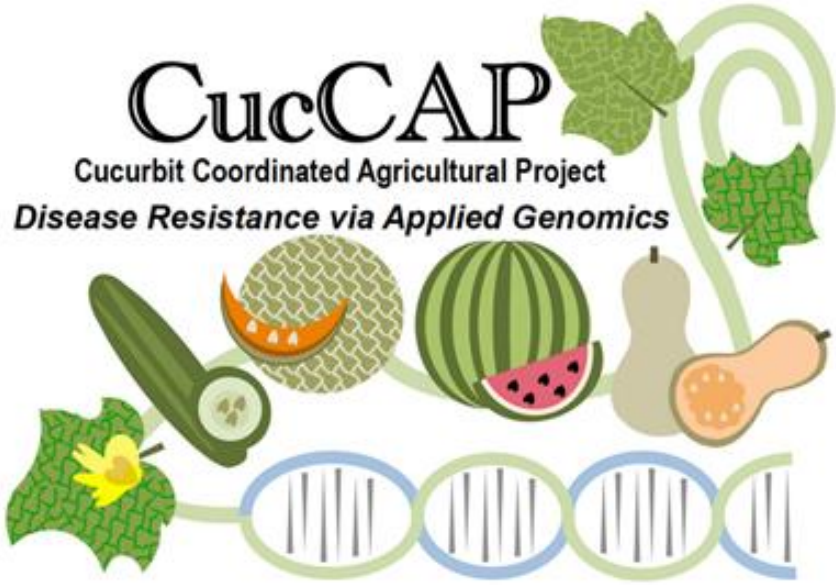
CucCAP2

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



CucCAP2 Team Meeting

October 27-29, 2021

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AGENDA

TIMES: a (Eastern, EDT) /b (Central) /c (Pacific) /d (Europe)

Wednesday October 27

1:00pm/12:00/10:00/7:00 Arrival, welcome, introductions
1:15pm/12:15/10:15/7:15 Overview of project progress, plans for meeting

Session I – Genomic Tools

Objective I: 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

1:30pm/12:30/10:30/7:30 Overview of progress: bioinformatics platforms, databases, pan-genomic analyses (Fei, Wu)
2:00pm/1:00/11:00/8:00 Status of core panels (seed stocks; resequencing)
watermelon (Levi)
melon (McCreight)
cucumber (Weng/Grumet)
squash (Mazourek)

2:20pm/1:20/11:20/8:20 Discussion and feedback from industry

2:40pm/1:40/11:40/8:40 – 3:30/2:30/12:30/9:30 break, lunch, dinner?

Session II – Breeding for disease resistance

Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.
- Utilize genomic approaches to map resistance loci for key cucurbit diseases (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

3:30pm/2:30/12:30/9:30 **Watermelon**: Status for each disease
Fusarium race 1 and 2, gummy stem blight, Phytophthora, powdery mildew, CYSDV, GCMMV, PRSV-W, ZYMV
(Levi, Branham, Kousik, Ling, McGregor, Reddy, Wechter)
4:30pm/3:30/1:30/10:30 Feedback/priorities from industry

Thursday October 28

Session II – Breeding for disease resistance- continued

11:00am/10:00/8:00/5:00 **Melon**: Status for each disease
powdery mildew, downy mildew, CYSDV, Fusarium
(McCreight, Kousik, Wechter, Wintermantel)
11:40am/10:40/8:40/5:40 Feedback/priorities from industry

11:55am/10:55/8:55/5:55 **Cucumber**: Status for each disease
downy mildew, Phytophthora, CGMMV
(Weng, Grumet, Keinath, Ling)
12:35pm/11:35/9:35/6:35 Feedback/priorities from industry

12:50pm/11:50/9:50/6:50 – 1:45/12:45/10:45/7:45 Break, lunch, dinner?

1:45pm/12:45/10:45/7:45 **Squash**: Status for each disease
C. moschata – powdery mildew, *Phytophthora*, *C. maxima* – *Phytophthora*,
C. pepo – powdery mildew, *Phytophthora*
(Mazourek, Hausbeck, Kousik, Meru, Ramirez, Smart)
2:45pm/1:45/11:45/8:45 Feedback/priorities from industry

Session III – Planning Sessions

3:00pm/2:00/12:00/9:00

A. **Genomics: resequencing, databases, pan-genomics (Fei lead)**

B. **Multi-location resistance trials (Lina lead)**

Friday October 29

Session IV – Integrated disease management and economic analysis

Obj. 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)

11:00am/10:00/8:00/5:00 Multi-location trials, pathogen population analyses, economic analyses, delivery of disease information
(Quesada, Hausbeck, Keinath, Kousik, Schulthies, Smart, Tregeagle, Lorscheider)

12:20pm/11:20/9:20/6:20 Discussion and feedback from industry

12:40pm/11:40/9:40/6:40 – 1:30/12:30/10:30/7:30 Break, lunch, dinner?

Session V – Planning Sessions and Wrap-Up Discussions

1:30pm/12:30/10:30/7:30

(note: other topics are welcome if someone would like to add a session)

A. RenSeq (Andres Salcedo and Camilo Parada, leads)
B. Metabolic catalogs (Amnon Levi, Larry Parnell, leads)
C. Synteny (Yiqun Weng, lead)
D. Integrated disease management and economics (Daniel Tregeagle, lead)

2:15pm/1:15/11:15/8:15 **Wrap up discussions, thoughts moving forward, feedback from external reviewers**

CucCAP Team

Project Director

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Stakeholder Advisory Board		
Organization	**Representative**	**Position**
Commodity Groups - Growers, Shippers, Processors, Marketing		
National Watermelon Promotion Board	Mark Arney	Executive Director
National Watermelon Association	Robert Morrissey	Executive Director
California Melon Research Board	Milas Russell	Former Chair, California Melon Research Board President, Sandstone Melon Company
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	Brian Bursiek	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	Darush Struss	Biotechnology Manager
Enza Zaden	Bart Kay	Research Molecular Biology
Hollar Seed Company	Bruce Carle	Plant Breeder
Johnny's Selected Seeds	Lindsay Wyatt	Squash and pumpkin breeder
Limagrain Vegetable Seeds	Kishor Bhattarai	Research Discovery Manager, HM Clause, Vegetable Seeds Division
Origene America	Eyal Vardi	Founder and CEO
Sakata Seeds	Nihat Guner	Senior watermelon breeder
Syngenta Seeds Inc.	Matt Kinkade	Team Lead, watermelon breeding
Voli Agri Group Inc.	Lakhwinder Randhawa	VP Research and Development

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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)	ARS-SC
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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Rebecca Grumet (Michigan State University)

CucCAP Affiliated Postdocs and Graduate Students
Jingyin Yu – Postdoc at Boyce Thompson Institute (Fei, Wu)
Honghe Sun – Graduate Student at Cornell Plant Biology (Fei, Wu)

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

Work in progress and plans
1.1. Develop genomic and bioinformatic platforms for cucurbit crops.
1.1.1. Development of high-resolution genotyping platforms for cucurbits.
Our main goal here is to construct single-base resolution genome variation maps (variomes) including both SNPs and SVs through genome resequencing of all accessions in the core collections of cucumber, watermelon, melon and squash, and make them publicly available to the community. Currently samples have been grown for the cucumber core collection, and tissues are being collected and DNA extracted.

Under CucCAP1, genome resequencing data for 21 accessions in the *Cucurbita pepo* core collection and for 133 accessions in the cucumber core at an average depth of ~15× were generated and processed.

1.1.2. Development of novel, advanced genome and pan-genome platforms for cucurbit species.
Due to the reduced sequencing cost and contributions of the genome resequencing and de novo genome sequencing by our Chinese collaborators, we have changed our plan from generating one or two additional reference genomes to developing multiple reference genomes (several dozens) for each crop (mainly cucumber and watermelon) using the HiFi sequencing technology.

For cucumber, we have selected 25 accessions including five wild *Cucumis sativus* var. *hardwickii*, four semi-wild Xishuangbanna and 16 cultivated cucumbers for HiFi sequencing. Ten of these 25 accessions are from the core collection. Plants of these 25 accessions are grown at BTI greenhouse. High molecular weight (HMW) DNA has been extracted for six accessions and sent out in early October to Mount Sinai for sequencing. In addition, in collaboration with the Chinese group, we have sequenced and assembled reference genomes for another 11 cucumber accessions using PacBio CLR reads, including seven cultivated, one Xishuangbanna and three wild cucumbers.

For watermelon, in collaboration with the Chinese group, we selected a total of 127 accessions for

reference genome development, including one *Citrullus naudinianus*, one *C. rehmii*, one *C. ecirrhosus*, five *C. colocynthis*, 13 *C. amarus*, five *C. mucosospermus*, eight landrace, and 93 cultivated lines. Eight of these accessions are in the core (five *C. amarus*, one landrace, and two cultivated). Sequencing of two accessions have been completed and leaf tissues of 122 accessions have been sent to the company for DNA extraction, library preparation and HiFi sequencing, while leaf tissues for the remaining three accessions should be be ready in a month. In addition, we have assembled a *C. mucosospermus* (USVL531-MDR) genome using PacBio CLR reads, resulting in 78 contigs with a total size of 365.3 Mb and an N50 contig size of 27.58 Mb; and 99.4% of the contigs were anchored and ordered to the 11 watermelon chromosomes. We have also assembled a Kordofan melon (*C. lanatus* subsp. *cordophanus) genome* using PacBio CLR reads. The assembled genome contained 86 contigs with a total size of 367.9 Mb and an N50 length of 9.34 Mb, and 98.94% of the contigs were clustered into 11 pseudomolecules.

For squash, in collaboration with the Chinese group, three accessions, two from *Cucurbita pepo* ssp. *texana* (also known as ssp. *ovifera*) and one from *Cucurbita pepo* ssp. *pepo*, have been selected for HiFi sequencing. The sequencing and genome assembly of these three accessions are expected to be done later this month (October, 2021). We are also in the process of generating improved reference genomes for *Cucurbita maxima* Rimu and *Cucurbita moschata* Rifu using the HiFi and Hi-C sequencing. Both HiFi and Hi-C sequencing had been done and genome assembling is underway.

1.1.3. De novo genome assembly and pan-genome construction

We have evaluated the efficiency of assembling cucurbit genomes with different depths of HiFi data for cucurbit genomes using watermelon as the example. We generated ~30× HiFi reads with an average length of 18.8 kb and 16.4 kb for watermelon cultivars LvWangTuo and SP5, respectively, and randomly selected different depths of HiFi reads and assembled the reads using HiCanu. We found that ~20× HiFi reads, which correspond to the throughput of a half SMRT cell of the Sequel IIe system, are good enough for a high-quality reference genome assembly. Using ~20× HiFi reads, we obtained assemblies with total sizes of 368.2 Mb and 367.6 Mb and N50 contig sizes of 14.0 Mb and 14.3 Mb for LvWangTuo and SP5, respectively. Quality evaluation using Merqury indicated that the two assemblies have high base accuracy (QV score of 47, corresponding to two errors in 100,000 bases) and completeness (99.7%). Using RagTag and the 97103 genome as the reference, ~99% of the assemblies could be anchored to the 11 chromosomes for both genomes. Based on these analyses, we propose to pool two samples and sequenced then on one SMRT cell of the Sequel IIe system.

We have established genome assembly, quality evaluation, pseudochromosome construction and genome annotation pipelines for the cucurbit species. Multiple high-quality reference genomes will be used to construct graph-based pan-genomes that can be further used to facilitate gene discovery and variant detection.

Genome resequencing data were generated for 29 *C. colocynthis* (30×), 30 *C. mucosospermus (30*×), 115 *C. amarus* (15×), and other 414 watermelon accessions. These data have been processed

and assembled to identify additional novel genes in the pan-genome. The data will also be used to identify novel genes and genome variants (SNPs and SVs) based on the constructed graph-based pan-genomes.

1.1.4. Breeder-friendly web-based database for phenotypic, genotypic and QTL information
During CucCAP1 we developed the Cucurbit Genomics Database (CuGenDB), a critical resource for cucurbit genomics that is widely used by the community. However, the current CuGenDB (v1.0) suffers from one big drawback: it takes too long (weeks to even more than one month) to add a new genome in the database. To accommodate the needs for the increasing numbers of cucurbit genomes developed during the past couple of years and many more in the near future, we are re-implementing CuGenDB (CuGenDB v2.0) with the updated Tripal module (v3.0) that only takes a couple of days to add a new genome. We have collected a total of 43 cucurbit genomes published to date, of which 31 from 25 different species/subspecies are included in CuGenDB v2.0, with nine also included in CuGenDB v1.0. Of the 12 genomes not included in CuGenDB v2.0, five are either of low quality or lack of the annotation files, and seven are old versions (all these seven are included in the current CuGenDB). We expect to officially release CuGenDB v2.0 by the end of 2021.

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 were evaluated for the melon core collection. For the cucumber core collection a combination of 15 external and internal characteristics are being collected for immature and mature fruit of plants grown in 2019 and 2021. Examples of the cucumber phenotypic data are shown in Figure 1. These phenotypic data are currently being used to develop visualization and analysis tools in CuGenDB v2.0. Developing a breeding information management module to integrate phenotypic and genotypic data will be the main focus of the CuGenDB v2.0 development in 2022.

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, five companies will help seed increases of the 399 lines in the core collection. Seeds have been shipped to three companies while seeds for the remaining two companies will be shipped within the next month.

For watermelon, HM.Clause are increasing the seeds for the 384 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. The first 80 accessions are being increased there at this time. HM.Clause are planning to provide us roughly 3-4 selfed seed lots per accession - enough to reach the 1,000 seed/accession target. The S1 and S2 seeds are being increase at the U.S. Vegetable Laboratory and at University of Georgia.

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

For *Cucurbita pepo*, we have selfed seed from 229 accessions that represent ~99% of the genetic diversity of the species. These will be increased starting this winter at Linda Vista in Costa Rica.

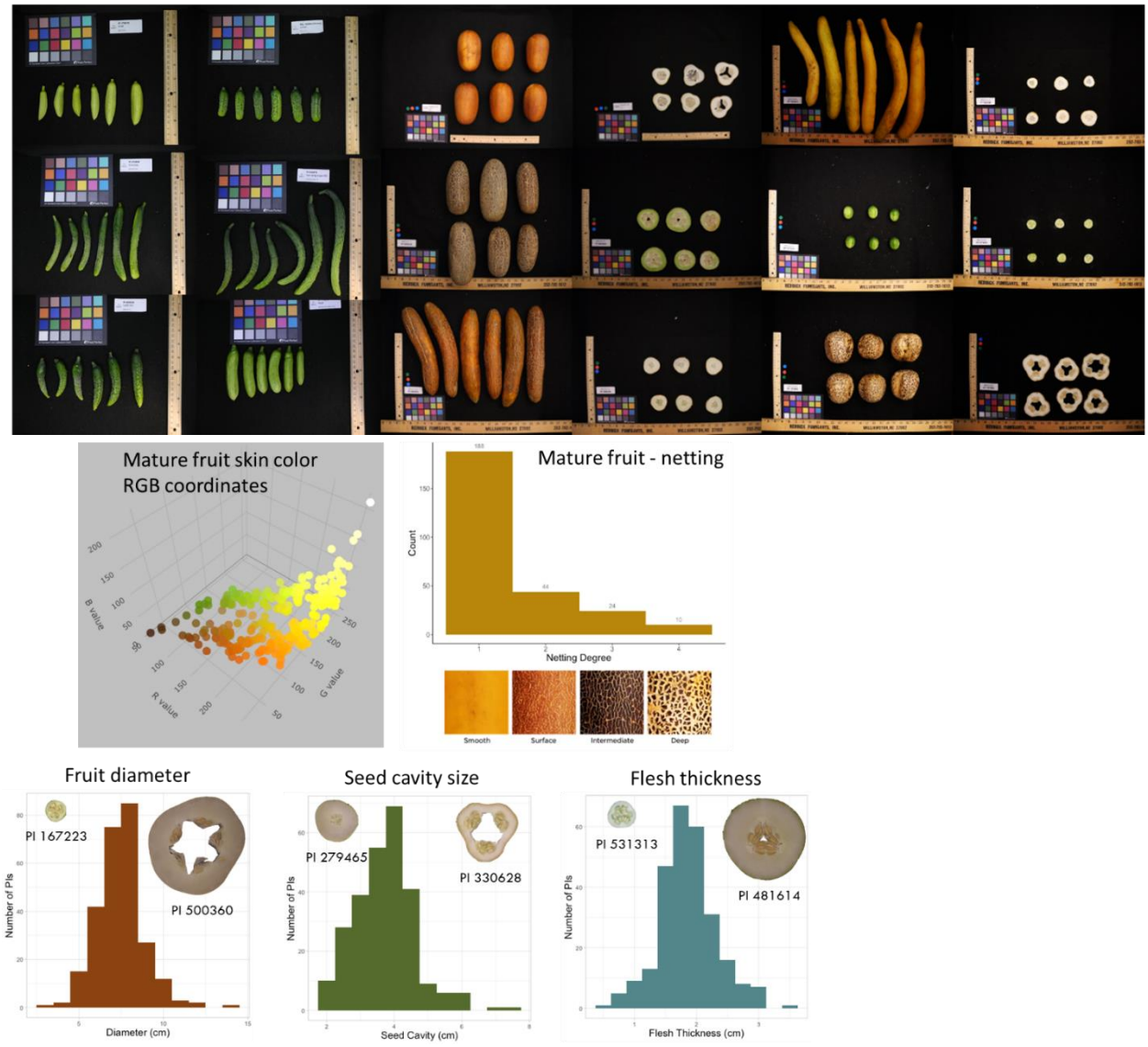

Figure 1.

1.2.2. Population genetics and phenotype-genotype association analysis
Nothing to report for this period.
1.2.2. Population genetics and phenotype-genotype association analysis
Nothing to report for this period.

Watermelon Team

Amnon Levi (AL), Shaker Kousik (SK), William Patrick Wechter (PW), Sandra Branham (SB), Kai-shu Ling (KL), Umesh Reddy (UR) and Cecilia McGregor (CM)

CucCAP Postdocs and Graduate Students
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Bidisha Chanda (BC) -Postdoc at USDA, ARS, U.S. Vegetable Laboratory (Kousik; Ling)
Gabriel Rennberger (GR) -Postdoc at USDA, ARS, U.S. Vegetable Laboratory (Wechter)
Puru Natarajan (PN) -Postdoc at WVSU (Reddy)
Yan Tomason (YT) - Postdoc at WVSU (Reddy)
Venkata Rao Ganaparthi (VG) -Graduate Student at Clemson (Branham)
Samikshya Rijal (SR) -Graduate Student at UGA (McGregor)
Lincoln Adams (LA) - Graduate Student at UGA (McGregor)
Subhash Mahankali (SM) -Graduate Student at WVSU (Reddy)

Obj 1. Develop genomic, bioinformatic, mapping approaches and tools for cucurbits
-Seed multiplication of core populations, AL, PW, SK and CM
We are preparing 384 *Citrullus* spp. accessions to be included in the core collection. Of these 305 are *Citrullus lanatus*, 23 are *C. mucosospermus*, 38 are *C. amarus*, 8 are *C. colocynthis,* and 10 are heirloom cultivars. We shipped to HM.Close 249 seed packs (accessions-PIs) with 50 S2 seeds in each pack. Prior to shipping the seeds to the HM.Close station in Davis, California, they were tested (Wechter's Lab) for presence of Bacterial fruit blotch using an RT-PCR procedure. HM.Close conducted seed health testing in California and the lots were shipped to the HM.Close station in Thailand. The first 80 accessions are being increased there at this time. HM.Close are planning to provide us roughly 3-4 selfed seed lots per accession – enough to reach the 1,000 seed/accession target. The S1 and S2 seeds are being increased at the U.S. Vegetable Laboratory in Charleston and by Cecilia McGregor and Team at University of Georgia, Athens, GA (UGA).

Sandra Branham (SB), William Patrick Wechter (PW), Shaker Kousik (SK), Amnon Levi (AL), and Dennis Katuuramu (DK)
Obj 2. Map and develop markers for disease resistance
2.1: Developing populations (P), phenotyping (Ph), QTL mapping (Q), Fine mapping (F)
-CGMMV, KSL/AL, P
-Fon race 2, AL/PW/SB, Ph
-GSB, PW/AL/SB/AK, Ph
-Downy mildew, DK/PW/AL/SB, Ph

2.2: Develop marker (M), verify (V)
-Fon race 2, AL/PW/SB, M
-PRSV-W, AL/KSL/SB, M
-ZYMV, AL/KSL/SB, M

Marker development and validation for *Fon* race 2 resistance in Watermelon

Fusarium oxysporum f. sp. *niveum (Fon)* is a soil born disease responsible for seedling wilt and death in watermelon. Among the four known pathogenic races, race 2 is considered as the most important soil-born pathogen in US by the National Watermelon Association. Lack of effective fungicides necessitates development and release of resistant cultivars. A *Fon* race 2 resistant inbred line, USVL246-FR2, was developed in CucCAP1 and will be used as the donor parent for resistance breeding. *Fon* race 2 resistance of USVL246-FR2 is polygenic and is controlled by five loci on chromosomes 2, 5, 8, 9 and 10. KASP markers have been developed across all of five QTL and tested in the original *C. amarus* mapping population (USVL246-FR2xUSVL114). An interspecific $F_{2:3}$ population is being generated to verify the effectiveness of the KASP markers and begin the breeding process. Commercial cultivar, 'Sugarbaby' was crossed with USVL246-FR2 to generate the interspecific population. The KASP markers were used to genotype the parents, and four markers were polymorphic for Q9, and 6 each for Q1, Q6 and Q8. The F_2 population will be genotyped with the polymorphic KASP markers. F_3 families from the self-pollinated, genotyped F_2 plants (N=240) will be screened for *Fon* race 2 resistance in replicated growth chamber trials. Genotype and phenotypic data taken together will be instrumental in validating the KASP markers developed, haplotype block or blocks imparting maximum resistance and to understand the influence of Sugarbaby genetic background in expression of resistance. [True breeding F_3 for *Fon* race 2 resistance will be utilized in further selections leading towards cultivar development]

Phenotypic screening for CDM resistance and marker-trait associations testing via GWAS in watermelon

Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is an emerging threat to watermelon production. We screened 122 *C. amarus* accessions for resistance to CDM over two tests. The accessions were genotyped with 2,126,759 single nucleotide polymorphic (SNP) markers. A genome-wide association study approach 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. Broad-sense heritability estimate was 55%, implying the presence of moderate genetic effect for resistance to CDM. The peak SNP markers associated with resistance to *P. cubensis* were located on chromosomes Ca03, Ca05, Ca07, and Ca11. The significant SNP markers accounted for up-to 30% of the phenotypic variation and were associated with candidate genes including disease resistance proteins, leucine-rich repeat receptor-like protein kinase, and WRKY transcription factor. This information will be useful in understanding the genetic architecture of the *P. cubensis-Citrullus* spp. patho-system as well as development of resources for genomics-assisted breeding for resistance to CDM in watermelon.

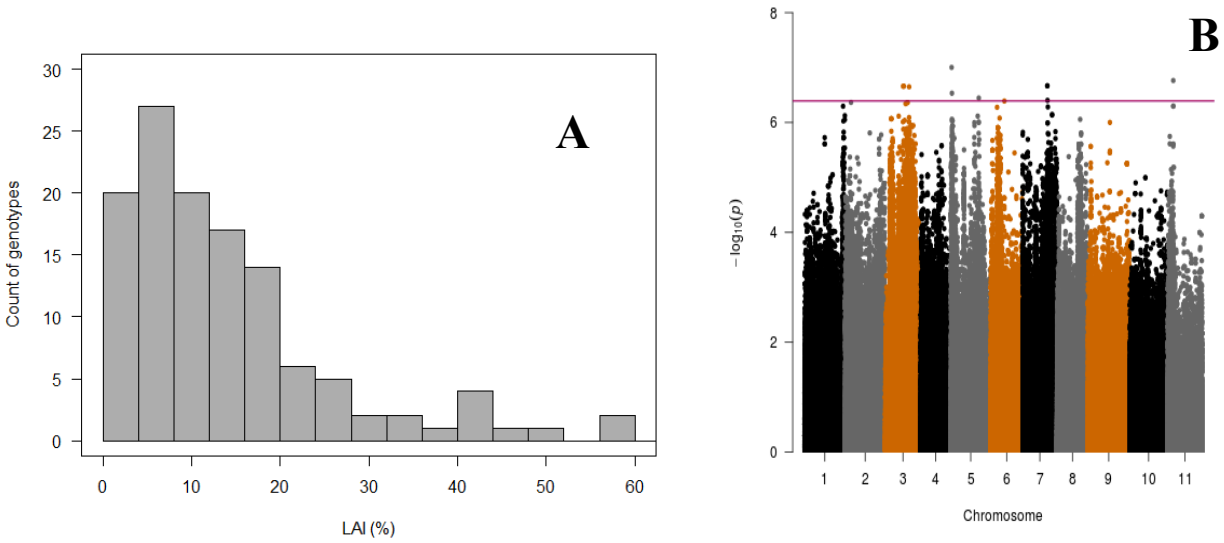

Fig. 1: Distribution of CDM leaf area infection (**A**); Manhattan plot depicting genome-wide marker-trait associations for CDM leaf area infection (**B**) across 122 *Citrullus amarus* genotypes across two combined screening tests.

Marker development and validation for Powdery mildew resistance

Historical data (Tetteh et al. 2010) for the USDA-NPGS *Citrullus* collection's (N=1148) response to inoculation with powdery mildew were used to choose resistant and susceptible bulks (N=50 each) for bulked segregant analysis. Whole-genome resequencing data of the bulks was input for an extreme phenotype-genome wide association study (XP-GWAS). Three QTL (chromosomes 2, 4 and 7) were significantly associated with powdery mildew resistance. The QTL on chromosome 2 was previously associated with resistance in a traditional QTL mapping study (Kim et al. 2015), while the other two QTL are novel. Sixteen KASP markers were designed across these QTL. We are currently testing the KASP markers in 300 of the most and least resistance accessions for validation of the markers.

Marker development and validation for ZYMV resistance

A BC4F2 Citrullus lanatus population (N=183) segregating for resistance to ZYMV was developed from a cross of PI595203 (R) and 'Charleston Gray' (S). The population was evaluated for response to ZYMV and 25 each of the most and least resistant individuals were bulked for QTL-seq analysis. QTL-seq using whole-genome resequencing data identified a single significant region on chromosome 3 that had been identified in previous studies. KASP markers (N=22) were developed across the QTL with 13 successful amplification in the population. The peak SNP was a non-synonymous SNP in the eIF4E gene. An independent population (BC6F2; PI 595203 x Charleston Gray-recurrent parent) have been developed for verification.

Marker development and validation for PRSV-W resistance

An F2:3 *C. amarus* population segregating for resistance to PRSV-W and Fon race 2 resistance was derived from the cross of USVL252-FR2 by USVL019. The population was genotyped with GBS and phenotyped for both diseases. A single QTL (chromosome 3) was associated with resistance to PRSV-W and KASP markers were developed across the region. Twenty-two KASP amplified in the population. An independent F2 population (PI 244019-PRSV-R x PI 596665) has been developed (by Dennis Katuuramu) for verification of the markers.

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

Develop breeding/germplasm lines (B), introgress resistance into cultivars (I), Develop and validate advanced lines (A), release lines to breeders (R)

-Fon races 1 and 2, AL/PW/SB, I

-PRSV-W, AL/KSL/SB, B

-ZYMV, AL/KSL/SB, B

-Pyramiding Fon1, Fon2, PM, PRSV, ZYMV, into advanced breeding lines are ongoing projects at the USDA, ARS, USVL (AL/PW/SK/KL/SB)

-RILs and MAGIC populations derived from crossing disease resistant *C. amarus* PIs and watermelon cultivars with desirable fruit quality are being developed and will be used to identify and select advanced lines having resistance and sufficient fruit quality. The MAGIC-RILs should be a useful germplasm resource with diverse allelic combinations to be exploited by the cucurbit/watermelon community for mapping quantitative trait loci (QTLs) and for watermelon varietal development.

Cecilia McGregor and Team at University of Georgia, Athens, GA (UGA)

2.1 Map resistances and identify QTL for key cucurbit diseases

The WPop GSB1 (PI 482276 x Crimson Sweet) F2:3 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 *Stagonosporopsi*s isolates.

2.2. Develop and verify markers for MAS

KASP marker assays were developed for Qgsb5.1 (syn. ClGSB5.1; Gimode et al., 2020), Qgsb5.2 (Adams and McGregor, unpublished), and Qgsb7.1 (syn. ClGSB7.1; Gimode et al., 2020) identified in our lab. In addition, KASP marker assays were developed and/or tested for Qgsb8.1 (Ren et al., 2020) and Qgsb8.2 (syn. qLL8.1 and qSB8.1; Lee et al. (2021) (Fig. 1).

F2:3 or F2:4 plants were selected from WPop GSB1 (PI 482276 x Crimson Sweet) population based on the presence of resistant alleles for Qgsb5.1 and Qgsb7.1. These lines were backcrossed to Crimson Sweet.

F2:3 plants were selected from WPop GSB2 (PI 189225 x Sugar Baby) population based on the presence of resistant alleles for Qgsb5.2 and Qgsb8.1 or Qgsb8.2. The lines were backcrossed to Sugar Baby.

Umesh Reddy, Todd Wehner, Padma Nimmakayala, Puru Natarajan, Yan Tomason, and Subhash Mahankali (WVSU)

Using historical data (Gusmini 2005; Todd Wehner) to identify QTL associated with resistance to gummy stem blight (GSB)

We have sequenced resistant and susceptible bulks (bulks of 30 individual RIL progeny) of the gummy stem blight phenotyped individuals from the MAGIC population. This sequencing produced 1346984333 (RB), 1693263106(SB), 1344506187 (RB) and 1681510931 (SB) mapped reads for resistant bulk (RB) and susceptible bulk (SB) upon mapping with USVL-246 and Charleston Grey (CG) reference genome sequences. These reads produced 6001242 (RB with USVL), 6119469 (SB with USVL), 1821568 (RB with CG) and 401496 (SB with CG) polymorphic SNPs between the bulks. QTL-seq was performed separately with USVL-246 and CG genomes (Fig 1, 2).

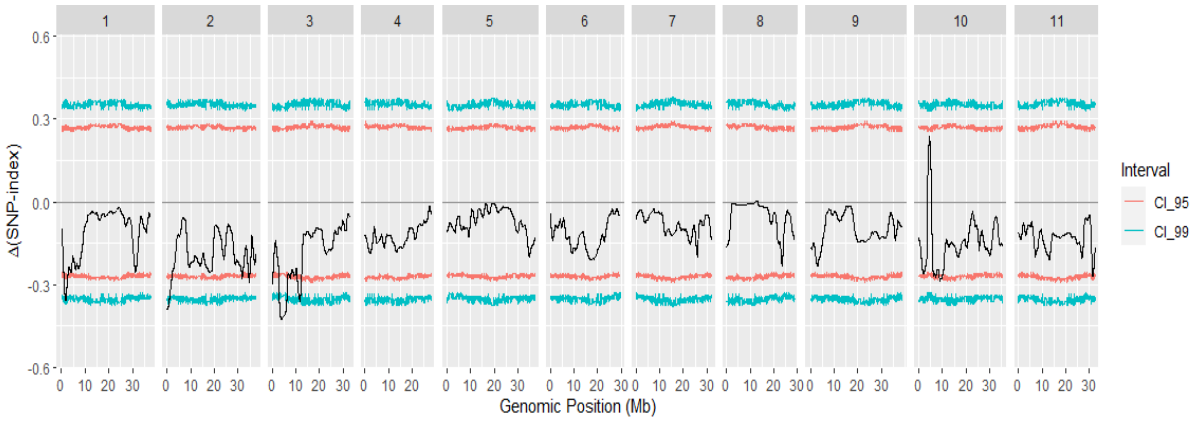

Fig 1: QTL-seq analysis using USVL-246 genome as reference

QTL-seq and G' analysis detected 16 homozygous loci in susceptible bulk and a mix of R and S loci in resistant bulk when counted respective BAM reads.

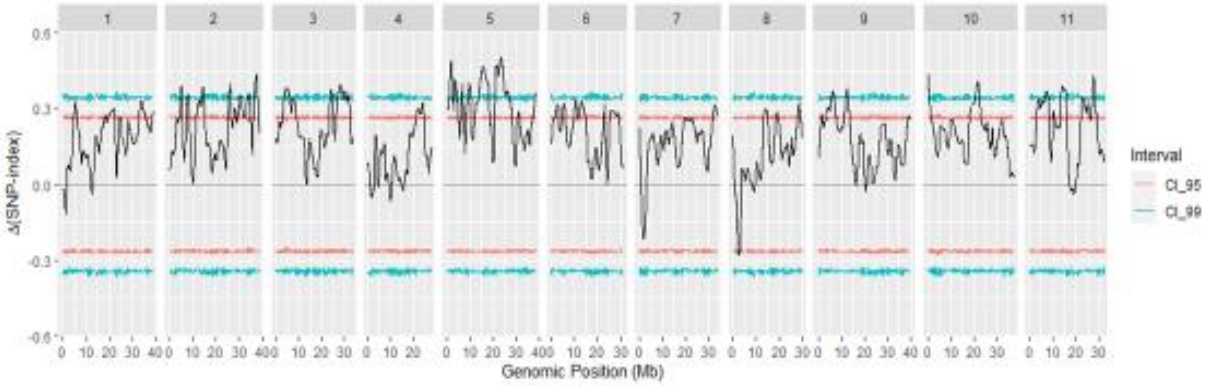

Fig 2: QTL-seq analysis using CG as reference

QTL peaks were detected for chromosomes 1, 2, 3, 4, 5, 7, 8, 10 and 11 chromosomes. Known resistant genes like NBS LRR motif containing, lipoxygenase, several kinases and other

important transcription factors were noted in the Delta peaks. A GWAS was conducted to detect robust associations with GSB resistance among 1345 collections using the publicly available GBS generated SNPs.

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

Powdery mildew of watermelon.

Powdery mildew (PM) of watermelon (*Citrullus lanatus*) and other cucurbits 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, and commercial watermelon cultivars with resistance are rare. 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.

Identifying and developing multiple disease resistant lines from accessions

Majority of the watermelon plant introductions (PI) considered as resistant or tolerant display varying levels of disease resistance. Hence it is important to screen and select for several generations to develop highly resistant lines from these PI. We have developed 36 lines with high levels of resistance to powdery mildew from various PI. Of these 13 are also resistant to Phytophthora fruit rot and can be considered as multiple disease resistant (MDR). These lines were evaluated for resistance to powdery mildew and Phytophthora fruit rot in the field in 2021 and displayed high levels of resistance compared to susceptible lines including Mickey Lee and USVL677-PMS. These lines will serve as useful sources of resistance for future studies.

Development of KASP markers for powdery mildew resistance in watermelon.

During the previous project we had identified as *ClaPMR2, Citrullus lanatus PM Resistance gene 2* {Chr2 : 26750001 .. 26753327 (-)}, a NBS-LRR resistance protein (R) with homology to the *Arabidopsis thaliana* PM resistance protein, RPW8 and developed CAPS markers that were validated using the parents, four PM resistant RILs and susceptible and resistant F2 populations using conventional DNA gel electrophoresis. Based on SNPs in Chr2we developed and evaluated eight KAPS markers on a limited set of DNA samples from 40 F_2 plants of the cross between USVL531-MDR X USVL677-PMR. Based on this evaluation we narrowed down our choices to four KASP markers and evaluated DNA from 179 F_2 plants. Significant correlation ($P \leq 0.0001$) between observed phenotype (PM rating) and genotype was observed. This was in relation to powdery mildew ratings recorded on hypocotyl (r=0.72), cotyledons (r=0.84) and true leaves (r=0.81). One of the KASP markers identified the correct resistant or susceptible Phenotype with 97.2% accuracy.

Advancing Powdery mildew resistant inbred lines.

Fruit from F_2 plants from a cross of USVL531-MDR and USVL677-PMS with powdery mildew resistance, uniform red flesh and decent brix (>8) were collected and have been advanced till $F_{8.}$ These lines were homozygous for resistance to PM. They will be further evaluated in the field in 2022 for PM resistance and fruit quality traits.

Phytophthora fruit rot of watermelon

Phytophthora fruit rot of watermelon has been a major problem in watermelon growing areas in the Southeastern U.S. (FL, GA, SC, NC and VA). In recent years it has also become a problem in watermelon growing areas in Maryland (MD), Delaware (DE) and Indiana (IN). The National Watermelon Association considered Phytophthora fruit rot as it's top research priority in 2017 as well.

Broad resistance to post-harvest fruit rot in USVL watermelon germplasm lines.

At the U.S. Vegetable Laboratory (USDA, ARS) in Charleston we have developed several germplasm lines with high levels of resistance to Phytophthora fruit rot. These germplasm lines were developed by phenotyping using a local isolate(RCZ-11) of *P. capsici* from South Carolina. The present study was undertaken to determine if these resistant lines had broad resistance to diverse *P. capsici* isolates collected from different states and crops. Five resistant germplasm lines (USVL020-PFR, USVL203-PFR, USVL782-PFR, USVL489-PFR and USVL531-MDR) and two susceptible cultivars Sugar Baby and Mickey Lee used as checks were grown in a field in 2014 and 2015 to produce fruit for evaluation. Mature fruit were harvested and placed in a walk-in growth chamber and inoculated with 20 different *P. capsici* isolates. The chamber was maintained at 26±2°C and high relative humidity (>95%) using a humidifier. 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*. A publication describing this research was recently published in Plant Disease.

QTLseq analysis of USVL003-MDR X Dixie Lee

We are completed phenotyping the populations from USVL003-MDR x USVL677-PMS for resistance to Phytophthora fruit rot and DNA was extracted from the most susceptible and resistant F2 plants and bulked. Bulked DNA was sequenced by Novogene. QTLseq identified a major QTL in Chr4 significantly associated with resistance. Several other QTL's significantly associated with resistance were also identified. We are currently developing KASP Markers based on SNP's in these regions to conduct marker analysis. In addition a recombinant inbred line developed based on the cross of USVL531-MDR X USVL677-PMS has also been completed and seeds have been increased. These will be evaluated in 2022.

Ling and Levi:

Characterizing watermelon for CGMMV resistance: Previously in CucuCAP1, we had identified a watermelon germplasm (*Citrullus colocynthis*, PI 537300) with resistance to CGMMV and another line (PI 195927) with susceptibility. Using these two parental lines, we generated a F2 population of 600 seeds. In 2022, we will conduct phenotyping analysis through bioassay, symptom observation and confirmation through virus testing to evaluate the properties

of genetic resistance in watermelon to CGMMV. In addition, in 2020, we evaluated a list of over 20 chemicals and identified several disinfectants that are effective against the mechanical transmission of CGMMV in watermelon.

Levi, Kousik, Wechter, McGregor, Branham and Dennis Katuuramu

Progress in developing a multi-parent advanced generation inter-cross (MAGIC) population in collaboration with seed companies

[USVL 246 x Sugar Baby]-4♂ x [NH Midget x Calhoun Gray]-2♀	X	[Crimson Sweet x PI 244019]-4♂ x [PI 392291 x Mickylee]-2♀
[Hungarian x USVL252]-3♂ x [Jenny x PI 595203]-3♀	X	[USVL 531 x PI 269677]-3♂ x [PI 189225 x PI 279461]-2♀
[PI 189225 x PI 279461]-6♂ x [Jenny x PI 595203]-6♀	X	[USVL 531 x PI 269677]-1♂ x [USVL 246 x Sugar Baby]-2♀
[NH Midget x Calhoun Gray]-1♂ x [Hungarian x USVL252]-3♀	X	[Crimson Sweet x PI 244019]-6♂ x [PI 392291 x Mickylee]-5♀

We have completed the inter-crosses in the table above and are proceeding to the final inter-crosses generation prior to self-pollinating and generation of F2 through F8/F9 RIL generations.

Wehner, Reddy, McGregor, Levi

Advancing a MAGIC-RIL population described in the Table below and derived from crossing resistant *C. amarus* lines with watermelon cultivars was developed by Todd Wehner and team at NCSU.

Generation	Breeding approach	Description
I_0	PI 482342 × PI 482283 PI 482342 × PI 189225 PI 189225 × PI 482342 PI 482374 × PI 189225 PI 526233 × PI 482283 PI 526233 × PI 189225	Crosses of the most resistant plant introductions
I_1 I_2 I_3 I_4	Four cycles of intercrossing	Intercrossing without selection
I_4F_1	Charleston Gray Calhoun Gray Mickylee Minilee Allsweet Crimson Sweet Petite Sweet	Crossing with susceptible elite lines of excellent fruit quality
$I_4F_1I_1$ $I_4F_1I_2$ $I_4F_1I_3$ $I_4F_1I_4$	Four cycles of intercrossing	Intercrossing without selection, while maintaining wild and elite types in the population
$I_4F_1I_4S_1$ $I_4F_1I_4S_2$ $I_4F_1I_4S_3$ $I_4F_1I_4S_4$ $I_4F_1I_4S_5$ $I_4F_1I_4S_6$ $I_4F_1I_4S_7$	Seven cycles of self-pollination	Self-pollination of plants at random to develop RILs

We have a few seeds of the F8 RILs and we currently advancing them to F9 generation.

Melon Team

Team members:
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Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances
2.1.2. Melon
- 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).
One or both of the two markers flanking the gene on Chromosome 5 were present in six of 10 other putative CYSDV resistance sources. Eight F2:3 lines with low virus titer resembled PI 313970 for the two flanking markers, which can, therefore, be utilized in marker assisted breeding of CYSDV-resistant melons.

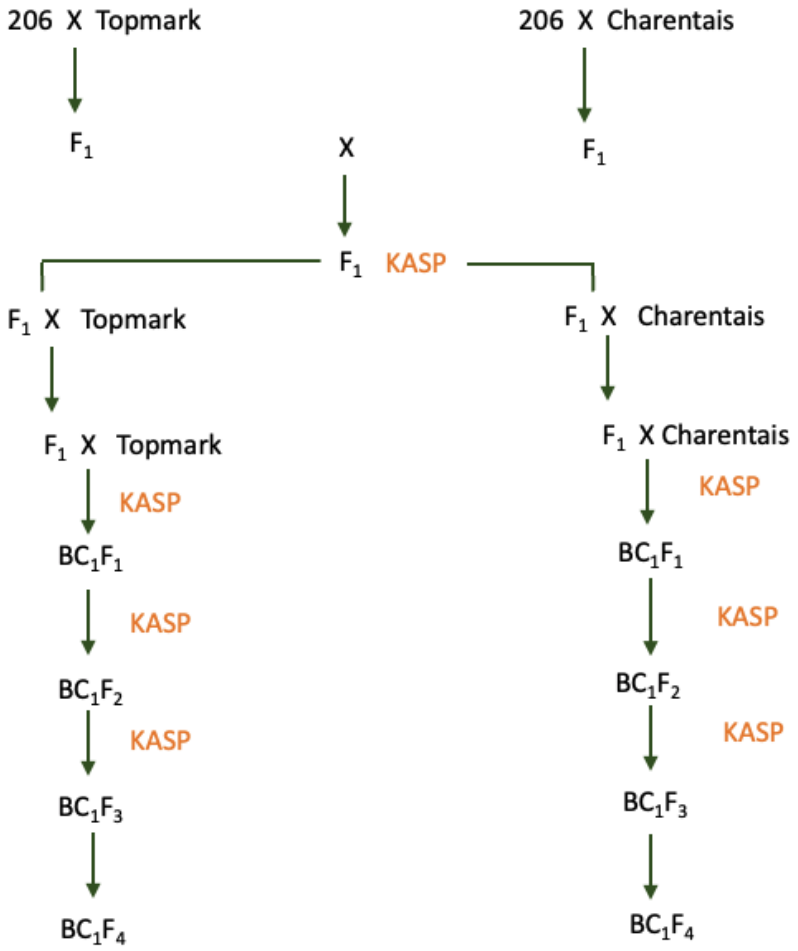
One of the eight F2:3 lines with low virus titer in 2019 has been evaluated to date for resistance reaction in a controlled inoculation, growth chamber test. Plants will be selfed and backcrossed with CYSDV-susceptible 'Top Mark'. The other seven F2:3 families are in preparation for screening. Testing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

2.2 Marker development and verification
2.2.2. Melon
- powdery mildew and Fusarium: Melon production is threatened by Fusarium wilt, powdery mildew, and downy mildew. Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *melonis* (Fom) and four races have been identified so far in North America. Resistance to races 1 and 2 were identified and well characterized. Powdery mildew (PM) and downy mildew (DM) are caused by biotropic pathogens *Podosphaera xanthii* and *Pseudoperonospora cubensis*, respectively. Resistances to PM and DM were identified and characterized. PM resistance identified is oligogenic in nature with a single QTL responsible for the majority of resistance.

DM resistance is complex, and all the significant loci together explained lesser than 50 % of resistance. Sulfur, effective against PM, is also effective against DM, and QTL imparting sulfur tolerance were identified. Sulfur application is a feasible strategy to control DM on sulfur-tolerant melons. Thus, sulfur tolerance is an important trait for breeding.

Recombinant inbred line (RIL) 206 is resistant to Fom races 1 and 2, PM and is sulfur-tolerant; its flesh is light-orange in color with low sugar content. Orange flesh, western shippers like 'Top Mark' are commonly grown in North America; their flesh is orange colored and fruit is heavily netted. 'Charentais' is a French melon with deep orange or salmon color flesh and little to no netting on the fruit surface.

With the objective to develop disease-resistant and sulfur-tolerant commercial melons (orange flesh, western shipper and Charentais), the following crossing scheme was designed to produce four-way [F_1(206 x TM) x F_1(206 x Charentais)] progenies for genetic analyses.



Four hundred, four-way progenies (see above) and their parents were planted, and total genomic DNA was extracted from 2-week-old seedling leaves following the modified Triticarte Pty.Ltd protocol (http://www.triticarte.com.au/). Quality and concentration of extracted DNA was

checked using nano drop. DNA concentration was later adjusted to 10-20 ng/ul. Markers flanking and markers within the QTL region of targeted resistances were utilized for genotyping. Three markers for Fom race 1, five markers for Fom race 2, and three markers for PM race 1 were used as proxies for the respective resistance QTL. PCR reactions (5 µl volume) consisted of 0.07 µl of primer mix (IDT technologies; fluorophore-labeled, allele-specific forward primers and a reverse primer), 2.5 µl of 2× master mix (IDT technologies) and 10-20 ng of sample DNA. A standard thermal cycler was used for a touchdown PCR reaction with a 94°C hot-start activation step for 15 min, then 10 cycles of 94°C (20 s) and a starting annealing temperature of 61°C that dropped by 0.6°C each cycle. Twenty-six additional cycles of 94°C for 20 s and 55°C for 60 s followed the touchdown steps. Fluorescence was quantified with a Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA) quantitative PCR system at 25°C. Fluorescence values were used to cluster samples into genotypes with MxPro v4.10 software associated with the qPCR machine. Selected genotypes were transplanted into 12-inch pots and moved to a greenhouse for crossing.

Two plants homozygous for all the targeted resistance loci were selected, one was crossed with 'Charentais' and other with 'Top Mark'. Five to 10 plants from each cross will be backcrossed with their respective recurrent parent. KASP markers will be used to select desired BC_1F_1 population and BC_1F_2 population. Disease screening and sulfur tolerance testing will be done on BC_1F_3 and BC_1F_4 based on availability of seeds. Horticultural trait assessment will be done on BC_1F_5 families.

Fom race 2 resistance

The *Fom-1* gene identified and cloned in 'Védrantais' imparts resistance against Fom races 1 and 2. Resistance from this source was not quantified. MR-1 is a treasure trove of disease resistance QTL in melon; it showed strong resistance against Fom race 2. Initial mapping with high density SNP map showed one strong peak explaining about 35% of phenotypic variation with Spanish genome as reference. Further, increasing marker density around the identified peak with same reference genome showed another peak, stronger than the first identified QTL and explained about 40% variation; on whole, phenotypic variation explained was 85% as opposed to 40% in the initial mapping experiment. These two peaks seem to be linked very strongly, and physical length of this whole region is 300 kb. *Fom-1* was located inside of the peak explaining 30-35 % of phenotypic variation. Oumouloud et al., (2010) in their inheritance study mentioned two independent genes controlling resistance, i.e., one dominant and one recessive in 'Tortuga'.

To better understand the genetic nature of resistance in MR-1, a large segregating four-way F_1 population developed with RIL-206, 'Top Mark' and 'Charentais' (see above) was phenotyped, and 170 selected individuals were genotyped with KASP markers on both side of the two peaks. Two genotypes showing evidence of crossover were selected and are being grown in a greenhouse. Further cohort phenotyping of F_2 families from selected plants, F_1 progenies with both resistant and susceptible parents along with parents will reveal the genetic nature of *Fom* race 2 resistance in MR-1.

- CYSDV: see 2.1.2 above

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

3.2. Melon

- powdery mildew: see 2.1.2 above
- Fusarium: see 2.1.2 above
- CYSDV: see 2.1.2 above

Literature Cited

McCreight, J.D. and W.M. Wintermantel. 2011. Genetic resistance in melon PI 313970 to *Cucurbit yellow stunting disorder virus*. HortScience 46:1582–1587.

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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
From previous work, a cucumber core was developed which is composed of 399 accessions from diverse taxonomic groups, geographic origins, and market groups. A dozen important US historical varieties were also included. Most lines have undergone at least two generations of self pollination and are morphically uniform. Seeds of the 399 lines have been or are being distributed to five industry collaborators for seed increase by self pollination. It is expected that least 2,000 seeds will be returned in two years.

Obj. 2. Map and develop markers for disease resistance

2.1 QTL mapping of resistances

2.1.1 Downy mildew (YW & AK)

In 2021, two $F_{2:3}$ populations, WI7747 and WI7769, were developed for QTL mapping of DM resistance in two cucumber inbred lines, WI7631 (Chinese Long type), and WI7773 (an introgression line with DM resistance derived presumably from *C. hystrix*), respectively. Ninety-six F_3 families of the two populations (two replications, eight plants per rep) were grown in open fields at Clemson, SC to exam inoculation responses to natural infection of the DM pathogen. Phenotypic data for general impression of DM symptoms were recorded at three time points. Data for yellowing, necrosis and sporulation were also collected from each plant at one time point. The frequency distribution of mean disease scores for the four parameter is shown in **Figure 1**.

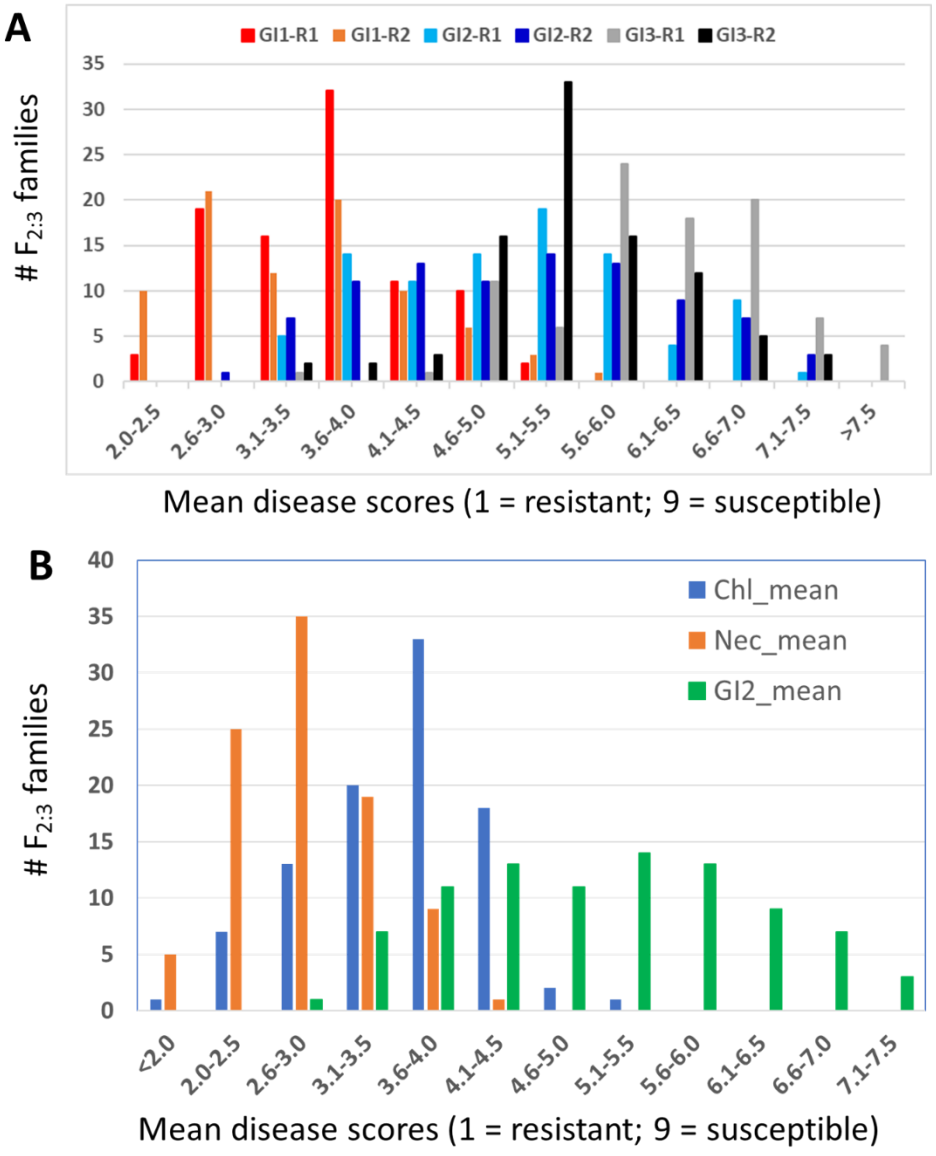

Figure 1. Bar graphs for downy mildew disease scores among 96 families of the WI7769 $F_{2:3}$ population. A. Disease scores for GI (general impression) from three time points in two replications. B. Distribution of mean disease scores for chlorosis (Chl), necrosis (Nec) and general impression (GI2).

Recombinant plants used for fine mapping of the *dm4.1* (from WI7120), and *dm5.3* (from PI 197088) major-effect QTL for DM resistance were also tested for DM inoculation responses. In 2022, these populations will continue to be tested in both field and growth chamber trials.

2.1.2 Phytophthora fruit rot (RG)

1) QTL mapping in PI 109483. QTL analysis on PFR resistance was conducted using segregating population from the cross between Gy14 and A4-3 (from PI 109483-DH). QTL-Seq identified two peaks on Ch5 and Chr6. Subsequent testing of F_2 progeny homozygous for either the Gy14 or PI 109843 allele verified a role for *qPFR5.1*, which spanned a 4.5 Mb region overlapping with two DM resistance loci, *dm5.2* and *dm5.3*. A4-3 also has been crossed with Poinsett 76 to verify the allele effect in a second genetic background. These plants are currently being grown in the greenhouse.

2) GWAS for PFR resistance. Of 395 lines in the cucumber core population, we planted 267, 20, and 379 lines in the field in 2019, 2020, and 2021 field season, respectively. Young fruit (20-40 per line from multiple harvests per season) were harvested for PFR testing at 5-7 days post-pollination. At least two seasons of data are available for the majority (70%) of lines. There was good correlation for disease scores (r=0.775) between lines tested in 2019 and 2020, indicating reliability of phenotyping (Figure 2). Data have not been processed yet for fruit tested in 2021.

Year	Number of lines planted	Number of lines tested for *P. capsici*[2]
2019	271	259
2020	30	26
2021 (full core)	379[1]	354
Tested two years		249 (70.3%)

[1] Seed was not available and/or did not germinate for 16 lines
[2] Lines with ≥10 fruit tested; for the great majority, 20-40 fruits were tested/line.

Figure 2. Correlation in phenotype scores between PI lines tested in 2019 and 2020

2.1.3 CGMMV (KL and YW)

We are screening 50 cucumber inbred lines for CGMMV resistance in a greenhouse in USDA-ARS Charleston, SC using a bioassay through mechanical inoculation. Symptom observation and testing for virus concentration will be conducted using a serological test (ELISA). In 2022, we will continue screening efforts on cucumber germplasm, first using the 400 core collections of cucumber and then with USDA cucumber germplasm collection.

2.2 Marker development and verification

2.2.1 Downy mildew (YW & AK)

For QTL mapping of DM resistances in WI7773 and WI7631, leaf samples from 96 F_2 plants in each of the WI7747 and WI7769 population were collected. DNA extraction is underway. In 2022, we plan to do genotyping-by-sequencing of the two populations for QTL analysis.

Our second objective is 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*). Phenotyping and genotyping of recombinants among NIL-derived F_2 and BC plants revealed four sub-QTL at the *dm4.1* locus in both WI7120 and PI 197088. Recombinants defining each sub-QTL are being identified. For *dm5.3*, extensive genotyping and phenotyping were conducted among segregating populations, which allowed to delimit the *dm5.3* locus into ~650 kb region on chromosome 5. In 2022, we will further narrow down the candidate gene regions for *dm4.1.1,* and *dm4.1.2B*, and *dm5.3*.

2.2.2 Phytophthora fruit rot (RG)

1) To refine map location of *qPFR5.1*, F_2 progeny (n=768) were screened with KASP markers flanking the QTL to identify recombinant individuals within the *qPFR5*.1 region, which were then self-pollinated to produce F_4 lines. Nine recombinant lines were planted in the field and 30-100 fruit were phenotyped for each family. KASP SNP genotyping narrowed the *pPFR5.1* QTL to a 1-2Mb region that falls between *dm5.2* and *dm5.5* (Figure 3)

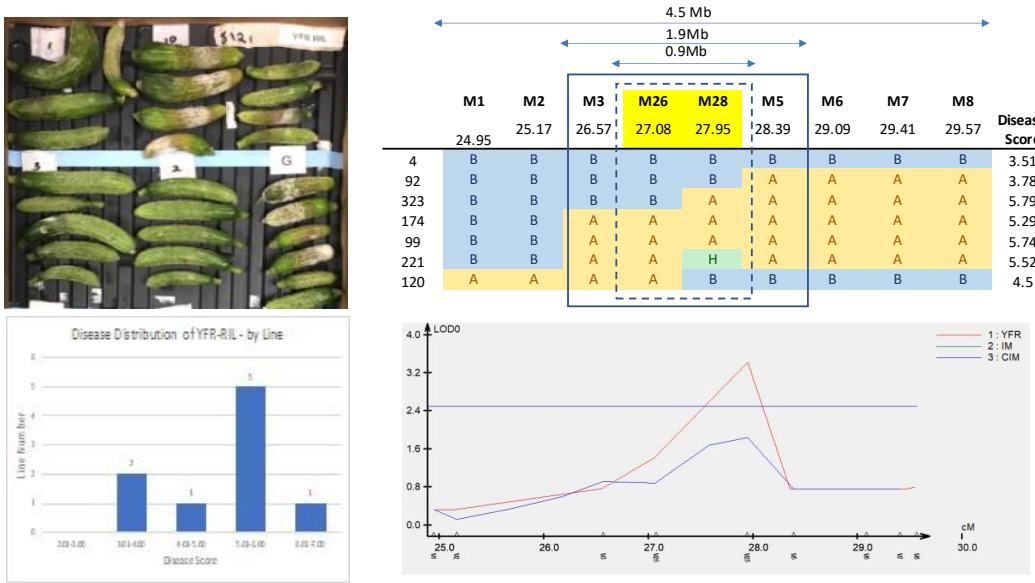

Figure 3. Testing F_4 lines recombinant in the QTL PFR5.1 region for young fruit resistance

2) Age-related PFR resistance. QTL-seq analysis among F_2 progeny and DH lines derived from Gy14 (ARR-) X Poinsett 76 (ARR+) identified a single strong QTL for ARR on chromosome 3. KASP markers flanking the QTL were used to genotype 768 F_2 seedlings. Selected recombinant and non-recombinant individuals were self-pollinated to produce F_4 lines. Phenotyping was performed in a replicated trial of 22 recombinant and 14 non-recombinant lines (5 plants/line; RCBD) in the greenhouses. The non-recombinant lines verified the effect of the QTL. Genotyping by KASP markers is being used to refine the QTL (Figure 4).

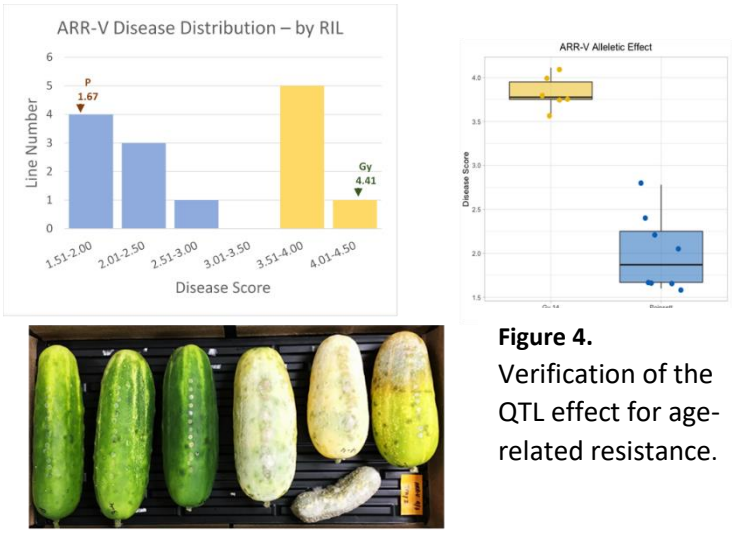

Figure 4. Verification of the QTL effect for age-related resistance.

Obj. 3. QTL introgression into breeding or advanced lines, and release to breeders

*3..1 Downy mildew (*YW & AK)

During fine mapping of *dm4.1* and *dm5.3*, plants carrying different combinations of *dm4.1, dm5.2* and *dm5.3* resistance alleles were identified and backcrossed with Gy14 aiming to develop Gy14 carrying all permutations of the three QTL. We have developed homozygous *dm4.1+dm5.2* QTL in Gy14 backgrounds (Gy14Q2). In 2021 summer field season, the Gy14Q2 inbred line was provided to the extension team for evaluation of its DM resistance and horticultural performance. In 2022, we plan to advance these plants to BC_2F_2 to identify those that are homozygous at all three loci.

3.2 QTL pyramiding of DM and PFR resistances (YW, RG and AK)

We have developed cucumber plants that carry DM resistance QTL *dm4.1, dm5.2* (both from WI7120), and *dm5.3* from PI 197088. Marker-assisted selection was practiced using four markers at the three loci (two for *dm5.3*) to select plants that were homologous at all three loci (Gy14Q3). A plant carrying homozygous *qPFR5.1* QTL for PFR resistance 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 5 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 will continue to do marker-assisted selection with more plants to identify desired allele combinations. The selected BC_1 plants will be backcrossed again with Gy14 to advance to BC_2.

Squash Team

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Objective 2: Map and develop markers for disease resistance

2.1.4 Squash: QTL mapping of resistance to *Phytophthora capsici* in *C. pepo* (GM-UF)

1. Methodology

A. **Population development and genotyping**

Resistant breeding line #181761-36P (resistance derived from USDA accession PI 181761) was crossed with a susceptible Acorn-type cultivar, Table Queen to generate F1 seed. A single F1 seed was selfed to generate F2 individuals which were individually selfed to generate F2:3 (n =83) families. DNA from the parents, the F1 and each of the F2 plants was extracted from the leaf tissue using a commercial kit (E.Z.N.A, Omega Biotek). The concentration and quality of the DNA was determined by absorbance measurements (NanoDrop 8000; Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel (0.8% w/v) electrophoresis. Six hundred and five publicly available SNP markers were selected for genotyping. These SNPs were within genic regions and evenly distributed across the genome. Among these, 83 SNP markers were unsuitable for probe design, thus only 523 markers were genotyped in the parents, F1 and F2 individuals using the targeted genotyping by sequencing platform. Briefly, the *C. pepo* reference genome was used to develop a library of oligo probes (average 60 bp) flanking each SNP of interest. Sequencing libraries (1 × 75 bp) were prepared and run on a NextSeq 500 Illumina Next Generation Sequencing platform. Sequence reads were mapped onto the *C. pepo* reference genome and SNP calling was performed using standard bioinformatic tools.

B. Phenotyping

Inoculum was prepared from a virulent isolate of *P. capsici* according to the method described by Krasnow et al. (2017). Briefly, 5-mm cornmeal mycelial agar plugs of *P. capsici* were transferred to 14% V8 agar plates (140 mL V8 juice, 3 g CaCO3, 16 g agar per liter) and cultured under constant fluorescent light at 28 °C. On the 7th day, the plates were flooded with cold sterile distilled water (4 °C), and chilled at 4 °C for 30 min prior to incubation at 21°C for 60 min to allow release of zoospores synchronously. Zoospores in the inoculum suspension were quantified with a hemocytometer and adjusted to 2.0 × 104 zoospores/mL. Twelve seeds, each of

the F2:3 families (n = 83); 40 seeds of each parent and 10 seeds each of the F1 individuals, were sown in the greenhouse in 4-inch diameter pots filled with sterilized Proline C/B growing mix (Jolly Gardener, Quakertown, PA, USA) amended with a slow-release fertilizer (14N-4.2P-11.6K). Twelve seeds of the resistant *C. moschata* breeding line #394-1-27-12 were also included in each experiment as checks. The experiment was arranged in an incomplete block design with 10 seeds of both parents included as controls in each block. At the third true-leaf stage, a hand spray bottle adjusted to release 0.5 mL volume per spray was used to deliver 1.5 mL of zoospore suspension at the crown of each plant. Visual recording of disease severity was done every three days from six days post inoculation (dpi) to 28 dpi using a scale of 0 to 5 whereby a rating of 0 was assigned to plants with no symptoms, 1 for plants with a small brown lesion at the base of the stem, 2 for plants with a lesion progressed up to the cotyledons causing constriction at the base, 3 for partially collapsed plants with apparent wilting of leaves, 4 for completely collapsed plants exhibiting severe wilting, and 5 for dead plants. Plants having a score of 1 or less at 28 dpi were classified as resistant, whereas those having a score ≥2 were classified as susceptible. Area Under Disease Progress Curve (AUDPC) values for the F2:3 families were determined using the trapezoidal integration method and used for QTL mapping. The experiment was carried out thrice.

C. Linkage mapping and QTL analysis

A genetic linkage map was constructed with Onemap package in R software with SNP markers polymorphic between the parents. SNP markers with significant segregation distortion from the expected Mendelian segregation (1:2:1) as determined through χ 2 test were excluded. Linkage groups were constructed using the Kosambi mapping function by exploiting recombination fractions. This was done by choosing three initial markers using rapid chain delineation and sequentially adding markers that map with a significant LOD threshold of three. Alternative marker orders were considered with the same LOD threshold before assembling the final linkage map QTL mapping was performed by Haley–Knott linear regression of AUDPC values against genotype probabilities calculated from the linkage map as implemented in the R/qtl2 package. QTL analysis was conducted independently for each experiment, while joint analysis was conducted using the mean data across experiments. Likelihood-odds (LOD) thresholds set by 1000 permutations ($\alpha = 0.05$) were used to determine the statistical significance of a QTL. Additive and dominance effects, as well as the proportion of total phenotypic variance explained by the QTLs were also estimated. The QTL were visualized using MapChart software.

2. Results

A. Phenotypic analysis

Breeding #181761-36P plants exhibited resistance to Phytophthora crown rot (mean DS = 0.55), whereas the susceptible parent (Table Queen) rapidly succumbed to the pathogen (mean DS = 5) (Figure 1). The resistant check breeding line #394-1-27-12 (*C. moschata*) remained asymptomatic throughout the experiment (mean DS = 0) (Figure 1).

Figure 1 Resistance to Phytophthora crown rot in breeding line (a) #181761-36P and (b) #394-1-27-12 and susceptibility in (c) Table Queen Acorn-type cultivar.

AUDPC values for the F2:3 families across the three experiments ranged from 21.18 to 40.69 and displayed a slightly left-skewed normal distribution (Pearson co- efficient of skewness = −0.7563) (Figure 2). Transgressive segregation was observed in one direction, with some F2:3 families showing higher susceptibility than the susceptible parent (Figure 2). Significant positive correlations ($p < 0.05$) were observed for AUDPC values among the three experiments and ranged between 0.57 to 0.65.

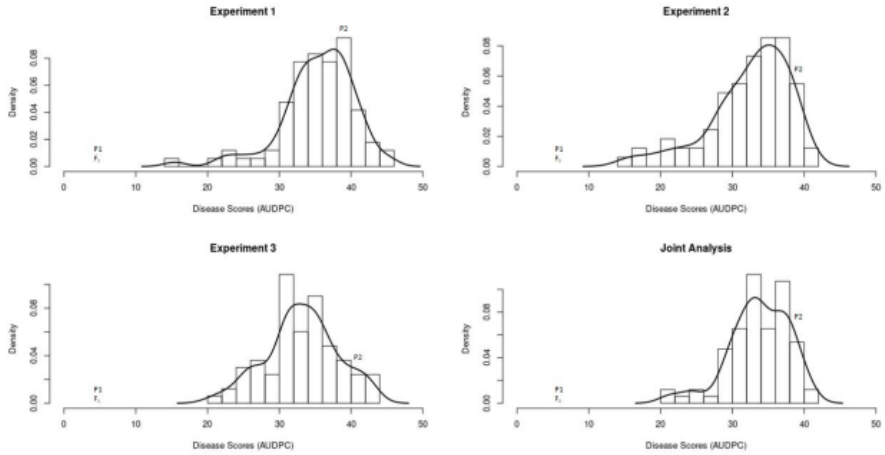

Figure 2. Frequency distribution for disease severity [area under disease progress curve (AUDPC)] in the F2:3 population for experiment 1, experiment 2, experiment 3 and joint analysis (mean across 3 experiments).

B.

C. Linkage mapping and QTL analysis

SNP Analysis and Map Construction Targeted genotyping by sequencing yielded 24,933,788 reads averaging approximately 129,858 reads per sample, effectively giving a 231× coverage for each target SNP. SNP markers that were heterozygous (n = 68) in the parents, monomorphic (n = 182) between the parents or those that deviated ($p < 0.00001$) from the expected segregation ratio of 1:2:1 (n = 29) was excluded from linkage mapping. The complete genetic map comprised 21 linkage groups encompassing 2068.96cM with a marker density of 8.1 SNP/cM. QTL analyses with phenotypic data from the three experiments, and from joint analysis, consistently detected a

significant QTL (QtlPC-C13) on chromosome 13 (Table 1 and Figure 3). This QTL explained 17.9% to 21.5% of the phenotypic variation observed in F2:3 families, with likelihood-odds values ranging from 3.1 to 5.9 (Table 1). The peak SNP (C002686) for QtlPC-C13 was consistent across the three experiments and the joint analysis. The interval for QtlPC-C13 spanned between 1.07 Mb (Experiment 2) and 1.85 Mb (Joint Analysis) and contained five SNPs (LOD = 3.65 to 5.9)

Table 1 Linkage group positions (cM) of the QTL associated with resistance to Phytophthora crown rot on chromosome 13 and the corresponding peak SNP positions in the #181761-36P × Table Queen F2:3 squash population.

Screen	Position (cM)	R^2	LOD	Peak SNP	Additive Effect	Dominance Effect
Experiment 1	89.3	17.92	3.66	C002686	4.14	1.75
Experiment 2	97.9	18.47	3.14	C002686	3.14	2.84
Experiment 3	90.5	21.47	4.04	C002686	3.81	2.78
Joint Analysis	89.7	20.79	5.92	C002686	3.88	2.41

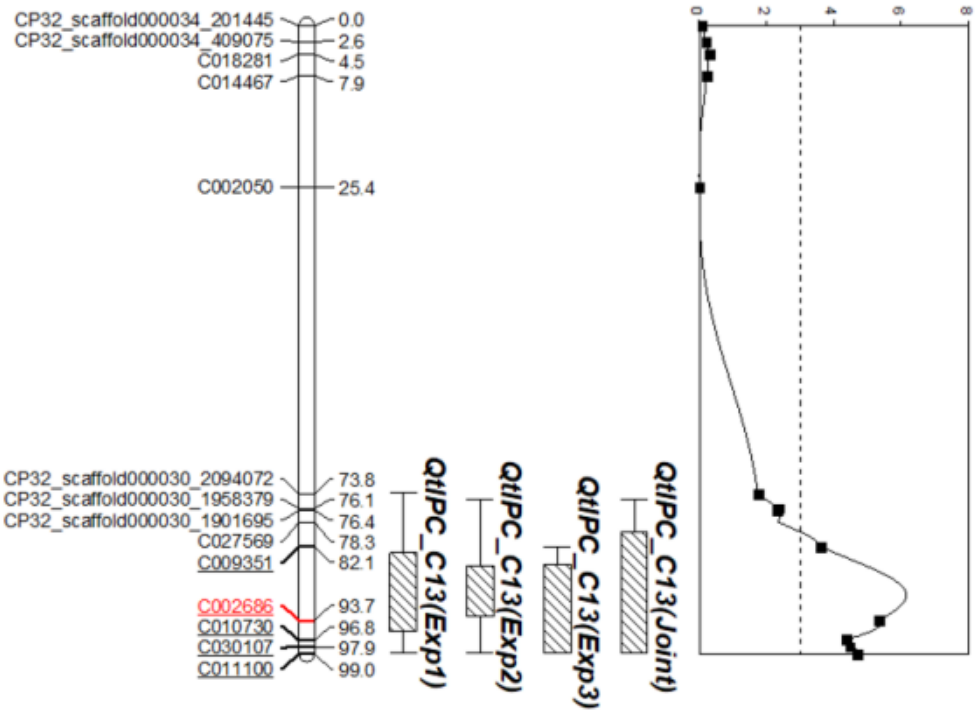

Figure 3 QTL associated with resistance to Phytophthora crown rot on LG (Chr) 13 in #181761-36P. Underlined markers are those within the QTL interval. The significant marker (C002696) is indicated in red font.

Objective 2.2 Marker development and verification

1. *Phytophthora capsici* in *C. pepo* (GM-UF)

A. Methodology

Five SNP markers (Table 2) within the confidence interval of the detected QTL (*QtlPC-13*) were converted into Kompetitive allele specific (KASP) PCR assays and genotyped in the F2 population. KASP oligonucleotides were designed using BatchPrimer3 software, and the PCR assays were performed in 10-µL reactions containing 5-µL of 2× low ROX KASP master mix, 0.16 µL each of forward primers (10 µM), 0.41 µL of reverse primer, 2 µL of genomic DNA (50 ng/µL) and 2.27 µL of H2O. The PCR conditions consisted of an initial incubation at 94 ◦C for 15 min, a touchdown PCR at 94 ◦C for 20 s, 61 ◦C for 60 s, with a 0.6 ◦C decrease per cycle for 10 cycles, followed by 26 cycles of 94 ◦C for 20 s and 55 ◦C for 60 s. Fluorescent end-point readings and cluster calling were performed using LightCycler® 480 Instrument II. Marker-trait associations were tested using the Kruskal-Wallis test ($p \leq 0.05$) in R statistical software. Candidate genes within the significant QTL interval were identified by scanning the corresponding genomic region for disease resistant homologs using the C. pepo reference genome.

Table 2 Five SNP markers genotyped in the F2 population

SNP	SNP Position	Reference Allele	Alternate Allele	KASP Primer	Sequence
C002686	8447660	C	A	C002686_FAM	GAAGGTGACCAAGTTCATGCTCCCAAGTTCTTGAAGAATCTATGAA
				C002686_VIC	GAAGGTCGGAGTCAACGGATTCCCAAGTTCTTGAAGAATCTATGAC
				C002686_R	GGATCAATCCGCTCGATAACCA
C009351	7368860	A	G	C009351_FAM	GAAGGTGACCAAGTTCATGCTTTTCCAATCAAGCCAGAACCA
				C009351_VIC	GAAGGTCGGAGTCAACGGATTTTTCCAATCAAGCCAGAACCG
				C009351_R	AACAACTTCAATGGCGCGTC
C010730	8697466	C	T	C010730_FAM	GAAGGTGACCAAGTTCATGCTCGTTGATACTGGATTTAACAATGGC
				C010730_VIC	GAAGGTCGGAGTCAACGGATTCGTTGATACTGGATTTAACAATGGT
				C010730_R	CAAGTCTCTCAGCTTCGACCA
C011100	9081265	C	T	C011100_FAM	GAAGGTGACCAAGTTCATGCTGCATAACCTTCTTTTAGTTTGTCCAC
				C011100_VIC	GAAGGTCGGAGTCAACGGATTGCATAACCTTCTTTTAGTTTGTCCAT
				C011100_R	CGCTTGAAGCAGAAAGTGGT
C030107	8898585	A	G	C030107_FAM	GAAGGTGACCAAGTTCATGCTATCGCCAAAACTGTCCGATTCCA
				C030107_VIC	GAAGGTCGGAGTCAACGGATTATCGCCAAAACTGTCCGATTCCG
				C030107_R	AGGGGTGATTGTGTTGGTCC

B. Results

Among the five markers, SNP marker C002686 was significantly associated with resistance to Phytophthora crown rot in the F2:3 population (Kruskal–Wallis rank sum test, p-value = 0.0009528).

Objective 3. Introgress resistance in advanced breeding lines
Crosses between #181761-36P and cultivars within various C. pepo market types have been completed. These include: #181761-36P x Zucchini, #181761-36P x Acorn, #181761-36P x Crookneck and #181761-36P x Straightneck. Most of these crosses have been advanced to BC1F1, and are currently undergoing screening in the greenhouse and using marker C002686.

Outcome: Our work on QTL mapping of Phytophthora crown resistance in #181761-36P has recently been published in Plants Journal (MDPI): Michael, V.N.; Fu, Y.; Shrestha, S.; Meru, G. 2021. A Novel QTL for Resistance to Phytophthora Crown Rot in Squash. Plants 10:2115. https://doi.org/10.3390/ plants10102115

QTL for Phytophthora resistance in C. pepo (MM, CS – CU)
Graduate student Greg Vogel combined linkage mapping and bulked segregant analyses for molecular mapping and QTL identification for resistance to the root and crown rot phase of Phytophthora in *Cucurbita pepo*. Through this process, SNPs were identified on five genomic regions (chromosomes 4, 5, 8, 12, and 16). These markers can be used as fixed effect markers in genomic selection or as a preliminary selection criteria when combined with phenotyping.

Obj. 3.4.1 Introgress, pyramid/stack resistances into advanced breeding lines – (MM, CS-CU)

Fruit Quality Determinations (CS-CU)
To determine the quality of newly bred squash cultivars for canning, we have begun experiments in collaboration with the Cornell Food Science Pilot Plant in Geneva NY. In 2021, we grew two industry standard cultivars Golden Delicious (*C. maxima*) and Dickinson (*C. moschata*). The squash will be harvested in the next several weeks (mid-October) and used in a preliminary canning experiment. In 2022, we will grow and can the two industry standard cultivars, Golden Delicious and Dickinson, along with new CucCAP2 cultivars that have been bred for resistance to powdery mildew and Phytophthora. We will then compare these cultivars as they go through the canning process as well as post canning. Texture, color, taste, and ease of canning will all be assessed.

Phytophthora (MM-CU)
Poor seed yields from interspecific crosses between ‘Dickinson’ and ‘Golden Delicious’ restricted the ability to conduct Phytophthora fruit rot assays. To remedy this we are trying three approaches in parallel. First, rather than rely on hand pollinations, we shifted to bee pollinations of isolated plots. Seed yield improved dramatically from ~10 seed per fruit to ~50 seed per fruit. Interestingly despite being BC1F1, fruit do not resemble the recurrent parent and pumpkin x pumpkin crosses resulted in some elongated butternut fruit shapes.

Figure #. Interspecific BC1F1 population (bottom two rows) derived from a cross between ‘Golden Delicious’ and ‘Dickinson’ (F1, top) backcrossed to ‘Golden Delicious’.

Second, we are initiating crosses between ‘Golden Delicious’ and *C. moschata* from other genetic clades, as an alternative if there may be fertility barriers between the ‘Dickinson’ clade specifically. While ‘Dickinson’ is a cultivated processing pumpkin, like ‘Golden Delicious’, age related Phytophthora fruit rot resistance is common in *C. moschata*. Third, as planned we increased seed from members of each *C. maxima* clade for a structured query into the potential for native genetic resistance to phytophthora fruit rot withing the *C. maxima* species.

PI #	**Variety Name**	**Group**
PI_458702	*Plomo ruso (Plaunorskja)*	1
PI_458678	*Tanca*	1
PI_458698	*Zapallito del Tronco*	1
PI_176527	Kestane	2
PI_135373	No. 5018	2
PI_143274	No. 7488	2
PI_357898	Amzibegovska	3
PI_370452	Golema	3
PI_357915	Ukrasna	3
G_30147	Mayo Blusher	4
G_32612	Crown Squash	4
PI_143284	No. 7735	4
G_23726	Alayo	5
PI_458688	*Maguilta*	5

PI_458673	*Tabalque*	5
PI_458683	*Vistalba*	5
PI_500529	Fipushi	6
PI_500508	ZM-2223	6
PI_234608	Queensland Blue	6

Powdery mildew (MM-CU)
The *Pm-0* allele was transferred to processing pie pumpkin, 'Dickinson'. BC3F1 plants were screened for the resistant allele with MAS and the resistant progeny were self-pollinated. Pending greenhouse availability, these will be advanced so BC3F3 seed can be distributed for multiregional resistance testing and canning assays.

Tropical conditions evaluation (AL-UPR)
Evaluation of cultivar/breeding lines resistance to powdery mildew (*Podosphaera xanthii* and *Golovinomyces cichoracearum*) was performed during summer in a randomized complete block design with three repetitions. The plants were sowed on June 9th, 2021 in the Lajas Experimental Station with a distance of 6 ft. between plants, 10 ft. between rows and 8 ft. between plots. Susceptible genotypes 'Taina Dorada', 'Soler', 'Verde Luz', 'Waltham', 'Dickinson', and tolerant breeding lines 20-1716-05 x 1720-05, 20-1716-02 x 1720, 20-1716-08 x 1720-02 and 20-1716-03 x 1720-02 were evaluated. Variables evaluated were flowering dates, resistance to PM, harvest date, fruit weight (kg) and yield (kg/ha).

In absence of natural powdery mildew infestation in the field, powdery mildew that was present in the greenhouse in susceptible Waltham genotype was used to inoculate in the field for screening. Infected leaves were cut in squares of 2.54 x 2.54 cm and overlaid on top of young leaves with a paperclip. One leaf per plant was inoculated per plot. The leaves were evaluated 14, 28 and 42 days after inoculation with the following scales:

1. Number of lesions per leaf
2. Incidence scale: 0 = without lesions, 1 = from 1-3 lesions, 2 = from 3-5 lesions, 3 = from 5-7 lesions, 4 = from 7-9 lesions, and 5 = more than 9 lesions
3. Severity scale: 0 = no damage, 1 = chlorosis on top of leaf, 2 = chlorosis and sporulation present on the bottom of the leaf, 3 = sporulation on both sides of the leaf, 4 = necrosis in most part of the leaf, 5 = dead leaf

Flowering data on genotypes assessed demonstrate that the 'Waltham' genotype was the first to flower for both male and females (Table 1). Harvest date data shows that 20-1716-05 x 1720-05 and 20-1716-03 x 1720-02 were the first genotypes to be harvested (Table 2). Fruit weight (kg) and yield (kg/ha) data indicate 'Taina Dorada' genotype had the best performance (Table 2). Since powdery mildew pathogen was not present by natural infestation, and mechanical inoculation was not successful, evaluation for powdery mildew resistance wasn't possible. However, presence of virus and other fungal pathogens such as *Fusarium* and *Alternaria* were detected.

Table 1. Flowering data for each genotype.

Genotypes	Male flowers (DAS*)			Female flowers (DAS)		
	Average	Min	Max	Average	Min	Max
Taina Dorada	52	51	53	53	51	55
Soler	51	49	53	53	52	53
Verde Luz	50	49	51	50	48	53
Waltham	41	41	41	41	34	45
Dickinson	43	42	43	51	51	52
20-1716-05 x 1720-05	41	41	41	46	41	53
20-1716-02 x 1720	44	42	45	51	46	62
20-1716-08 x 1720-02	41	41	41	52	49	55
20-1716-03 x 1720-02	44	43	45	51	49	52

*das= days after sowing

Table 2. Harvest date, fruit weight (kg) and yield (kg/ha) data for each genotype.

Genotypes	Ave. Harvest Date	Average weight (kg)	Number of Fruit per hectare	Yield (kg/ha)
Taina Dorada	101	5.78	2093	12100.54
Soler	98	5.12	2541	11490.58
Verde Luz	99	2.65	2392	5944.12
Waltham	84	0.38	448	170.55
Dickinson	78	4.21	1046	4403.65
20-1716-05 x 1720-05	73	3.20	1196	3821.49
20-1716-02 x 1720	77	3.71	1046	3887.40
20-1716-08 x 1720-02	79	3.32	1046	3708.78
20-1716-03 x 1720-02	73	4.11	598	2458.39

Given the flowering data, Cornell and temperate varieties seemed to produce and open male flowers earlier than the local or tropical varieties (Table 1). An explanation for this could be that the temperate varieties are under stress because of the change in temperature. In addition, even when powdery mildew was mechanically inoculated into the field, there was no presence of the pathogen in susceptible 'Waltham' cultivar. This effect indicates that factors such as temperature, humidity and such weren't favorable for the pathogen to establish and disperse. Moreover, harvest results suggest that genotypes 'Taina Dorada' and 'Soler' produced on average the heaviest fruits for local materials. Meanwhile, 'Dickinson' and 20-1716-02 x 1720 produced the heaviest fruits for temperate materials (Table 2). However, one should consider that even though all cultivars are the same species, different varieties come in variable shapes and sizes. Finally, yield data presented similar results (Table 2). Nevertheless, yield could've been severely affected given the abundant presence of fungal and viral pathogens in the field as mentioned previously.

The current summer temperature (20-34 ºC) can affect adversely local and temperate pumpkin cultivars by influencing in flowering, harvest (kg) and yield (kg/ha) given that it is not vegetable season in Puerto Rico. In addition, presence of other fungal and viral pathogens can affect the variables evaluated. Evidently, the temperature recorded in the past summer was not favorable for powdery mildew pathogen for expression in the field. However, one must emphasize that powdery mildew was present in the greenhouse. It is recommended for

experiments to be conducted in greenhouse conditions or in the field in alternate seasons for powdery mildew screening in the future.

Integrated Disease Management Team

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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: Cucurbit disease factsheets, crop production manuals, and integrated pest management resources for the Northeast, Southeast and the Midwest are maintained and updated on the website. Notices of regional commodity meetings and Extension education sessions are posted on the CucCAP website events calendar. News from CucCAP researchers is reported on the website and in the CucCAP Chronicle, the monthly newsletter. The CucCAP website shares weekly reports from The Cucurbit Downy Mildew Forecast and Melcast throughout the growing season. Since the beginning of the project, the website includes 37 CucCAP Team News posts, 12 CucCAP Featured Article posts, 77 Integrated Crop and Disease Management posts (cucurbit crop production news and disease outbreak news), and 27 Upcoming Event posts.

Since the start of the project, Quesada has provided diagnostics and disease management recommendations for 11 cucumber, 37 watermelon, 6 melon, 15 squash, and 7 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: 4,021 (lab) + 2,278 (Quesada) followers, LinkedIn: 2,408 followers), and generating disease management resources such as the NC Agricultural and Chemicals Manual and the Southeastern US Vegetable Crop Handbook.

Schultheis has provided cultivar recommendations through oral presentation and specifically generating cultivar results for a zucchini cultivar evaluation study. In addition, cultivar and fertilizer recommendations have been provided for cucurbits overall publications such as the Southeastern US Vegetable Crop Handbook

Keinath has provided management recommendations through oral presentations and generating disease management resources such as the Southeastern US Vegetable Crop Handbook.

Smart provided disease alerts through email, Cornell Cooperative Extension weekly publications, and social media. She responded to about 50 text message questions to provide recommendations for cucurbit disease control (mostly Phytophthora but some bacterial issues as well), received 25 samples for diagnosis, and provided disease control recommendations to extension educators throughout the season. She also had two in person field days focused on cucurbits, along with virtual talks at the NY and Minnesota winter expos.

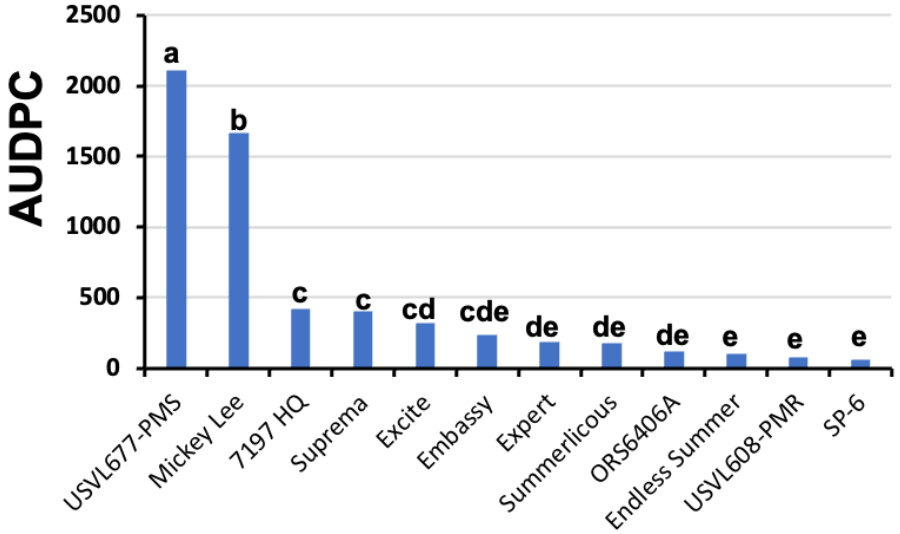
Hausbeck maintains a dedicated downy mildew page (945 page visits May 2021-Sept 2021, accounting for 27% of website traffic; with peak usage in July 18-July 24) on her website that includes weekly spore trapping (228 downloads between May 2021-Sept 2021) and disease updates (207 downloads between May 2021-Sept 2021), fact sheets, information on identifying (41 downloads between May 2021-Sept 2021), monitoring (21 downloads between May 2021-Sept 2021), managing (103 downloads between May 2021-Sept 2021), and testing for downy mildew, reference articles, and links to information about other cucurbit diseases. The website also contains fact sheets for Phytophthora on cucurbits and links to plant disease management reports, extension bulletins, extension news articles, and other relevant publications.

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):

Evaluation of watermelon varieties for tolerance to powdery mildew in Charleston, SC, 2021



This experiment was conducted at the U.S. Vegetable Laboratory farm in Charleston, SC. The soil was Yonges loamy fine sand. This study was undertaken to determine the performance of new commercial watermelon varieties for tolerance to powdery mildew (PM) as it is becoming more prevalent in the U.S.A. The experimental design was a randomized complete block with four replications for each variety. Watermelon varieties were seeded in 50-cell jiffy trays on 5 Apr. Seedlings were transplanted on 10 May onto raised beds with 40-in centers. Beds were spaced 4.6 m 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. General watermelon production practices recommended for the southeastern U.S. were followed. Each variety plot was a single row of 5 plants spaced 46 cm apart with 2.7 m 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 PM was used as the resistant control. After bedding but before transplanting, the row middles were sprayed with Roundup Pro (1.17 liter/ha), Dual Magnum (1.17 liter/ha) and Sandea (70.05 g/ha) for weed management. Weeds in the beds were controlled during the season by hand weeding. PM occurs naturally at this location every year and hence plots were not inoculated. Plant foliage for each variety plot was rated for powdery mildew 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). During each rating period the rating was recorded on lower leaves in the canopy. The underside of five lower leaves for each plot was observed to provide a 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 PM over time was observed. The appearance of PM on these varieties was confirmed by the presence of conidia of the pathogen on the abaxial surface of the leaves. 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. All commercial varieties and the germplasm line USVL608-PMR were significantly more resistant compared to the susceptible controls. Details of disease reaction of each variety evaluated are provided in the table below.

Reaction of commercial watermelon varieties and USVL germplasm lines to powdery mildew in Charleston, SC, 2021.

Cultivar/ Germplasm	PM rating on July 6 (%)[z]	PM rating on July 13 (%)[z]	AUDPC
USVL677-PMS[y]	92 a[x]	77 a	2107 a
Mickey Lee	77 b	82 a	1663 b
7197 HQ	19 cd	21 b	415 c
Suprema	25 c	19 b	398 c
Excite	14 de	16 bc	321 cd
Embassy	11 def	14 bcd	234 cde
Expert	7 efg	11 bcd	181 de
Summerlicous	5 fg	11 bcd	175 de
ORS6406A	5 fg	5 cd	121 de
Endless Summer	2 g	6 cd	98 e
USVL608-PMR	2 g	4 d	75 e
SP-6	3 g	4 cd	62 e
P=	<0.0001	<0.0001	<0.0001
LSD0.05			203

[z]Powdery mildew ratings were recorded 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). The mid percentage points were used in the analysis. PM rating on lower leaves on 6 and 13 July and Areas Under Disease Progress Curves (AUDPC) are presented. AUDPC was calculated based on 5 weekly ratings.

[y]USVL677-PMS and USVL608-PMR were developed at the USDA, ARS, US Vegetable Laboratory in Charleston, SC.

[x]Means followed by the same alphabet are not significantly different (*P*=0.05).

Evaluation of varieties for control of powdery mildew of watermelon, Goldsboro NC 2021.

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

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

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

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

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

Evaluation of watermelon cultivars for resistance to Fusarium wilt in SC and NC, 2021.

Two replicated studies were conducted in fields that contained the pathogen *Fusarium oxysporum* forma specialis *niveum* to assess how susceptible and resistant 10 watermelon cultivars were to Fusarium wilt. One study was conducted in Charleston, SC, the other in Clayton NC in 2021. Cultivars were obtained from four international seed companies and are listed in the table below.

The majority of these cultivars have not been tested in a public study for Fusarium wilt susceptibility, tolerance, or resistance. All cultivars in the replicated study were triploid watermelons. All plants were scored weekly for Fusarium wilt symptoms. Plants in the Charleston, SC were evaluated for 7 weeks while plants were evaluated for 10 weeks in the Clayton, NC study.

Percentage of watermelon plants of each cultivar with Fusarium wilt symptoms at 4, 7 and 10 weeks after planting (WAP) and area under the disease progression curve (AUDPC) in South Carolina (SC) and North Carolina (NC), 2021.

Cultivar	Percentage Incidence Fusarium wilt								AUDPC			
	4 WAP		7 WAP				10 WAP		7 WAP			
			SC		NC				SC		NC	
7197 HQ	30.0	ab[Z]	24.2	cd	65.0	a	95.0	a	24.2	cd	15.0	a
El Capitan	35.0	ab	48.6	abc	72.5	a	80.0	a	8.7	abc	19.4	a
Embassy	27.8	ab	14.6	c	69.1	a	92.5	a	3.2	c	16.4	a
Fascination	17.5	ab	24.5	cd	47.5	a	75.0	a	4.5	bc	10.6	ab
Gr. Fascination	0.0	b	0	e	0	b	0	b	0	d	0	b
Joy Ride	35.0	ab	62.4	ab	70.0	a	100	a	11.9	ab	17.8	a
Power House	35.0	ab	22.8	cd	92.5	a	92.5	a	4.6	bc	17.1	a
Shoreline	22.5	ab	67.8	a	97.5	a	97.5	a	16.4	a	15.6	a
S. Nevada (SC)	-	-	41.4	abc	-	-	-	-	9.8	abc	-	-
S. Madre (NC)	45.0	a	-	-	70.0	a	95.0	a	-	-	21.3	a
Tri-X-313	40.0	a	34.1	abc	65.0	a	95.0	a	8.7	abc	18.3	a

[Z]Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Tukey LSD).

Percentage plants with Fusarium wilt symptoms ranged from 0 to 67.8% in the SC location 7 weeks after planting. No Fusarium wilt symptoms were detected in the grafted plants in which Carolina Strongback was used as the rootstock. The greatest incidence of Fusarium wilt was detected on Shoreline. Two cultivars had over 60% Fusarium occurrence, 2 cultivars had over 40 to 50 % occurrence, 1 cultivar had 34% occurrence, 3 cultivars between 23 and 25% occurrence, and Fascination had 15% occurrence. The AUDPC correlated over 90% with the percentage incidence of Fusarium wilt in a given cultivar. Yields correlated as well with percentage Fusarium wilt as a high percentage Fusarium wilt resulted in low yields, and reduced or no Fusarium wilt resulted in high yields.

The response of cultivars in the NC location was very different than what occurred in the SC location. All cultivars, regardless of 4, 7, or 10 weeks after planting had a similar percentage of plants with Fusarium wilt symptoms at each given time after planting. Unlike the SC location, no cultivars showed more resistance to Fusarium wilt than another. Like the SC location, the use of the rootstock Carolina Strongback in NC resulted in no plants exhibiting Fusarium wilt symptoms. AUDPC data confirmed a similar response to the Fusarium wilt symptom data at the various time intervals after planting. Best yields were obtained when Fascination was grafted to Carolina Strongback rootstock.

Fusarium wilt races 1 and 2 have been confirmed in the SC location. Race 1 has been used as an inoculum in the NC location. We are working closely with some seed companies to see if only race 1 is present in NC or if there are other races present.

As a note, USVL 380A and USVL 380b were obtained as a part of the CucCAP project from the USDA Vegetable laboratory in Charleston, SC. These two lines are diploid lines. One observational plot was planted in the NC location and there was 0% incidence of Fusarium wilt.

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

Evaluation of cucumber cultivars for resistance to downy mildew in MI, 2021.

A trial with 11 cultivars, plus 3 cultivars in strip trial, were established at the Michigan State University Plant Pathology Farm in Lansing, MI, in a field of Capac loam soil previously planted to cucumber. The field was plowed on 5 May and disced 17 May. Discing also occurred on 20 May to incorporate the preplant fertilizer (100 lb/A urea, 180 lb/A potash, 25 lb/A of 95% sulfur, 20 lb/A boron). On 25 May, raised beds were formed in the field with black plastic mulch 6 ft apart, and drip tape for irrigation and in-season fertilization. Weed control was performed biweekly via mechanical cultivation.

The 11 cultivar trial was planted on 30 July and the strip trial was planted on 13 August, both from seed. The treatments were arranged in a completely randomized block design with 4 replications. Each replication was 20 ft with a 5 ft buffer between each, and the strip trial was a single 100 ft row. Each week during the growing season the cultivars were fertilized with 28% N liquid fertilizer at 1 gal/A through the drip tape. Quadris F 15.5 fl oz/A and Quintec SC 6 fl oz/A were sprayed on 27 August to control Alternaria leaf spot and powdery mildew, and 10.5 fl oz/A of Admire Pro through the drip lines on 13 August for insect control. The cultivar trial was evaluated for foliar infection on 23 and 30 August and 2, 8, and 17 September. The strip trial had a single foliar evaluation on 18 September.

All cultivars had significantly less disease than the 'Straight Eight' cucumber that was included as a control and WI720402 according to the last observation date (17 September). According to the Area Under the Disease Progress Curve (AUDPC), WI720402 and 720403 were similar to the susceptible standard Straight Eight. Two of the cultivars, PI197088 and Peacemaker, had less than 10% of the foliage with downy mildew symptoms on the last observation date. However, the AUDPC indicated that PI197088 had significantly less disease over the course of the season compared to all other cultivars included in this study.

In the strip trial, the Encounter cultivar had less disease and foliar necrosis than the other two cultivars.

Cucumber cultivars evaluated for downy mildew resistance in a replicated trial.

Cultivars	Foliage (%) with Symptoms of Downy Mildew[z]					
	23 Aug	30 Aug	2 Sep	8 Sep	17 Sep	AUDPC
Straight Eight	8.0 c[y]	25.0 ab	35.0 a	46.0 a	70.0 a	972.4 a
PI197088	5.0 bc	0.5 d	0.3 e	5.0 e	4.0 g	76.6 g
Peacemaker	10.3 bc	18.7 bc	22.5 dc	11.0 e	7.5 g	349.0 f
Zircon	7.5 c	15.0 c	18.8 d	18.0 d	32.5 f	463.1 ef
Citadel	11.8 ab	22.5 a-c	23.8 b-d	15.0 d	33.8 f	524.9 e
Gy1402	1.0 c	20.0 bc	26.3 a-d	30.0 c	53.8 de	688.5 d
Gy14D4	5.5 bc	15.0 c	20.0 d	35.0 bc	58.8 cd	711.1 cd
WI7204	12.3 ab	25.0 ab	30.0 a-c	45.0 a	50.0 e	756.6 cd
Liszt	13.3 ab	22.5 a-c	26.3 a-d	35.0 bc	63.8 bc	826.4 bc
720403	20.3 a	30.0 a	32.5 ab	40.0 ab	57.5 d	925.9 ab
WI720402	13.0 ab	26.3 ab	22.5 dc	35.0 bc	68.8 ab	958.6 ab

Based on a visual estimation of the percentage of the foliage with downy mildew symptoms.
[y]Column means with a letter in common are not significantly different (LSD t Test; *P*=0.05).

Cultivars evaluated for downy mildew resistance in a strip trial.

Cultivars	Foliar Infection (%)[z]	Foliar Necrosis (%)[y]
Hyper C	22.5	4.3
Jumbo Green	13.0	2.5
Encounter	2.5	0.0

[z]Based on a visual estimation of the percentage of foliage with downy mildew symptoms on 18 September.

Evaluation of varieties for control of downy mildew on cucumber, Clinton NC 2021.

The experiment was conducted at the Horticultural Crops Research Station in Clinton, NC. Plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. In 2020, the field was planted with cucumber. Cucumber was direct 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 and 23 Sep as percent leaf area with necrosis per plot. Fruit were harvested on 22 Sep. 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. The varieties Encounter, Chaperon, Hyper C, Citadel, Zircon, Peacemaker and Gy14Q2 controlled *P. cubensis*. In the table, varieties are sorted by the disease severity rating on 23 Sep.

	Disease Severity[z] (%)	
Variety	14-Sep	23-Sep
PI-197088	5.0g[y]	9.3f
Encounter	12.0de	26.3e
Chaperon	9.5ef	28.8de
Hyper C	16.5c	30.0cde
Citadel	9.8def	32.5b-e
Zircon	12.3d	33.8b-e
Peacemaker	8.8f	33.8b-e
Gy14Q2	16.8c	36.3bcd
Gy14	17.3c	38.8abc
7204Q3	29.3a	40.0ab
Jumbo G/L	16.5c	40.0ab
Liszt	20.3b	47.5a

[z] Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.
[y] Varieties followed by the same letter(s) within a column are not statistically different (*P*=0.05, Fisher's protected LSD Test).

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

Evaluation of squash cultivars for resistance to powdery mildew in NY, 2021.

Six cultivars were evaluated, planted on 6/22 in a randomized complete block design with four replications: Bugle, Waltham, Butterfly (all *Cucurbita moschata*) and TNK-157, TNK-163, and Golden Delicious (all *C. maxima*). Powdery mildew was observed in the trial, from natural inoculum, starting in mid-August and was rated six times from 8/9 to 9/10 for disease severity. Area Under the Disease Progress Curve (AUDPC) was calculated and is reported below. Fruit were harvested, counted, and weighed on 9/24, with five representative fruit per plot measured for size, flesh firmness, and Brix.

Bugle and Butterfly had the lowest disease severity while the remaining cultivars each had significantly higher ratings that are indistinguishable from one another. Bugle produced the greatest number of marketable fruit (50.75), while TNK-163 and Golden Delicious produced the fewest (23.50 and 15.25, respectively). Golden Delicious yielded the heaviest fruit on average (2.087 kg), while Bugle yielded the smallest (0.412 kg). The remaining cultivars produced fruit with comparable numbers and weights. Fruit were different in length and width across cultivars, but there were no differences in soluble solids content or flesh firmness. All cultivars will be included in the repeated trial during Year 2.

Comparison of powdery mildew disease severity on six squash cultivars. Plots were rated six times to calculate the Area Under the Disease Progress Curve. The fourth, fifth, and sixth individual ratings are also displayed.

	Foliar disease severity (%)			
Variety	**30-August**	**3-September**	**10-September**	**AUDPC**
TNK-157	1.6865 a[x,y]	4.2462 a	6.4312 a	16.6215 a
TNK-163	1.6453 a	5.6148 a	7.8495 a	20.8563 a
Waltham	1.2015 a	4.9455 a	7.2287 a	18.5173 a
Golden Delicious	1.1282 ab	4.1519 a	6.2531 a	15.8676 a
Butterfly	0.3349 bc	0.8852 b	2.3149 b	5.0466 b
Bugle	0.0791 c	0.4081 b	0.8598 b	1.8843 b
P value[z]	> 0.001	> 0.001	> 0.001	> 0.001

[x]Disease severity data were square root transformed to meet the assumptions of parametric analysis.
[y]Means separations achieved using the post hoc Tukey's HSD adjustment for multiple comparisons.
[z]Probability values reported from analysis of variance output using RStudio (version 4.0.4).

Comparison of the yield parameters of six squash cultivars. Fruit were harvested once, counted, and weighed to determine parameters, calculated per five plants.

Variety	**Marketable fruit[x]**	**Total fruit**	**Marketable yield (kg)**	**Total yield**	**Average marketable fruit wt (kg)**
Bugle	50.75 a[y]	51.25 a	78.74	78.85	0.412 d[z]
Butterfly	41.50 ab	42.75 ab	92.90	93.25	0.756 cd
Waltham	33.75 abc	33.75 abc	85.09	85.09	0.926 bc
TNK-157	23.50 bc	23.75 bc	73.78	73.99	1.109 bc
TNK-163	18.50 c	20.00 c	66.00	66.81	1.263 b
Golden Delicious	15.25 c	16.00 c	124.67	124.89	2.087 a
P value[w]	> 0.001	> 0.001	0.542	0.439	> 0.001

[x]Marketable fruit, marketable yield, and average marketable fruit weight included fruit with standard dimensions for each fruit type.
[y]Means separations achieved using the post hoc Tukey's HSD adjustment for multiple comparisons.
[z]Average marketable fruit weight data were logarithmically transformed to meet the assumptions of parametric analysis.
[w]Probability values reported from analysis of variance output using RStudio (version 4.0.4).

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
2. Alzohairy, S.A., Hammerschmidt, R., and Hausbeck, M.K. (2021) Antifungal Activity in Winter Squash Fruit Peel in Relation to Age Related Resistance to *Phytophthora capsici*. Physiological and Molecular Plant Pathology 114:101603. NIFA acknowledged.
3. Alzohairy, S.A., Hammerschmidt, R., and Hausbeck, M.K. (2020) Changes in winter squash fruit exocarp structure associated with age-related resistance to *Phytophthora capsici*. Phytopathology 110(2):447-455. NIFA acknowledged.
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Book Chapters

1. D'Arcangelo K., Quesada-Ocampo L. M., and Hausbeck M. K. (2021) Diseases of cucurbits: Cucurbit downy mildew. In: Handbook of Vegetable and Herb Diseases. Editors: Elmer W., McGrath M. T., and McGovern R. Springer.
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EXTENSION PUBLICATIONS and OUTREACH RESOURCES

1. Adams M. L., Collins H., and Quesada-Ocampo L. M. 2020. Evaluation of fungicides for control of downy mildew on cucumber, Clayton, 2019. Plant Disease Management Reports 14:V116.
2. Adams M. L., Collins H., and Quesada-Ocampo L. M. 2020. Evaluation of cultivars in combination with fungicides for control of downy mildew and yield effects on cucumber, Clinton, 2019. Plant Disease Management Reports 14:V117.
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15. Hausbeck, M.K. and Linderman, S.D. 2020. Managing Phytophthora on Summer Squash and Zucchini. Fact Sheet.
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17. Hausbeck, M.K., Linderman, S.D., and Higgins, D.S. 2020. Monitoring cucurbit downy mildew. Fact Sheet.
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23. Keinath, A.P., DuBose, V. B., and Zardus, S. H. 2021. Evaluation of several fungicides to manage foliar and fruit anthracnose on seedless watermelon, 2020. Plant Dis. Manag. Rep. 15:V089.
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28. Keinath, A.P. July 26, 2021. Fungicides to manage gummy stem blight in fall cucurbits. https://scgrower.com/2021/07/26/preparing-for-gummy-stem-blight-in-fall-cucurbit-crops/.
29. Keinath, A.P. and Silva, F. D. August 24, 2021. Not Planting to Manage Phytophthora Blight by Reducing Disease Risk. https://scgrower.com/2021/08/24/not-planting-to-manage-phytophthora-blight-by-reducing-disease-risk/
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31. Kenny, G.E., Engfehr, C.L., and Hausbeck, M.K. 2020. Evaluation of 9 alternating programs of fungicides for control of downy mildew on pickling cucumbers, 2019. Plant Disease Management Reports 14:V216.
32. Kenny, G.E., Engfehr, C.L. and Hausbeck, M.K. 2020. Evaluation of single product treatments for control of downy mildew on pickling cucumbers, 2019. Plant Disease Management Reports 14:V183.
33. Lukasko, N.T., Engfehr, C.L. and Hausbeck, M.K. 2020. Evaluation of fungicides for the control of powdery mildew on butternut squash, 2019. Plant Disease Management Reports 14:V159.
34. Peterson, D. 2020. Phytophthora control is a long-term commitment. Interview, Vegetable Grower News: Jan: 14-15.
35. Prieto-Torres M., Purayannur S., Adams M., and Quesada-Ocampo L.M. Squash downy mildew found in North Carolina. Extension Plant Pathology Portal. August 3, 2021.
36. Prieto-Torres M., Adams M., Purayannur S., and Quesada-Ocampo L. M. Cucumber downy mildew found in North Carolina. Extension Plant Pathology Portal. June 16, 2021.
37. Quesada-Ocampo L.M., Meadows I., and Gorny A. 2021. Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Contributed 11 tables total).
38. Schultheis, J.R., K.D. Starke, and M.D. Collins. 2021. 2020 Zucchini squash cultigen evaluations. Dept. of Horticultural Science. North Carolina State University. Hort. Series No. 237, 35 pp. https://cucurbits.ces.ncsu.edu/about-cucurbits/growing-cucurbits/variety-trials/2020-zucchini-squash-cultivar-evaluations/

39. Smart, C.D. 2021. Managing Phytophthora in pumpkins & melons – the New York experience. Proceedings of the 2021 Minnesota Fruit and Vegetable Convention.
40. Smart, C.D. 2021. Phytophthora of cucurbit crops Disease fact sheet https://www.vegetables.cornell.edu/pest-management/disease-factsheets/phytophthora-blight/
41. Smart, C.D. 2021. Cucurbit Downy Mildew Disease fact sheet https://www.vegetables.cornell.edu/crops/cucurbits/downy-mildew/
42. Tekip, A. 2020. Monitoring and managing cucumber downy mildew. Interview, Michigan State University AgBioResearch, Futures: Summer 2020.
43. Walker, K. 2021. Downy mildew in cucumbers this season. FarmWorld. Interview, 30 Jul.

SCIENTIFIC CONFERENCE and UNIVERSITY PRESENTATIONS

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. D'Arcangelo, K. N., Rahman, A., Miles, T. D., and Quesada-Ocampo, L. M. 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.
7. 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/
8. Dennis N. Katuuramu, Sandra E. Branham, Amnon Levi, and W. Patrick Wechter. 2021. Genome-wide association analysis of downy mildew resistance in a pre-breeding watermelon (Citrullus amarus) collection. Eucarpia Cucurbitaceae Symposium Proceedings.
9. 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.
10. 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.
11. 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.
12. 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.
13. 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

14. Kenny, G. 2020 Cucumber field data. Departmental seminar, Department of Plant Pathology, Michigan State University, East Lansing, MI. Virtual.
15. 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
16. 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.
17. 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.
18. 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.
19. 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.
20. Mandal M. K., Thompson, D., Harris, R. and Kousik C.S. 2021. Bacterial Biocontrols in Sustainable Management of Phytophthora Crown and Fruit Rot in Pepper and Watermelon. Annual Meeting of the American Phytopathological Society.
21. 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).
22. 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.
23. 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.
24. 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.).
25. 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
26. 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
27. 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.
28. 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.
29. 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.

30. 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.
31. 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.
32. 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.
33. Schultheis, J. and K. Starke 2021. Melon cultigens and their adaptation in the southeastern United States when grown in North Carolina (abstr.)
34. 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
35. 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.).
36. 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.)
37. 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.
38. 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.

EXTENSION PRESENTATIONS

1. Grumet R (2021) The CucCAP2 project. BASF. January 2021.
2. Grumet R, Lin YC (2020) Resistance of cucumber fruit to *Phytophthora capsici*. Pickle Packers International. Virtual conference, October 2020.
3. Hausbeck, M.K. 2021. A Partnership to protect Michigan's Cucumber Industry. Farm Lane Society meeting, Virtual, 5 Mar.
4. Hausbeck, M.K. 2020. Management of Phytophthora Blight in Processing Squash. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 10 Dec. 167 attendees.
5. Hausbeck, M.K. 2020. Downy Mildew Management in Pickling Cucumbers. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 8 Dec. 174 attendees.
6. 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
7. Hausbeck, M.K. 2020. 2021 Spray Program. Southeast Vegetable Meeting. Virtual, 4 Nov. 90 attendees.
8. 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.
9. 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.
10. 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.
11. 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.
12. 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
13. 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/
14. 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/
15. 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
16. 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
17. McGregor, C. & G. Boyhan (2020) Breeding better Cucurbits. Vegetable & Specialty Crop News, September 2020: 16-17
18. Meru G. 2021. Progress towards breeding resilient cultivars for Florida. Presented at the Southeast Florida Extension Meeting, held virtually April 8, 2021.

19. 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.
20. Quesada-Ocampo L.M. Pickles in a pickle: trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Pickle Packers International Spring Meeting. Raleigh, NC, April 2021*. (*Cancelled due to COVID-19)
21. Quesada-Ocampo L.M. Management of Fusarium wilt and anthracnose in watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
22. Quesada-Ocampo L.M. Cultural and chemical control options for Phytophthora fruit rot of watermelon. NC Watermelon Convention. Virtual meeting, February 2021.
23. Quesada-Ocampo L.M. Never a dill moment when managing cucumber downy mildew. 2020 Eastern NC Certified Crop Adviser Training. Virtual Meeting, December 2020.
24. 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.
25. Schultheis, J.R. 2021. Hollow heart considerations and pollenizer cultivar comparisons. North Carolina Watermelon Growers Association. Virtual meeting, January, 2021. 65 attendees
26. 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.
27. 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/
28. Weng Y, Chen FF, Tan JY (2020) Marker-assisted QTL pyramiding for downy mildew (DM)
29. and phytophthora fruit rot (PFR) resistances in pickling cucumber. Pickle Packers International. Virtual conference, October 2020.
30. Weng Y (2021) The Gy14v2.0 cucumber draft genome. Chinese Cucumber Breeders Association 2021 Annual Meeting and Variety Show. April 2021. Virtual.
31. Weng Y (2021). Disease resistances in cucumber. SIPS seminar. Cornell University, Ithaca, NY. Virtual.