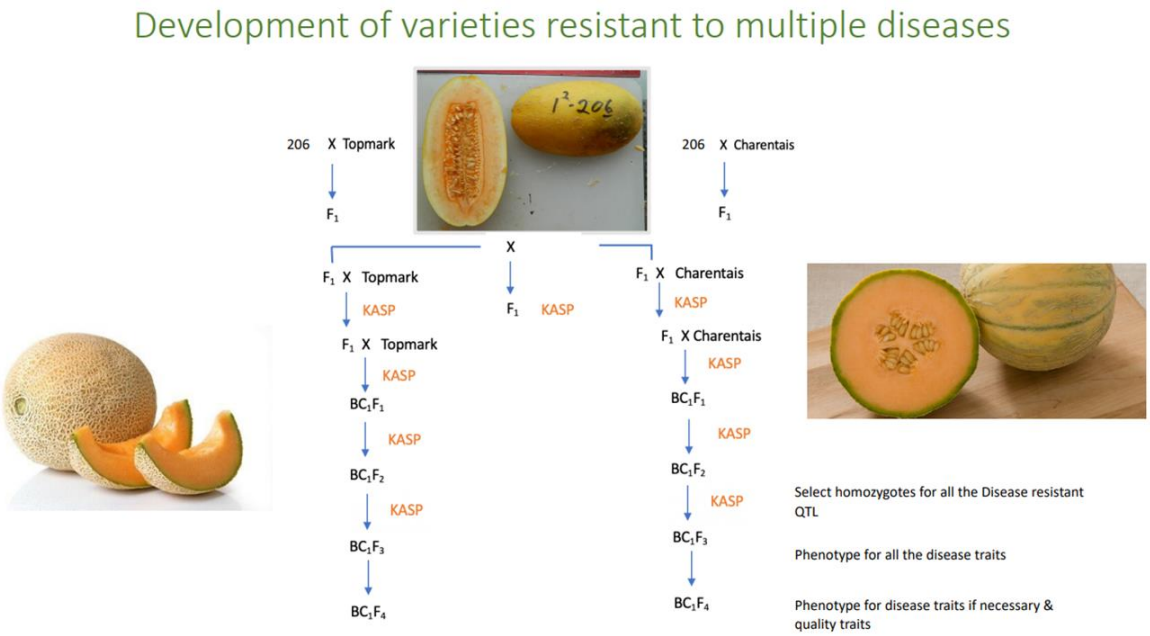
Eight F2:3 lines with low virus titer as determined by RT-qPCR (Mondal et al. 2023) resembled PI 313970 for the two flanking markers, which can, therefore, be utilized in marker assisted breeding of CYSDV-resistant melons. The eight F2:3 lines were evaluated to date for resistance reaction in a controlled inoculation, growth chamber test. The lines segregated for resistance reactions and flanking markers. Some of the high-titer plants (low CT value) were frequently asymptomatic or exhibited mild yellowing. Low-titer plants with one or both PI 313970 markers and asymptomatic/mild symptoms been advanced to next generation.

2.2 Marker development and verification
-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 Fusarium wilt races 1 and 2 resistance were developed in the MR-1xAY RIL population. Manuscript accepted in *Theoretical and Applied Genetics*.

- CYSDV: see 2.1.2 above

Obj. 3. Introgress, pyramid/stack resistances into advanced breeding lines
3.2. Melon
-Fusarium wilt races 1 and 2/Powdery mildew race 1, B,I,A. Using KASP markers developed under this project and a backcross/ phenotype/genotype strategy, we are currently making seed for BC2F3 populations from the three-way cross of [Top Mark x Majik Melon (resistance donor) x Charentais]. Selections have been made in the early phases of the project for a Charentais-type line and a Top Mark-type line with subsequent backcrosses to either Charentais or to Top Mark. Current lines have high brix, firm flesh, powdery mildew race 1 resistance, sulphur tolerance, and Fusarium race 1 and 2 resistance.



- CYSDV: see 2.1.2 above

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Tamang, P., K. Ando, W.M. Wintermantel, and J.D. McCreight. 2021. QTL mapping of Cucurbit yellow stunting disorder virus resistance in melon accession PI 313970. HortScience 56:424–430. doi: https://doi.org/10.21273/HORTSCI15495-20.

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 2022 by five industry collaborators. As of March 28, 2023, seed increase of 106 accessions has completed (>1000 seeds each). The goal under this sub-objective is expected to be accomplished by 2023.

Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances

2.1.1 Downy mildew (YW & AK)

One goal under this objective is to conduct QTL mapping of DM resistance in WI7773 (an introgression line, PT108, with DM resistance derived presumably from *C. hystrix*) and WI7631. In 2022, 91 WI7769 $F_{2:3}$ families from the cross between WI7773 and susceptible line '9930' (two replications, eight plants per rep) were grown in open fields at Clemson Univ., SC to examine inoculation responses to natural infection of the DM pathogen. Phenotypic data for general impression (GI) of DM symptoms were recorded at two time points. Based on the two years' DM data in two populations (WI7747 and WI7769), we performed bulked segregant analysis (BSA)-Seq aiming to identify sub-chromosomal regions harboring DM resistance QTL in the parental lines. Genome-wide delta SNP-index curves for the two populations are shown in Figure 1 which suggest a major-effect DM resistance QTL on chromosome 2 and two on chromosome 5 in the WI7769 and WI7747 populations, respectively.

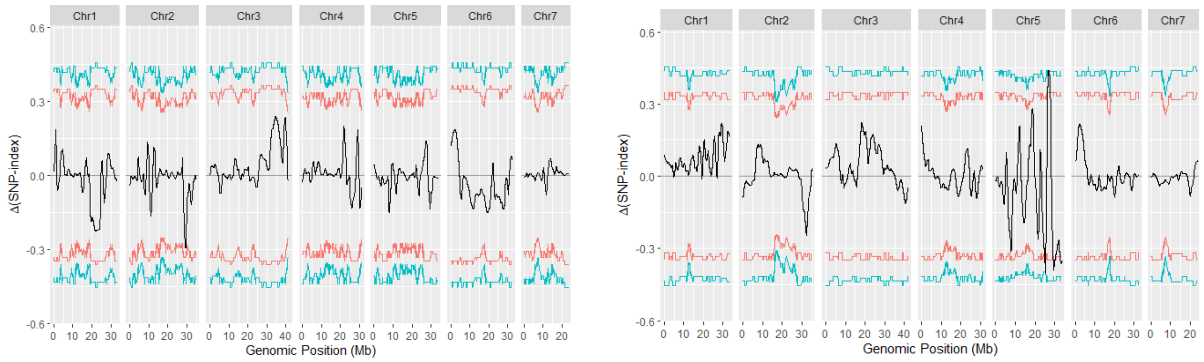

Figure 1. Genomewide delta SNP-index curve for the WI7668 (left) and WI7747 (right) populations. The red and green lines in each graph indicate 95% and 99% confidence interval, respectively. Based on BSA-Seq, a major-effect DM resistance QTL on chromosome 2 and two on chromosome 5 seem to present in the two populations, respectively.

In 2022 field trials at Clemson Univ. we also screened many other materials for DM responses including 120 inbred lines (for GWAS), recombinant plants used for fine mapping of the *dm4.1* (from WI7120) and *dm5.3* (from PI 197088) major-effect QTL (see below), as well as multiple introgression lines for DM resistance QTL. In August 2022, there was an epidemic of downy mildew at the University of Wisconsin Hancock Agriculture Research Station where DM occurrence is usually very rare. We were able to observe DM responses for 204 cucumber accessions. Many known sources of DM resistance were confirmed, and some new sources of

resistance were identified. The epidemic also confirmed the DM resistance of introgressed lines with varying numbers of DM resistance QTL.

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

1) QTL mapping of resistance derived from PI 109483. The QTL on chromosome 5 identified from the cross with pickling cucumber breeding line Gy14 and DH line A4-3, *qPFR5.1*, was tested in a second genetic background, the fresh market cucumber variety, Poinsett 76. F_2 plants (Poinsett 76 x DH A4-3; n=768) were genotyped and individuals homozygous for either Poinsett 76 or DH A4-3 alleles at *qPFR5.1* were self-pollinated. The resulting 25 F_3 families were grown in the greenhouse and field. Fruit were harvested 2-3 times a week at the age of 5-7 dpp (7-10 cm long) to provide 10-50 fruits/plant for replication and brought to the lab for inoculation with *P. capsici*. Consistent with the Gy14 background, the DH A4-3 allele was associated with resistance in the Poinsett background in the greenhouse and field (Figure 2).

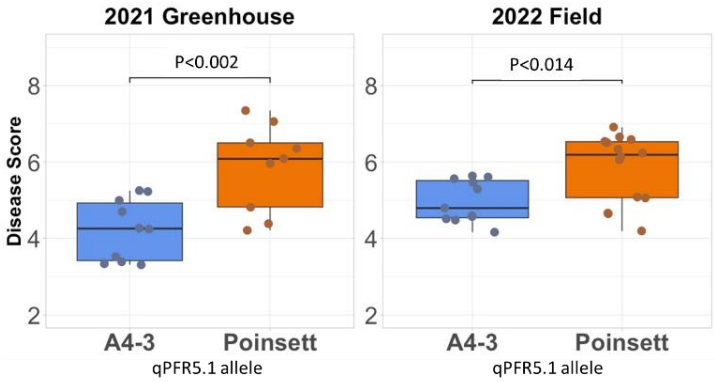

Figure 2. Allelic effect of *qPFR5.1* in fresh market cucumber background. F_3 families of Poinsett 76 x A4-3 possessing either the A4-3 or Poinsett allele at *qPFR5.1*. Each point is the mean of >20 fruits/family from the greenhouse and > 50 fruits/family from the field.

2) *Association analysis for PFR resistance.*
GWAS. The cucumber core collection was screened from 2019 to 2021, the number of accessions grown each year varied depending on seed availability. Phenotypic data was obtained from 378 accessions with 1-4 years of disease scores per accession; 70% of the accessions had at least two years of data. Disease scores for the population were normally distributed, consistent with a quantitative trait. The correlation between years ranged from 0.48-0.80 (Figure 3).

GWAS was performed using the resequencing data obtained for the cucumber core collection. SNP data was downloaded from CucGenDB and filtered using VCFtools (Danecek et al., 2011) with the following criteria: bi-allelic, GQ scores > 15, maximum read depth within one standard deviation of the mean read depth, and minor allele frequency > 0.1. GWAS marker-trait association analyses were carried out for the phenotypic data from each year, as well as the estimated genotypic best linear unbiased predictor (BLUPs) to correct effects from different environments. BLUPs were calculated using the R package lme4. Association analysis was performed using the R package GAPIT 3.0 with its implanted MLM, FarmCPU, and BLINK models (Wang & Zhang, 2021). The Manhattan plots and Quantile-Quantile (Q-Q) plots were

graphed using R package gwaspr. The genome-wide significance threshold was determined using the Bonferroni correction at $\alpha = 0.05$.

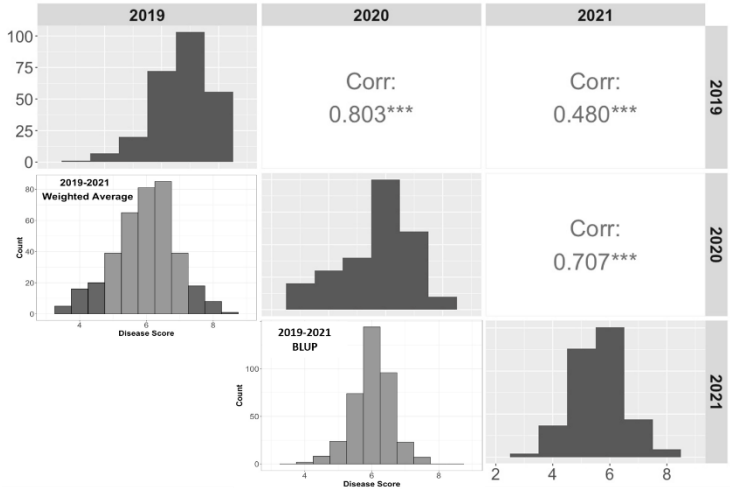

Figure 3. Disease score distribution and correlation from 2019-2021, and distribution of weighted average and BLUP values over the three years. The value for each accession in each year is the mean of 20-50 fruit. Darkly shaded bars in weighted average represent lines selected for XP-GWAS.

Though variations were observed across phenotypic data and models, significant SNPs on chromosome 1 at 21.11 and chromosome 2 at 10.22 Mb were detected consistently (Figure 4).

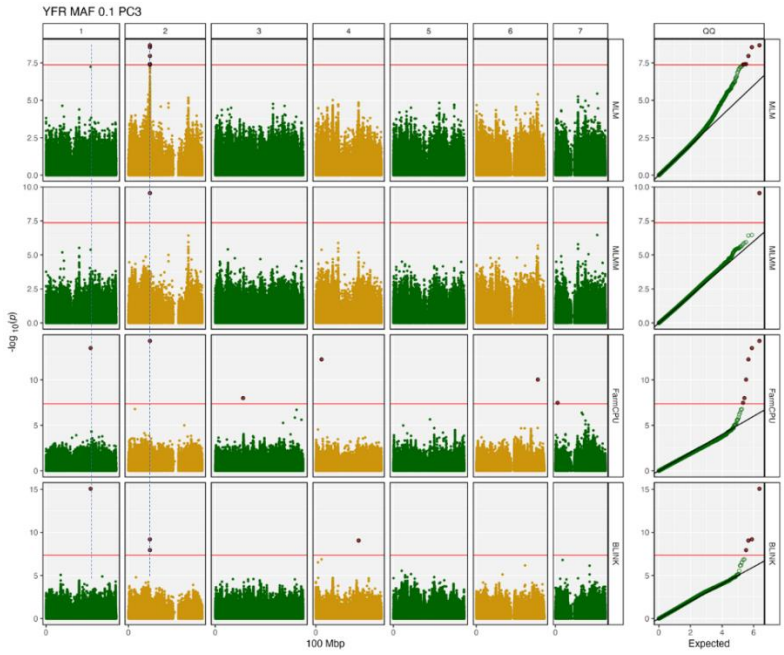

Figure 4. Manhattan and QQ plots of young fruit resistance in the cucumber core collection using combined (BLUP) data from 2019-2021. The GWAS analyses were performed using MLM, MLMM, FarmCPU, and BLINK models. The consensus SNPs identified from multiple models are indicated by vertical lines.

XP-GWAS. To enable additional replication of phenotypic data, we are also performing extreme phenotype (XP) GWAS. The weighted disease scores from 2019-2021 data were used to select the 30 most resistant and susceptible accessions (Figure 2). These genotypes were tested in an additional replicated trial in 2022 (Figure 5). XP-GWAS analysis is in progress.

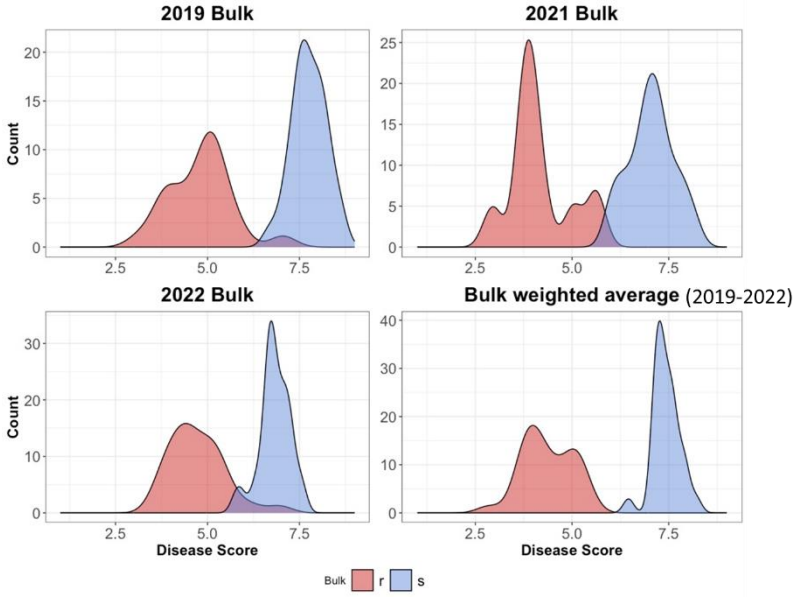

Figure 5. Distribution of disease scores of selected resistant and susceptible lines in 2019, 2021, their performance in replicated trial in 2022 and weighted average (2019-2022). Scoring for 2019-2021 is as described in Figure 2. Values for each line in 2022 are the mean of 50-100 fruits/line.

Several of the most resistant lines identified in 2019 were also tested in replicated trials in 2021 and 2022. Three lines showed reproducibly lower disease scores than Gy14 (Figure 6)

AM #	Accessions	Names	Country of origin
AM032	PI 105340	Kuai Huang Kwa	China
AM280	NSL 197095	Wautoma	United States
AM185	PI 481614	Gagon	Bhutan

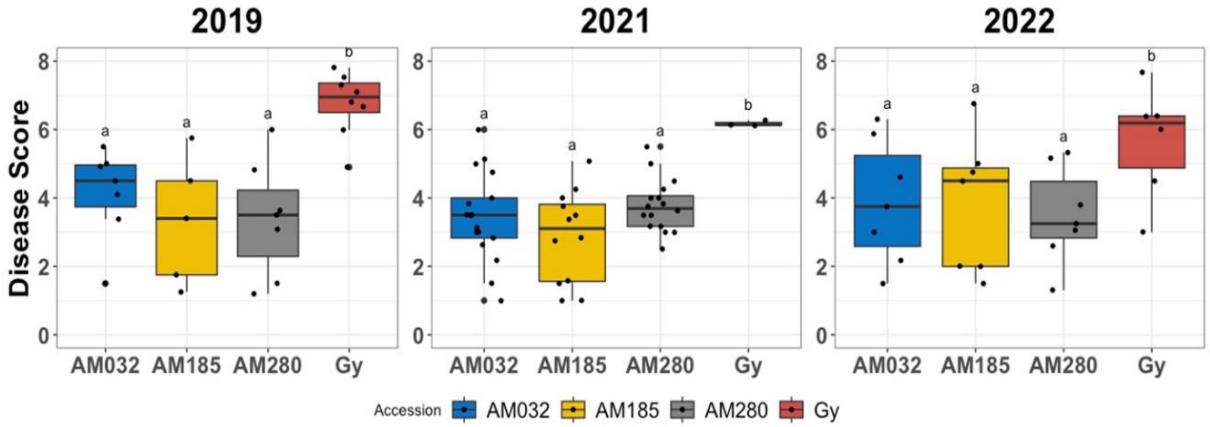

Figure 6. Young fruit response of selected lines to inoculation with *P. capsici* in 2019, 2021, and 2022.

2.1.3 CGMMV (KL and YW)

Cucurbit green mottle mosaic virus (CGMMV) is a seed-borne virus that can be introduced to new areas via infected plant material. It is easily spread through mechanical means, agricultural practices, and plant to plant contact. Due to its contagious nature, it is important to devise permanent solution to manage CGMMV. 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 50 cucumber lines were screened to assess phenotypic reactions to the CGMMV infection. As a result, three lines were identified as tolerant with no phenotypic symptoms but intermediate serological reactions. The three tolerant lines all belong to the Chinese Long type. The selected tolerant lines were crossed with susceptible ones to develop segregating populations, which together with previously developed recombinant inbred line (RIL) population were subjected to screen for phenotypic reactions. With the systemic virus infection, the phenotype scoring was not that straightforward. We used 0, 1, 2, and 3 to rate and calculate disease severity index (DSI) (Figure 7). The resistance class with the rating 0 showed no visible mottle or mosaic symptom on leaves and the rating 1 had mild mosaic and plant recovery. The susceptible class included inoculated plants with the rating 2 with mosaic and leaf deformed and the rating 3 on plant stunting, leaf deformation or dying plant (Figure 8).

Results in the screening of two F_2 populations (WI7182F2 and WI7814F2) developed between the resistant and susceptible parents showed segregation ratios that seem to be consistent with a simply inherited gene underlying the resistance in both populations (Table 1). We also tested 20 RILs (WI7326 population) derived from the cross of PI 197088 × WI7156 which showed segregation to CGMMV inoculation (Figure 9). Test of CGMMV inoculation responses on more RILs from this population is underway. Bulked segregant analysis (BSA)-Seq will be performed with data from the two F2 populations aiming to map the resistance loci to a sub-chromosomal region.

Figure 7. Rating classes of cucumber infected by CGMMV, rating 0: no symptom, rating 1: mild mosaic symptom, plant recovery; rating 3: severe mottle mosaic and rating 4: severe mottling and plant stunting.

Table 1. Segregating of CGMMV inoculation responses in two F_2 populations of cucumber.

	# Plants under each rating				
Rating scale	0	1	2	3	Total
WI7182F2	0	22	88	1	111
WI7814F2	0	18	94	0	112

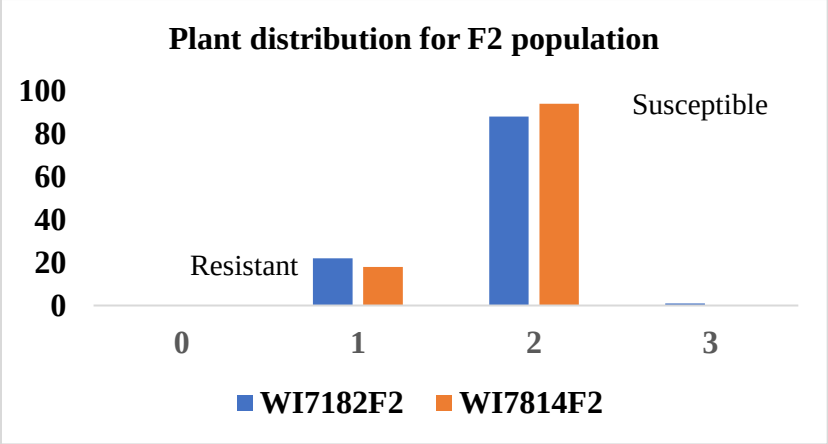

Figure 8. Distribution of individuals two F2 populations (WI7182F2 and WI7814F2) segregating for resistance to CGMMV based on disease severity index rating (R in rations 0 and 1, S in ratings 2 and 3).

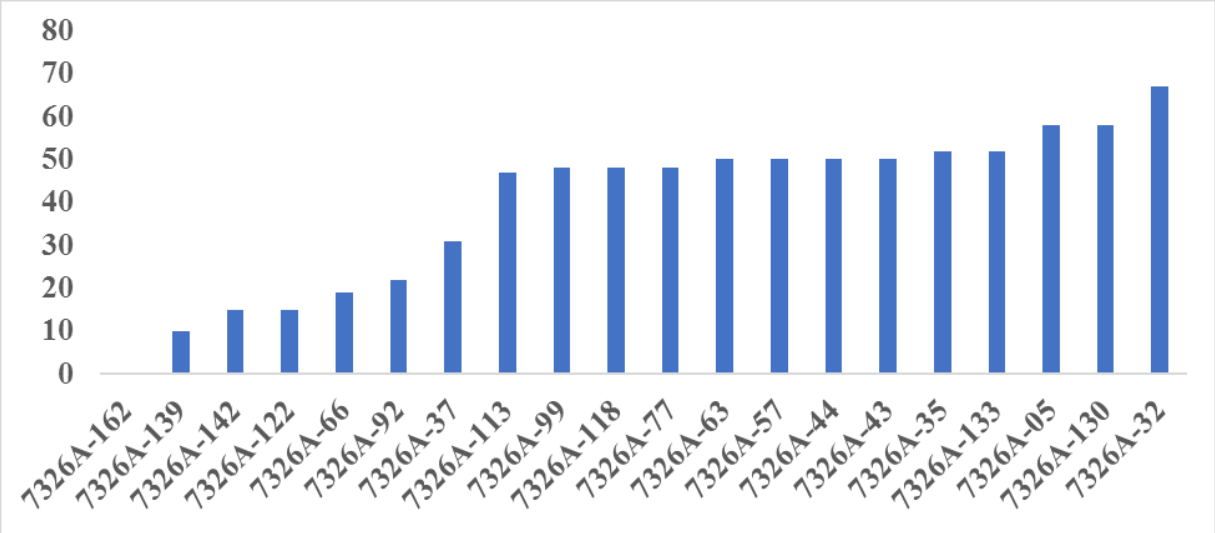

Figure 9. Disease index (DSI, Y-axis) of CGMMV inoculation responses among 20 RILs from PI 197088 × WI7156 cross.

2.2 Marker development and verification

2.2.1 Downy mildew (YW & AK)

We proposed to conduct 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. For fine mapping, we have developed near isogenic lines (NILs) for *dm4.1* and *dm5.3* in two backgrounds: the Chinese Long inbred line 9930 and the US pickling cucumber line Gy14 that also carries *dm1* (*CsSGR*). Genotyping and extensive phenotyping of these NILs and recombinants from NIL-derived F_2 and backcross progeny revealed 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*. DM resistance of NILs with different combinations of sub-QTL was evaluated in both growth chamber (Figure 10) and field conditions.

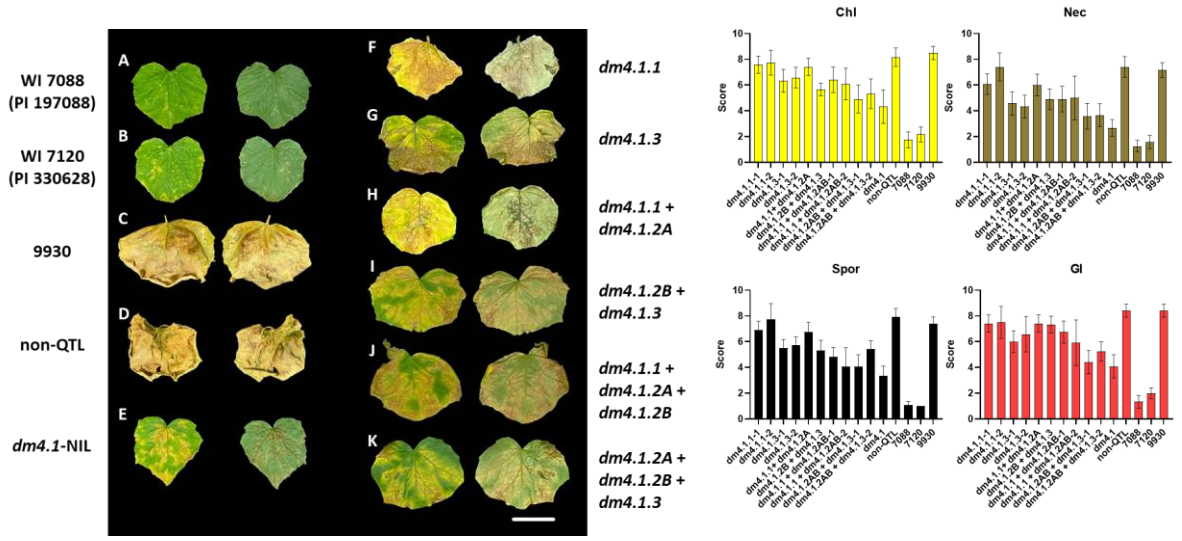

Figure 10. Performance of inoculation responses by DM pathogen (*P. cubensis*) on NILs carrying combinations of different sub-QTL (*dm4.1.1, dm4.1.2A, dm4.1.2B,* and *dm4.1.3*) at the *dm4.1* locus of WI7120. DM resistance was scored by four criteria: anti-chlorosis (Yel), anti-necrosis (Nec), anti-sporulation (Spor), and general impression (GI), which is a composite trait including Ye, Nec and Spor.

The candidate genes for *dm4.1.2A* and *dm4.1.3* in PI 197088 have been identified by another group (Berg et al. 2020, 2021). We examined allelic diversity in the *dm4.1* region between WI7120 and PI 197088 and did not find any DNA sequence variation indicating both lines have the same resistance alleles at the four sub-QTL. We focused on fine mapping and cloning of *dm4.1.2B* using NIL-derived segregating populations. Phenotypic characterization of DM resistance of the NILs in both field and growth chambers revealed anti-chlorosis nature of *dm4.1.2B* (Figure 11).

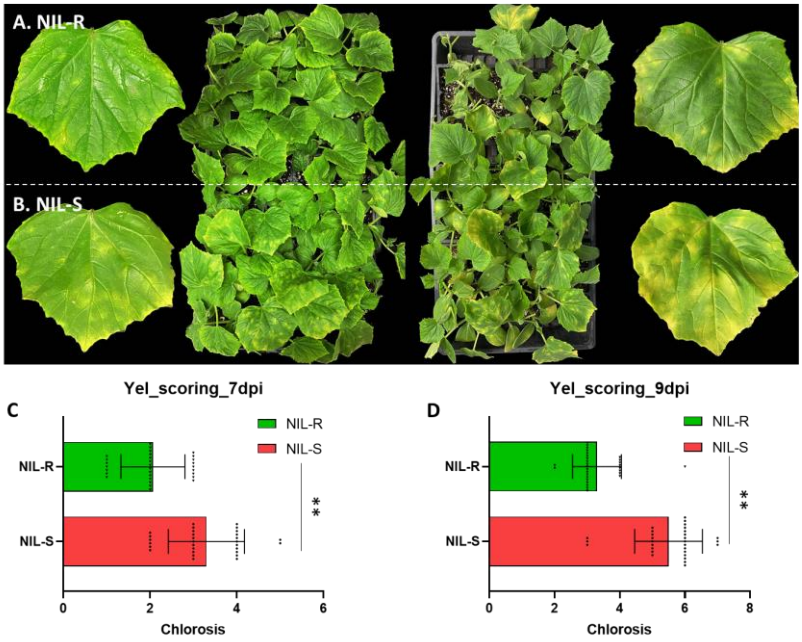

Figure 11. Anti-chlorosis effect conferred by *dm4.1.2B* in response *to P. cubensis* infection. At both 7 and 9 days post infection (dpi), the resistant RIL (NIL-R, A) shows less chlorosis than NIL-S (B) with significantly lower mean yellowing (Yel) scores (C and D).

We conducted fine genetic mapping of *dm4.1.2B* locus from WI7120 which delimited *dm4.1.2B* into a 36.2 kb region on Chromosome 4 (Figure 12) with four predicted genes. Confirmation of the candidate gene for *dm4.1.2B* is underway.

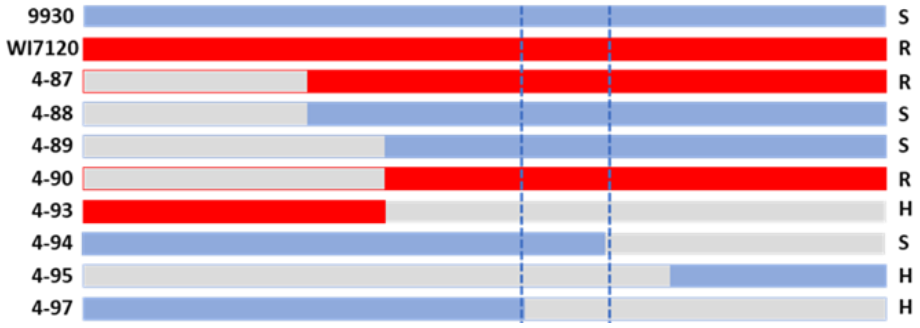

Figure 12. Map-based cloning of the *dm4.1.2B* sub-QTL. Eight recombinants in NIL-F2 defined by 11 markers loci which delimit *dm4.1.2B* into a 36.3 kb interval on Chr 4. R (red), S (blue), and H (gray) indicates resistant, susceptible and heterozygous (segregating) alleles, respectively.

For map-based cloning of *dm5.3* in PI 197088, NILs were developed for this major-effect QTL (Figure 13). GBS of the NILs suggests uniform genetic background (9930, the susceptible recipient of resistance allele from PI 197088, orange color in Figure 13A) except the *dm5.3* region that has ~5.24 Mb introgression from the donor line (WI7088D, green color in Figure 13A). Disease screening of NILs and relevant lines revealed moderate DM resistance contributed by *dm5.3* with both anti-chlorosis and anti-necrosis effects.

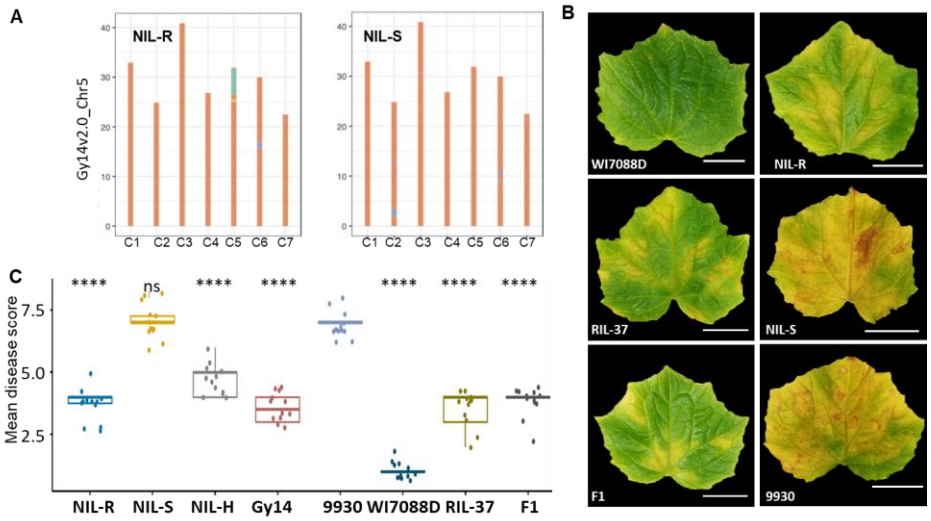

Figure 13. Development and characterization of DM resistance of NILs for *dm5.3*. The NIL-R carries ~5.24Mbp fragment from PI 197088 (A), which confers both anti-chlorosis and anti-necrosis in 9930 background (B and C).

Fine genetic mapping with recombinants from NIL-derived segregating populations allowed to narrow down the *dm5.3* locus into 144 kb region on chromosome 5 (Figure 14). Multiple lines of evidence support *CsSIB1* (*cucumber sigla factor binding protein1*) as the most possible candidate gene.

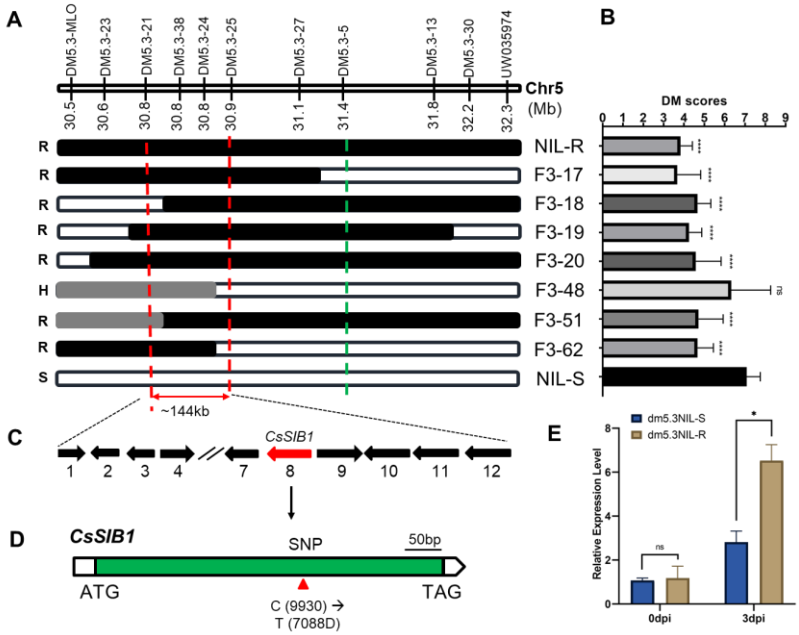

Figure 14 Fine mapping of *dm5.3* locus. A. Haplotypes of critical recombinants and DM ratings. B. Bar graph showing mean DM scores of recombinants. C. *dm5.3* was narrowed down to a 144kb region containing 12 predicted genes. D. Multiple lines of evidence suggests *CsSIB1* as the most possible candidate for *dm5.3*. The SNP between 7088D and 9930/Coolgreen is in the exon. E. Relative expression of *CsSIB1* in NIL-R and NIL-S at 0, 3 dpi with qPCR.

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

The *qPFR5.1* initially identified by QTL-seq spanned ~5 Mb on chromosome 5. To facilitate fine mapping, we used a recombinant inbred line (RIL) population and F_3 families selected for enriching recombination within the region for fine mapping. The prior tested RIL populations refined the region to 1.31 Mb, located between markers M26 and M5 (27.08-28.39 Mb). An additional set of F_3 families was tested in 2022; 99 individuals from 33 families that were homozygous recombinant in the *qPFR5.1* region (A4-3–Gy14 or Gy14 – A43) were grown in the greenhouse. Cuttings also were taken from each plant and transplanted to the field. The results from F_3 families suggested an overlapping but shifted region that varied somewhat between the greenhouse and field results [M26 – M5 (27.08Mb – 28.39Mb) in the greenhouse and M2-M26 (25.17- 27.08) in the field)] (Figure 15). Additional markers between M2 and M3 and M28 and M5 are being tested.

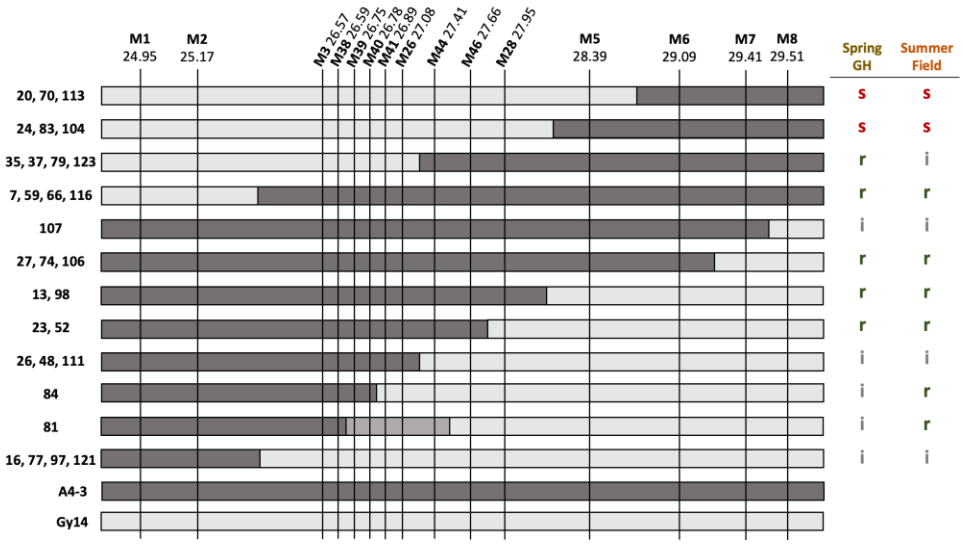

Figure 15. Fine mapping of the *qPFR5.1* QTL in recombinant F_3 families. Color bars refer to different genotypes: dark grey - DH A4-3 allele; light grey - Gy 14 allele. Number on the left indicates the name of the lines/families sharing the same genotypes, and the letters on the right indicates phenotypes of each genotype: r – resistant lines with score < 4.5; s – susceptible lines with score > 6.0; and i – intermediate lines with scores between 4.5 to 6.0.

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

During fine mapping of *dm4.1* and *dm5.3*, plants carrying different combinations of *dm4.1*, *dm5.2* (both from WI7120) and *dm5.3* (from PI 197088) resistance alleles were identified and backcrossed with Gy14 aiming to develop Gy14 carrying all permutations of the three QTL. In 2022, homozygous introgression lines carrying all three QTL (Gy14Q3) were obtained. DM resistance of Gy14Q3 was tested in both growth chamber and field trials at multiple locations (South Carolina, North Carolina, Michigan, Wisconsin). The three QTL were also introgressed

into the Chinese Long (9930) and beit alpha (WI7204) backgrounds, which revealed some background effects on DM resistance conferred by the three QTL.

We also aim to develop inbred lines with both DM and PFR resistances through marker-assisted QTL pyramiding. In 2021, a plant carrying homozygous *qPFR5.1* QTL for PFR resistance (from PI 109483) was crossed with Gy14Q3. The resulting F_1 plant carrying all four QTL (*dm4.1, dm5.2, dm5.3,* and *qPFR5.1*) was further backcrossed with Gy14 to advance to BC1, which were subjected to marker-assisted selection. Since *dm5.2-qPFR5.1-dm5.3* were located in a ~9 Mbp block in repulsive phase on cucumber Chromosome 5, ideal recombinants combined with expected alleles at three loci were not identified so far. In 2022, we revised the strategy by identifying recombinants between *dm5.1* and *1PFR5.*1 first, then recombinants between *qPFR5.1* and *dm5.3* in the next BC generation. With this strategy, we successfully identified recombinants carrying resistance alleles for all four resistance loci (*dm4.1, dm5.2, qPFR5.1*, and *dm5.3*). Notably, the *dm4.*1 locus contains all four sub-QTL from WI7120. In 2022 summer filed trail at Hancock, Wisconsin, the Gy14 plants carrying three homozygous DM QTL but heterozygous at the *qPFR5.1* locus (Gy14Q4) exhibited better resistance than Gy14 for natural epidemic of DM. Plants in the F_2 population that was segregating for the *qPFR5.1* locus were tested in the field in Michigan in 2022 and young fruit examined for PFR inoculation responses. Plants with the *qPRF5.1* allele showed intermediate resistance to the resistant and susceptible parents, respectively (Figure 16).

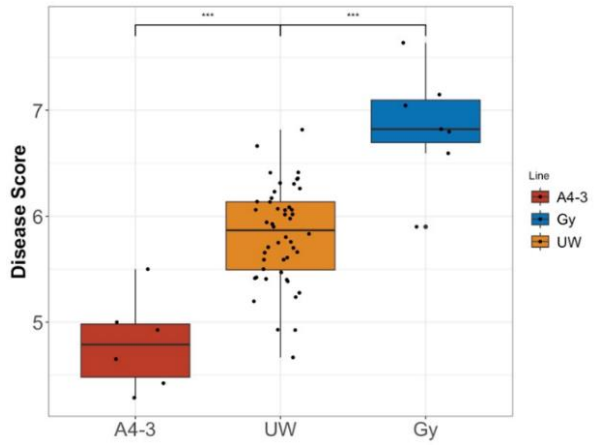

Figure 16. Values shown are *P. capsici* disease ratings for young fruit from: A4-3; F_2 family containing *PFR5.1, DM5.2,* and *DM5.3* in Gy14 background; and Gy14.

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*

To date, no natural source of powdery mildew resistance has been reported in *C. pepo*. Although a single gene resistance (designated *PM-0*) has been described in a wild *Cucurbita* species (*C. okechobeensis*), its sole utilization in current cultivars increases the chance of resistance breakdown which could lead to yield loss. We evaluated 207 USDA germplasm accessions of *C. pepo* for PM resistance under greenhouse conditions at Cornell University (CU) and under field conditions at Michigan State University (MSU). 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. At the 6th true-leaf stage, symptom severity data were collected on a scale of 0-100% and 0-5 at CU and MSU, respectively, based on visible pathogen sporulation on the surface of top 4th leaf, bottom 4th leaf, stem above 4th leaf, and stem below 4th leaf. For CU, 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%). For MSU, the bottom 4th leaf had the highest disease severity (40%) followed by stem above the 4th leaf (28%). Success PM (R) showed consistent resistance across reps, with a mean disease severity of 0% in the stem below 4th leaf. However, Early Prolific (S) was susceptible with mean disease severity of 20% in stem below 4th leaf. Data from CU showed that accessions 189, 55, 70, 48, 205, 80, 144, 107 and 71 were the most resistant and accessions 22, 7, 120, 108, 104, 92, 99 and 56 were the most susceptible. Accession 189 showed the highest resistance in both CU and MSU datasets, with a mean disease severity of 7% in the stem below the 4th leaf. Further evaluation of the germplasm will be continued in FL and NY in 2023, and the data will be used to conduct GWAS to identify novel resistance alleles for summer squash breeding.

- Powdery Mildew– *C. moschata*

A natural source of resistance has been identified in *C. moschata*. In contrast to the wild species derived resistance, *PM-0*, that was mapped in CucCAP 1, this resistance is recessive and protects the upper leaf surface. Powdery mildew resistant and susceptible bulks (n = 22 per bulk) of the F_2 along with the parents were sequenced using NextSeq 500/550 and reads analyzed with the QTL-seq bioconda package. The qtl-seq delta SNP index output indicated the novel PM

resistance lies in a 2 Mb region of the genome. Using PCR markers developed from the vcf file output of the snpEff package, we scanned the region for SNP calls associated with the resistant and susceptible parents and the resistant and susceptible bulks. We have created 11 PCR markers spread across a now smaller 1.3 Mb region and have seven that can differentiate resistant from susceptible individuals; we were able to shrink the region down to 1 Mb based on three individuals in the resistant bulk that have crossovers on the downstream side and three resistant and one susceptible individual that have crossovers on the upstream side of the region.

- Phytophthora – *C. pepo*

Resistance to Phytophthora crown rot in UF breeding line #394-1-27-12 is controlled by three complementary dominant genes (R1R2R3). Introgression of these loci into *C. pepo* background is needed to complement the low-moderate level of resistance available in this species. To facilitate interspecific resistance transfer, an RNA-seq study was conducted to identify potential causal R genes that would facilitate the development of functional markers for marker-assisted selection. Breeding line #394-1-27-12 (R) and Butterbush (S) were challenged with a virulent isolate of *P. capsici*, and crown tissues were sampled at 0, 12, 24, 72 and 120 hours post infection (hpi) with three technical and three biological replicates for each time point. On average 90% of the genes were uniquely mapped to *C. moschata* var. Rifu reference genome. Transcript data for #394-1-27-12 and Butterbush showed strong correlation among replicates (85%). Overall, there were 4,377 differentially expressed genes (DEG) across the two genotypes, of which 1,116 and 613 were upregulated or downregulated in #394-1-27-12, respectively. The DEG were highest at 72 and 120 hpi, but lowest at 24 hpi. Co-expression analysis revealed that 213 and 48 genes overlapped in expression at 72 and 120 hpi in #394-1-27-12, respectively. Gene ontology and pathway enrichment analysis revealed that most of the upregulated genes were involved in 'oxidation-reduction', 'response to stress' and 'response to wounding' networks. Among these, ten genes involved in 'stress / redox' response were co-expressed at 72 and 120 hpi, including defensin-like proteins, respiratory burst proteins, peroxidases, MYB transcription factors, and cytochrome P450 protein. Interestingly, several genes, including CmoCh04G003840 (MYB transcription factors), CmoCh04G001860 (Subtilisin-like serine endopeptidase) and CmoCh11G009740/ CmoCh11G009720 (Germin-like proteins) were within confidence intervals of previously resistance QTLs in #394-1-27-12. Functional characterization of these genes will determine their role in disease resistance against Phytophthora crown rot in UF breeding line #394-1-27-12.

-Phytophthora – C. maxima

The Hausbeck Lab (MSU) (Section 3) identified cultivars that are promising for native *Phytophthora capsici* resistance in *C. maxima*. The Mazourek group has made F_2 populations from crosses between the susceptible, commonly grown cultivar Golden Delicious and these other cultivars with promising resistance. Future work will focus on seedling screens for crown and root rot resistance and genotyping for mapping.

2.2 Marker development and verification

2.2.4 Squash

-Powdery Mildew –*C. moschata*

For recessive mildew resistance in *C. moschata*, one marker in the middle of the 1Mb region harboring the resistance was found to contain a 9bp insertion in the resistant individual and can be used as a polymorphic PCR marker that can be visualized during gel electrophoresis. This should help us to identify resistant individuals more quickly and cost-effectively. This spring we plan to run the indel marker on 800 F_2s and then run flanking markers on those individuals homozygous for the resistant parent allele for the indel marker. Individuals that have crossovers will be selected to go to the field and their resistant genotype and phenotype verified and the haplotype through this region will be explored through PCR markers and resequencing as necessary.

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

3.4 Squash (*C. pepo, C. moschata*)

- powdery mildew

Breeding lines for processing squash

Two field trials were conducted to evaluate resistance to CPM in processing squash breeding lines in 2022 in NY. One trial evaluated CPM resistance in improved 'Dickinson' lines. 'Dickinson' is a large-fruited industry standard processing cultivar that is related to butternut squash and susceptible to CPM. The two parents 'Bugle' (butternut, CPM resistant) and 'Dickinson' (CPM susceptible) were assessed alongside four of the most promising progeny from the breeding lines generated by this original cross. Each of the four progeny contains two copies of the CPM resistance gene *Pm-0*. **Of the four progeny tested, Accession 3 had significantly less disease than 'Dickinson' and was no different from 'Bugle' (Table 1).** This demonstrates that CPM resistance has been bred into a commercially desirable variety. The number of fruit did not significantly differ across the progeny and 'Dickinson' (Table 1); 'Bugle' had significantly more marketable fruit per ten-plant plot, and the fruit are smaller, which is normal and expected for this genotype. Accession 3 had internal fruit and processing qualities that resemble 'Dickinson' in canning trials conducted in the fall of 2022. Further evaluations of Accession 3 are planned for the 2023 field season.

The second trial included 15 accessions of *Cucurbita maxima*, representing multiple market classes, to search for genetic resistance that could be bred into more susceptible cultivars. **The squash accessions 'Tabalque', 'Zapallito de Tronco', and 'Plomo Ruso' had numerically the least disease, but did not have statistically less disease than any other variety except 'Mayo Blusher' (Table 2, Figure 2).** Currently, *C. maxima* cultivars do not carry the *Pm-0* gene that is common in butternuts (*C. moschata*) and are generally susceptible to CPM. Breeders are working towards incorporating resistance into commercially desirable *C. maxima* varieties.

Fruit processing

A protocol was developed in consultation with Olga Padilla-Zakour (Cornell University Department of Food Science) to process fruit at the lab-scale. For each accession in both 'Dickinson' and *C. maxima* trials, subsets of representative fruit were cooked for 30 minutes in a 3-tier, 9 qt electric steamer. Cooked fruit was then processed in a Victoria Food Mill and Sauce Maker (Model VKP250) using the pumpkin screen. Cheese cloth was used to press out 10% of free water by weight for products with excess

moisture. Accessions that required water removal are noted in Tables 3 and 4. Final products were canned in triplicate using 4 oz Ball Mason jars in a 6 qt Instant Pot Max under high pressure for 115 minutes. Excess products were frozen for later pressure canning with an industrial retort. Processing was completed between 10/19 – 10/28/22. Sections of raw fruit weighing approximately 20 g were lyophilized in triplicate to estimate dry matter. Jars were opened on 1/11/23 to assess water separation, measured as the weight (kg) of free water that strained from each jar after 30 seconds. Because each accession was only processed and canned once, canning yield data is not replicated.

Among 'Dickinson' breeding lines, dry matter in fruit from each progeny was no different from 'Dickinson' (Table 3). 'Bugle' fruit was significantly drier, with no water separation and dry matter comprising 18.40% of its fresh weight, compared to 9.38% in Accession 3. Canning yield ranged from 36.68% (Accession 6) to 49.48% (Accession 3) of fresh weight. Accessions 3, 4, and 5 had significantly less free water separate compared to 'Dickinson', while Accession 6 was no different from Accessions 3, 4, and 5, and 'Dickinson'. Overall, all progeny performed comparably to 'Dickinson' but not 'Bugle', which is promising for their advancement.

Fruit quality differed substantially within *C. maxima* accessions (Table 5). 'Kestane' fruit was among the moistest, with dry matter comprising only 5.22% of its fresh weight, and was no different from 9 other accessions with dry matter below 14.44%. Fruit from 'Thunder F1' were the driest; interestingly, this was among the most challenging to cut and prepare for processing. Canning yield spanned large numeric differences, from 34.62% ('Kestane') to 69.61% ('Zapallito de Tronco') of fresh weight. Seven accessions had no water separation. 'Buttercup Burgess Strain', 'Amzibegovska', and 'Golden Delicious' had significantly less free water separate compared to 'Plomo ruso', No. 7488, 'Kestane', and 'Zapallito de Tronco'. 'Dickinson' had the highest proportion of water separation. Though we lacked a quantitative method to measure texture, the consistency of processed 'Fipushi' resembled 'Dickinson' the most (Figure 4). Comparatively, the majority of *C. maxima* fruit evaluated were significantly drier than 'Dickinson'.

Table 1. Results of winter squash (*Cucurbita moschata*) breeding line field evaluations. Yield parameters were calculated per ten plants. 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.

	Foliar Disease		**Marketable Fruit[y]**			
Pedigree	**Severity (%)[x]**		**Number**		**Weight (lbs)**	
Bugle	24.2	a	42.0	b	8.2	a
Accession 3	25.0	a	10.7	a	8.7	a
Accession 6	31.7	ab	20.0	a	9.4	ab
Accession 4	51.7	ab	11.7	a	9.6	ab
Accession 5	55.0	b	16.3	a	9.2	ab
Dickinson	55.0	b	22.0	a	10.6	b
P value	< 0.01		< 0.001		< 0.05	

[x]Percent disease severity on adaxial leaf surfaces estimated per plot on 9/20/22

[y]Marketable fruit were free from developmental issues and abiotic or biotic damage

Table 2. Results of field evaluations of a winter squash (*Cucurbita maxima*) diversity panel and commercial controls. Yield parameters were calculated per ten plants. 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	Foliar Disease Severity (%)[x]		Marketable Fruit[y]			
			Number		Weight (lbs)	
Tabalque	18.3	a	17.0	abcd	18.6	a
Zapallito de Tronco	22.5	ab	30.0	d	39.4	ab
Plomo ruso (Plaunorskja)	23.3	ab	7.5	ab	94.6	abc
Golden Delicious	35.8	abc	12.3	abcd	99.4	abc
Amzibegovska	36.7	abc	10.0	abc	92.8	abc
Queensland Blue	37.5	abc	19.3	abcd	146.1	bc
Fipushi	38.3	abc	21.7	abcd	131.7	abc
No. 7488	38.3	abc	7.3	ab	79.1	abc
Kestane	40.8	abc	15.3	abcd	193.0	c
Ambar	46.7	abc	8.0	ab	41.1	ab
Golema	48.3	abc	5.7	a	52.3	ab
Autumn Cup F1	53.3	bc	16.7	abcd	46.2	ab
Thunder F1	54.2	bc	23.7	bcd	77.6	ab
Buttercup Burgess Strain	54.2	bc	27.3	cd	64.4	ab
Dickinson	55.0	bc	20.3	abcd	342.1	d
Mayo Blusher	65.0	c	11.0	abc	99.0	abc
P value	< 0.001		< 0.01		< 0.01	

[x]Percent disease severity on upper leaf surfaces estimated once on 9/20/22
[y]Marketable fruit were free from developmental issues and abiotic or biotic damage

Table 3. Results of *C. moschata* breeding line processing evaluations. 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.

Pedigree	Dry Matter (%)[x]		Canning Yield (%)[y]	Water Removed[z]	Water Separation (%)[w]	
Dickinson	7.28	a	47.83	+	4.7	b
Accession 6	7.71	a	36.68	+	4.0	ab
Accession 4	7.88	a	42.47	+	3.1	a
Accession 5	8.36	a	47.95	+	3.2	a
Accession 3	9.38	a	49.48	+	3.4	a
Bugle	18.40	b	40.39	-	0	
P value	< 0.001		--		< 0.01	

[x]Dry matter calculated as a percentage of fresh weight after freeze-drying
[y]Canning yield reported as a percentage of the weight of whole fruit (kg) after processing
[z]Ten percent of free water by weight was removed with cheesecloth before canning for hydrous products
[w]Water separation calculated as a percentage of free water strained (kg) from product after canning. Products with no separation were excluded from the analysis. Data was square root transformed

Table 4. Results of *C. maxima* breeding line processing evaluations. 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.

Pedigree	Dry Matter (%)[x]		Canning Yield (%)[y]	Water Removed[z]	Water Separation (%)[w]	
Kestane	5.22	a	34.62	+	3.6	e
Zapallito de Tronco	5.48	a	69.61	+	3.4	e
No. 7488	8.33	ab	40.69	+	2.8	d
Dickinson	8.85	abc	46.94	+	4.6	f
Tabalque	11.42	abcd	64.57	-	0.6	bc
Fipushi	11.61	abcd	46.67	-	0	
Plomo ruso (Plaunorskja)	12.13	abcd	47.95	-	1.0	c
Queensland Blue	13.92	abcde	55.51	-	0	
Amzibegovska	14.29	abcde	54.43	-	0.3	ab
Golden Delicious	14.44	abcde	51.77	-	0.5	ab
Mayo Blusher	16.22	bcde	48.03	-	0	
Buttercup Burgess Strain	18.56	cdef	59.08	-	0.1	a
Ambar	18.62	cdef	49.00	-	0	
Golema	20.12	def	51.34	-	0	
Autumn Cup F1	23.23	ef	46.95	-	0	
Thunder F1	26.95	f	52.24	-	0	
P value	< 0.001		--		< 0.001	

[x]Dry matter calculated as a percentage of fresh weight after freeze-drying
[y]Canning yield reported as a percentage of the weight of whole fruit (kg) after processing
[z]Ten percent of free water by weight was removed with cheesecloth before canning for hydrous products
[w]Water separation calculated as a percentage of free water strained (kg) from product after canning. Products with no separation were excluded from the analysis. Data was square root transformed.

Figure 1. Representative fruit from the powdery mildew resistant parent 'Bugle', the susceptible parent 'Dickinson', and the progeny with promising disease resistance and canning qualities. *Photos: Elizabeth Indermaur, Cornell AgriTech*

Figure 2. Representative fruit from *Cucurbita maxima* breeding lines with promising powdery mildew tolerance. *Photos: Elizabeth Indermaur, Cornell AgriTech*

Figure 3. Jars from ‘Dickinson’, ‘Bugle’, and Accession 3 with Libby’s Canned Pumpkin for comparison. *Photos: Elizabeth Indermaur, Cornell AgriTech*

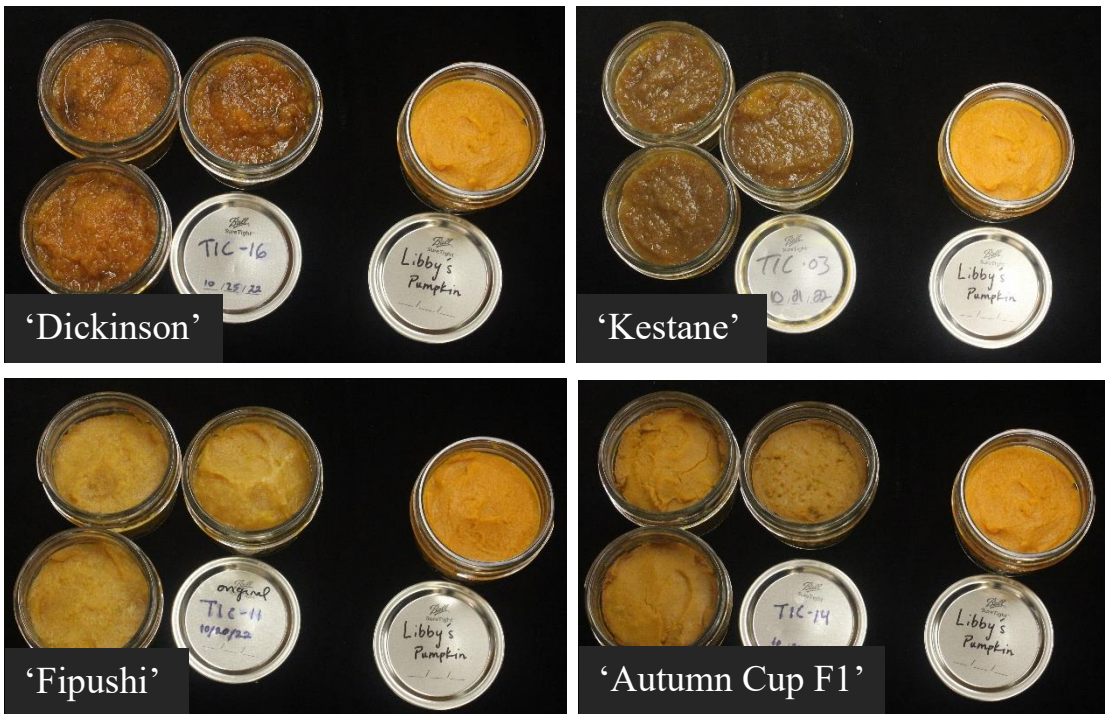

Figure 4. Jars from ‘Dickinson’, ‘Kestane’, ‘Fipushi’, and ‘Autumn Cup F1’ with Libby’s Canned Pumpkin for comparison. *Photos: Elizabeth Indermaur, Cornell AgriTech*

Honeynut is a miniature butternut noted for its quality, but because it is late maturing, it's quality can be impacted by powdery mildew that infects the plant during carbohydrate filling. Previously, crosses with a source of *Pm-0* for powdery mildew resistance were selected for several generations for quality and improved storage. This resulted in multiple new cultivars, however they lack the characteristic dark rind color of 'Honeynut' that functions as both a control for quality and consumer recognition. We used the *Pm-0* marker to select for homozygous resistant individuals within BC_2F_2 populations, assayed them for storage, aesthetics and eating quality and will select within these homozygous PMR breeding lines going forward.

- Phytophthora

To identify segregating interspecific squash populations which had captured Phytophthora crown and root rot resistance from 'Dickinson', a seedling stage, 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 populations (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.

	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 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).
**Values are the means of back transformed data using "rank minus 1".

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 than 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) shown 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).

UF lines were further screened in the greenhouse with an aggressive isolate of *P. capsici* (isolate STK-5A) from New York by Smart Lab. Overall, Line SS69-72 (interspecific hybridid) was very promising and was significantly less susceptible that all other lines included in the experiment, with the exception of 333-7. Additionally, lines 358_174, 358_195 and Early Prolific were significantly more susceptible than the other lines.

Line Evaluated	**AUDPC**
SS69-72	10 a
SSS333-7	22 ab
SS2147	29.5 b
SS333-8	30 b
PI181761-36P-LOT1	31.75 b
PI181761-36P-LOT3	36.25 b
358_164	36.5 b
SS2071	36.75 b
SS2061	37 b
SS2078	37.5 b
SS792-2	38 b
358_174	38.25 c
358_195	38.5 c
Early Prolific	40 c

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

To evaluate breeding lines for Phytophthora fruit rot Age-Related Resistance (ARR), fruits from a total of 16 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. capsisci* (strain SP98) was placed onto the surface/epidermis of each disinfected fruit 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 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 resistant 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

[Z] Average incidence (value) is based on 10 fruits harvested. No Fruit (NF) means no fruit available at harvest.

[Y] Average incidence (value) is based on 3 fruits harvested at 21dpp and 2 fruits at 28dpp.

[X] Average incidence (value) is based on 4 fruits harvested at 28dpp.

The main causal agent of powdery mildew in *C. moschata* in Puerto Rico was identified as *Podosphaera xanthii*. through a combination of molecular and morphological approaches, amplification of the ITS 5.8 rDNA region and conidia with had elliptical shape, presence of fibrosin bodies and either forked or apically swollen germination tubes.

Resistance to powdery mildew (*Posphaera xanthii*) was evaluated in Cornell *Pm-0* derived resistant lines and local tropical pumpkin (*Cucurbita moschata*) genotypes. These included 'Taína Dorada', 'Soler', 'Verde Luz', 'Waltham', 'Dickinson', 20-1716-05 x 1720-05, 20-1716-02 x 1720, 20-1716-08 x 1720-02 and 20-1716-03 x 1720-02. The field trial was a replicated complete bock design with 3 reps and inoculation was perfomed with infected leaf suash applied to the top of young leaves. Disease severity and incidence were recorded in addition to agronomic traits. Vigor and vine length was greater in tropical pumpkins. 'Taína Dorada' and 'Soler' had significantly more fruit ($p<0.05$) 'TD' had significantly more yield ($p>0.05$). The Incidence of PM was highest (50%) in 'Taína Dorada' and 'Waltham' ($p<0.05$) while the severity of PM was highest in 'Waltham', 20-1716-02x1720 and Dickinson ($p<0.05$). In a greenhouse trial, the incidence of PM was highest (80-89%) in temperate genotypes ($p>0.05$) and severity was highest in 'Ponca' and all temperate genotypes ($p>0.05$).

A breeding program was initiated at UPR for PMR tropical *C. moschata*. Parental genotypes were assayed for resistance and intermated to create different breeding populations. Greenhouse trial results indicate that under high pathogen pressure Ponca and all Cornell genotypes had significantly higher PM infection than local genotypes. Local genotypes, seem to be more tolerant to PM (*P. xanthii*).

Integrated Disease Management Team

Personnel Working on Project

- Lina Quesada-Ocampo (NC State)
    - Mike Adams (technician)
    - Yara Rosado (technician)
    - Mary Lorscheider (extension communicator)
    - Mariana Prieto (PhD student)
    - Andres Salcedo (Postdoc)
    - Ziaur Bhuyian (Postdoc)
- Mary Hausbeck (MSU)
    - Carmen M. Medina-Mora (technician)
    - Matthew Uebbing (graduate student)
    - David Perla (graduate student)
- Chris Smart (Cornell)
    - Colin Day (lab manager)
    - Taylere Herrmann (technician)
    - Libby Indermaur (PhD student)
    - Emma Nelson (PhD student)
    - Janna Fladd (high school intern)
- 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)

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

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

CucCAP website: From Sept. 1, 2021 until Feb. 7, 2023, the CucCAP website was visited by 24,933 users including 24,707 new users with 29,720 sessions and 48,432 pageviews. A total of 158 news items were posted on the website including 57 posts of news from CucCAP researchers and 112 crop and disease reports. These posts were shared in a monthly newsletter

sent to 160 subscribers and in 125 posts on social media including Facebook with 168 followers, Twitter with 130 followers, and LinkedIn with 18 followers. Cucurbit disease factsheets and links to integrated pest management resources are maintained and updated annually on the website. Five of the top ten pages visited on the website were Cucurbit disease management factsheets in Spanish. The CucCAP website events calendar shared notices of 10 regional commodity meetings, 22 education sessions, and 22 Scientific meetings.

Quesada: Since the start of the project, Quesada has provided diagnostics and disease management recommendations for 28 cucumber, 52 watermelon, 12 melon, 16 squash, and 12 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: 6,578 (lab) + 3,762 (Quesada) followers, LinkedIn: 2,869 followers), and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US Vegetable Crop Handbook.

Smart: Smart has provided diagnostics and disease management recommendations for cucumber, melon, squash, and pumpkin samples received across New York State (n=47). The Smart lab has also provided disease management recommendations through oral presentations, extension publications, and social media (Twitter: 2,088 followers, @ChristineSmart6).

Hausbeck: Hausbeck maintains a page dedicated to downy mildew (Downy Mildew News), which gets frequently updated during the cucurbits growing season (late May to late Sept.) maintaining the scientific community and growers informed with current information about the disease. This page includes a weekly summary of the results from spore trapping used for tracking, monitoring, and forecasting the disease in the state. This year's results corresponded to seven spore traps deployed in counties with commercial production of cucurbits along Michigan's lower peninsula. In addition, the webpage includes a Michigan map updated daily as mildew symptoms are confirmed in the state, facts sheets, illustrative identification of mildew on cucurbits, and reference articles and links to main diseases threatening crop production of cucurbits.

Schultheis: Since the start of the project, Schultheis has been involved in cultivar evaluations on melon, zucchini, pumpkin, and watermelon. Watermelon cultivar response to Fusarium wilt has been the main focus and this information has been conveyed through oral and poster presentations.

4.2. 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: 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: 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: 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: 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: 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: 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: 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 (Week 6).
[y] Disease rating scale based on percent necrotic foliage caused by *P. cubensis*. / Data point 6 Oct (Week 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: 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: 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: 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.

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

Hausbeck: 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.

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

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

	Plant death (%)					AUDPC	
Line Evaluated	**12-Sep**		**20-Sep**		**27-Sep**		
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: 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.

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 and Keinath have not started with this activity since it is planned for years 3 and 4.

Cucumber downy mildew (Lead: Hausbeck, Secondary Site: Quesada): Quesada has not started with this activity since it is planned for years 3 and 4.

Hausbeck 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 cucumber. The field was plowed on 20 May and disced 1 June. Preplant fertilizer (220 lb./A urea and 180 lb./A potash) was applied and incorporated on 1 June. On 9 July, raised beds were formed in the field with black plastic mulch 8-ft apart, and drip tape for irrigation and in-season fertilization. Biweekly mechanical cultivation and hand weeding was used for weed control. Planting occurred on 26 July from seed. The cultivar used for this experiment was 'Citadel'. The treatments were arranged in a completely randomized block design with four replications. Each replicate 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/A through the drip tape. Quadris F (15.5 fl oz/A) and Torino SC (3.4 fl oz/A) were sprayed on 26 August to control the incidence of *Alternaria* spp and powdery mildew and Admire Pro (10.5 fl oz/A) was applied on 10 August through the drip lines for insect control. Spray treatments were applied on 16 and 23 August using a CO2 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. Foliage was evaluated for infection on 18, 24, and 29 August by visually estimating the area of foliage with symptoms (%). Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).
Disease severity in the untreated control progressed from 10% on 18 August to 25% on 29 August. A significant increase in disease occurred from 18 August to 24 August in all treatments. There were no statistical differences among treatments at any of the rating dates, though Trillium had numerically less disease (17.5%) compared to all other treatments (21.3-26.3%) on the final rating date (29 August). According to the area under the disease progress curve (AUDPC), no treatment differed from the untreated control. Trillium did have significantly less disease compared to Kocide and MBI-121 alternated with Kocide. No phytotoxicity or tank mix incompatibilities were observed during the duration of the trial.

Treatment[z] and rate/A, applied at 7-day intervals	Foliar Infection (%)[y]						AUDPC[x]	
	18 Aug		24 Aug		29 Aug			
Untreated control	10.0	a[w]	21.3	a	25.0	a	209.4	ab
Trillium EC 1% V/V	7.5	a	17.5	a	17.5	a	162.5	b
MBI-121 EC 3 qt	8.8	a	20.0	a	25.0	a	198.8	ab
Theia WP 3 lb + Activator 90 0.125% V/V -*alt*- Kocide 3000 WP 0 1.25 lb	10.0	a	20.0	a	26.3	a	205.6	ab
Theia WP 3 lb + Activator 90 0.125% V/V	10.0	a	25.0	a	21.3	a	220.6	ab
Kocide 3000 WP 1.25 lb	12.5	a	23.8	a	25.0	a	230.6	a
MBI-121 EC 3 qt -*alt*- Kocide 3000 WP 1.25 lb	12.5	a	26.3	a	26.3	a	247.5	a

[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).

Squash powdery mildew (Lead: Smart, Secondary Site: Hausbeck):
Smart : **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. **Year 3-4:** Smart will conduct an integrated trial with three squash cultivars and Kocide 3000-O, Theia, and Curezin.

Hausbeck: 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	0.369	0.110	0.017		0.022		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).

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

Hausbeck: 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: 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 2×10^6 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.

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

Phytophthora capsici (Smart): 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.

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).

A draft pickling cucumber budget for North Carolina has been prepared. The remaining budgets will be prepared in 2023. Economic impact analysis will be conducted in 2024, combining the prepared budgets and the results of the other PIs field trials.

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

The literature regarding vegetable producer willingness-to-pay for crop traits was reviewed by Tregeagle and team, who performed a meta-regression analysis on existing estimates of producer willingness-to-pay for crop traits, comparing these results to existing consumer estimates. This analysis confirmed that estimates for producer willingness-to-pay for cucurbits are not available in the current literature, but that, in general, producers are willing to pay a premium for disease-resistant varieties.

Techniques identified in the existing literature are being adapted to develop surveys for the three crop-pest pairs below. The cucumber/downy mildew survey has been the focus of development, being used as a test case to refine the survey contents, distribution methods, and statistical design, which will then be applied to the other crop-pest pairs.

MI – cucumber/downy mildew (Hausbeck): The cucumber-downy mildew survey and related materials are currently being prepared for submission to NC State's IRB for review – the final step before deployment of the survey.

MI – squash/phytophthora (Hausbeck): Draft attributes and levels for squash/phytophthora survey have been developed from a review of current seed catalogs published by a variety of commercial seed distributors. The draft attributes and levels will be refined by discussions with other PIs and industry members, preparing the survey for deployment in 2023/2024.

NC – cucumber/downy mildew (Quesada): Quesada and Tregeagle have met several times to collaboratively develop this survey. However, the pandemic has delayed survey efforts due to cancellation of many extension events and grower meetings. We have plans to deploy this during in-person grower events in 2023. The cucumber-downy mildew survey and related materials are currently being prepared for submission to NC State's IRB for review – the final step before deployment of the survey.

NC – watermelon/fusarium (Schultheis): Draft attributes and levels for watermelon/fusarium survey have been developed from a review of current seed catalogs published by a variety of commercial seed distributors. The draft attributes and levels will be refined by discussions with other PIs and industry members, preparing the survey for deployment in 2023/2024. Schultheis and Tregeagle have met several times to discuss the design of the survey.

NY – squash/phytophthora (Smart): Smart has collected data on the value of winter squash in NY from producers and field scouts. The average price for winter squash in NY is $31.00 per cwt. Average yield in NY is 12 Tons/A with yields in 2022 ranging from 8-20 Tons/A. Average loss per year due to Phytophthora is 10%. In wet years losses will be higher, up to 35% (of total acreage) which would be 80-100% in some fields and 0-20% in other fields.

Draft attributes and levels for squash/phytophthora survey have been developed from a review of current seed catalogs published by a variety of commercial seed distributors. The draft attributes and levels will be refined by discussions with other PIs and industry members, preparing the survey for deployment in 2023/2024.

SC – watermelon/fusarium (Keinath): Draft attributes and levels for watermelon/fusarium survey have been developed from a review of current seed catalogs published by a variety of commercial seed distributors. The draft attributes and levels will be refined by discussions with other PIs and industry members, preparing the survey for deployment in 2023/2024.

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS

REFEREED PUBLICATIONS, BOOK CHAPTERS, CONFERENCE PROCEEDINGS

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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EXTENSION PUBLICATIONS and OUTREACH RESOURCES

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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. 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.
3. Branham SE. 2022. Marker-assisted vegetable breeding for production in the Southeastern US. Cornell University, School of Integrative Plant Science Spring Seminar Series.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. Chen FC 2022. Genetic architecture of the downy mildew resistance locus *dm4.1* in PI 330638 (WI7120). Cucurbitaceae 2022, Naples, FL
10. 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
11. Culp C, Chen YC, Grumet R. 2022. Testing Cucumber Accessions for Phytophthora Fruit Rot Resistance. MSU Undergraduate Research Forum. East Lansing MI
12. 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.
13. 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
14. 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.
15. 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

16. 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
17. 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/
18. Fei Z. 2023. Genomic basis of watermelon origin, domestication, and breeding. School of Plant Integrative Science, Cornell University. February 2023
19. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. The 9th International Horticulture Research Conference. Wuhan, China. November 2022
20. Fei Z. 2022. A super-pangenome of cultivated and wild watermelon species. Cucurbitaceae 2022. Naples, FL. October 2022
21. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. University of North Carolina. October 2022
22. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. Shandong Academy of Agricultural Sciences. October 2022
23. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. The 2022 International Symposium of Horticulture and Plant Biology. Wuhan, China. August 2022
24. Fei Z. 2022. Genomic basis of watermelon origin, domestication, and breeding. BTI PGRP Summer Intern. July 2022
25. Fei Z (2021) Genomic analyses shed light on watermelon origin and the genetic history of domestication and agronomic traits. Zhejiang University
26. 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
27. 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
28. Fei Z. The origin, history and future of watermelon. BTI Breaking Ground series. November 2021
29. 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
30. Frank A, Lin YC, Grumet R. 2022. Measurement and Analysis of Cucumber Fruit Curvature. MSU Undergraduate Research Forum. East Lansing MI
31. 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
32. 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.
33. 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.
34. Grumet R. 2022. Grumet R. 2022. Leveraging applied genomics to increase disease resistance in cucurbit crops. Corteva Symposium, Cornell University. March 2022

35. Grumet R. 2022. Grumet R. 2022. Cucurbit germplasm - genomic tools and disease resistance. National Association of Plant Breeders. Ames Iowa. August 2022
36. 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.
37. 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.
38. 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.
39. 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.)
40. 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.
41. 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.
42. 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.
43. 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
44. 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.
45. 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
46. Kenny, G. 2020 Cucumber field data. Departmental seminar, Department of Plant Pathology, Michigan State University, East Lansing, MI. Virtual.
47. 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
48. 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.

49. 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.
50. 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.
51. 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).
52. 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)
53. 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
54. 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
55. 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.
56. Levi A. 2022. Challenges and progress in genetic research and in enhancing disease resistance in watermelon. South Korean Society of Plant Breeders and Geneticists.
57. Lin YC, Grumet R. 2023. QTL Mapping of Young Fruit Resistance to *Phytophthora capsici* in Cucumber. Plant Animal Genome Conference, San Diego CA
58. Lin YC, Grumet R. 2022. QTL Mapping of Young Fruit Resistance to *Phytophthora capsici* in Cucumber. Cucurbitaceae 2022. Naples FL
59. Lin YC. 2022. 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
60. 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.
61. 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.
62. 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.
63. Luckew, A. and C.E. McGregor. 2022. Identifying resistance to whitefly transmitted viruses in Cucurbita. Cucurbitaceae, Naples, FL.
64. 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.
65. 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.

66. 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.
67. 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.
68. Mazourek M. 2022. Combining Resistance with Quality in Squash. Asia Pacific Seed Association Cucurbinar. Sept 29, 2022.
69. Mazourek M, Frost E 2022. Combining Cucurbits for Downy Mildew Resistance and More. OSSI Webinar Series. May 11, 2022.
70. 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)
71. 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
72. 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).
73. 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.
74. Meru, G. 2022. Advancing the cucurbit industry through a genomics-enabled breeding and extension program. Horticultural Sciences Department Seminar, University of Florida, Gainesville, FL.
75. 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.
76. 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.
77. 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.
78. 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.
79. Michel S., Schultheis J., Keinath A., and Quesada-Ocampo L. M. Incidence and yield response of seedless watermelon cultivars affected with Fusarium wilt. Cucurbitaceae, Naples, Florida, November 2022.
80. Mondal S, Wintermantel WM, McCreight J. 2022. Development of CYSDV-resistant lines using marker-assisted selection. Cucurbitaceae 2022 Naples, FL
81. 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
82. 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
83. 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.

84. 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.
85. 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.
86. 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.
87. Parada-Rojas C. H. and Quesada-Ocampo L. M. *Phytophthora capsici* populations structure by host, geography, and fluopicolide sensitivity. Cucurbitaceae, Naples, Florida, November 2022.
88. Parada-Rojas C. H. and Quesada-Ocampo L. M. (2022) populations structure by host, geography, and fluopicolide sensitivity. Phytopathology 112: S3.102.
89. 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.).
90. 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
91. 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.
92. 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
93. 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
94. 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.
95. 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.
96. 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
97. 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.
98. 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.
99. Quesada-Ocampo L. M. 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.

100. Quesada-Ocampo L. M. Translational research for detection and management of diseases of vegetable crops. Universidad Nacional Mayor de San Marcos. Lima, Peru, November 2022.
101. 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.
102. 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.
103. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Biology Seminar, University of Massachusetts, September 2022.
104. Quesada-Ocampo L. M. Disease management in vegetable crops. AgBiome seminar, Durham, NC, July 2022.
105. Quesada-Ocampo L. M. Translational strategies to improve management of re-emerging pathogens of vegetable crops. Australasian Plant Pathology Society, Australia, November 2021.
106. 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.
107. 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.
108. 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.
109. 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.
110. 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.
111. 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
112. Rett-Cadman S, Hammar S, Grumet R. 2022. Isolation and Characterization of Lipid Droplets in Cucumber Fruit. Cucurbitaceae 2022, Naples FL
113. Rijal, S. And C.E. McGregor. 2022. Marker-Assisted Breeding for Gummy Stem Blight Resistance in Watermelon. Cucurbitaceae, Naples, Florida.
114. 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.
115. 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
116. Rijal, S. 2022. Watermelon's Wild Friends: Introgressing Important Traits in a Favorite Fruit. IPBGG Research Seminar, UGA.
117. 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.

118. Salcedo, A., Parada-Rojas C. H., Purayannur S.,Quesada-Ocampo L. M. Accelerating Resistance Breeding in Cucurbits. CucCAP2 meeting, Virtual Meeting, October 2021
119. 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
120. 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
121. Schultheis, J. and K. Starke 2021. Melon cultigens and their adaptation in the southeastern United States when grown in North Carolina (abstr.)
122. 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.
123. Sun H. The Citrullus genus super-pangenome. BTI Monday Morning Seminar Series. March 2023.
124. 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
125. 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.).
126. 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.)
127. 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.
128. 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.
129. 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.
130. 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
131. 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)
132. 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, virtual)
133. 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).

134. 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).
135. Weng, Y. 2022. The Gy14v2.0 pickling cucumber genome. Cucurbitaceae 2022 international meeting (Naples, FL, November 2, 2022)
136. 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)
137. 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.
138. 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
139. Wintermantel WM. 2022. Seasonal prevalence and spread of whitefly-transmitted viruses in California production regions. Cucurbitaceae 2022. Naples FL.
140. 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.
141. Wintermantel WM. 2022. Emerging viruses threatening cucurbit crops. Annual Meeting of the American Phytopathological Society, Pittsburgh, PA, August 6-10, 2022
142. Wu S. (2023) Super-pangenome of wild and cultivated watermelons. Plant & Animal Genome Conference. San Diego, CA. January 2023.
143. Wu S (2022) Pan-genome of wild and cultivated watermelons. University of Georgia
144. 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
145. 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 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.
5. Grumet R., Weng Y. Breeding for disease resistance in pickling cucumber. Great Lakes Fruit, Vegetable, Farm Market Expo. December, 2021
6. Grumet R, Lin YC. 2020. Resistance of cucumber fruit to *Phytophthora capsici*. Pickle Packers International. Virtual conference, October 2020.
7. 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.
8. 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.
9. Hausbeck, M.K. 2022. Putting together a Phytophthora program that works. MSU Extension meeting. Oceana County, MI, Mar. 25 attendees.
10. 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.
11. 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
12. 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.
13. Hausbeck, M.K. 2021. A Partnership to protect Michigan's Cucumber Industry. Farm Lane Society meeting, Virtual, 5 Mar.
14. Hausbeck, M.K. 2020. Management of Phytophthora Blight in Processing Squash. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 10 Dec. 167 attendees.
15. Hausbeck, M.K. 2020. Downy Mildew Management in Pickling Cucumbers. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 8 Dec. 174 attendees.
16. 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
17. Hausbeck, M.K. 2020. 2021 Spray Program. Southeast Vegetable Meeting. Virtual, 4 Nov. 90 attendees.
18. 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.
19. 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.

20. 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.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. Keinath, A. P. 2023. Tebuconazole Resistance in the Gummy Stem Blight Fungus in South Carolina. 35th Southeast Vegetable & Fruit Expo. November 29, 2022.
28. 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.
29. 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.
30. 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.
31. 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.
32. 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.
33. Keinath, A. P. 2022. Cucurbit Disease Update with 2021 Clemson Trial Results. Clemson Extension Cucurbit Grower Meeting (virtual). February 17, 2022.
34. 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.
35. 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)
36. 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
37. 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)
38. 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)
39. 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.
40. 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
41. 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/
42. 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/
43. 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
44. 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
45. Levi A. 2023. History, Genomic Tools and Enhancing Disease Resistance in Watermelon. Giant Watermelon and Pumpkin Grower Group. March 25th, 2023, Elkin, NC.
46. 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.
47. Levi A. 2022. Presentation to Minister of Agriculture from Qatar. May 11th, 2022.
48. 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)
49. 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.
50. Mazourek M. 2022. Vegetable Breeding Institute Field Days. August 29-20, 2022. Ithaca and Freeville, NY.
51. Mazourek M. 2021. Winter Squash Background, Diversity and Breeding. Winter Squash Sagra. Culinary Breeding Network. January 25, 2021
52. McGregor, C. & G. Boyhan (2020) Breeding better Cucurbits. Vegetable & Specialty Crop News, September 2020: 16-17
53. Meru G. 2021. Progress towards breeding resilient cultivars for Florida. Presented at the Southeast Florida Extension Meeting, held virtually April 8, 2021.
54. 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.
55. 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.
56. 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.
57. Quesada-Ocampo L. M. Management of watermelon diseases. North Carolina Watermelon Production Meeting. Virtual, February 2023.

58. 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.
59. 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.
60. 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.
61. 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.
62. Quesada-Ocampo L.M. Management of Fusarium wilt and anthracnose in watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
63. Quesada-Ocampo L.M. Cultural and chemical control options for Phytophthora fruit rot of watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
64. Quesada-Ocampo L.M. Never a dill moment when managing cucumber downy mildew. 2020 Eastern NC Certified Crop Adviser Training. Virtual Meeting, December 2020.
65. 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.
66. Schultheis, J.R. 2021. Hollow heart considerations and pollenizer cultivar comparisons. North Carolina Watermelon Growers Association. Virtual meeting, January, 2021. 65 attendees
67. 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.
68. Schultheis, J. and S. Michel. Watermelon cultigen yield and quality results, North Carolina, 2022. Mar-Del Watermelon Association meeting. Cambridge, MD, February 3, 2023.
69. 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.
70. 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/
71. 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.
72. 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.
73. Smart C. Winter Squash Cultivar Evaluations for Resistance to Powdery Mildew. 2022. NY State producers expo.
74. 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.
75. 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/
76. 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.

77. 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.
78. 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.
79. Weng, Y. 2022. Genetic basis of downy mildew resistances in cucumber. Asia Pacific Seed Association. September 2022
80. 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)
81. 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)
82. Weng Y (2022) Marker-assisted QTL pyramiding for multiple disease resistances in cucumber. Midwest Pickle Association annual meeting (December 6, 2022, Grand Rapids, MI)
83. Weng Y (2021) The Gy14v2.0 cucumber draft genome. Chinese Cucumber Breeders Association 2021 Annual Meeting and Variety Show. April 2021. Virtual.
84. Weng Y (2021). Disease resistances in cucumber. SIPS seminar. Cornell University, Ithaca, NY. Virtual.
85. Weng 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.
86. Wintermantel W. 2022. California Melon research Board, January, 2022. Online presentation to ca. 70 Board members growers and seed company personnel.
87. 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.