CucCAP:

Leveraging applied genomics to improve disease resistance in cucurbit crops



Fourth Annual CucCAP Team Meeting

April 12-13, 2019

Hosted by Boyce Thompson Institute and Cornell University Ithaca NY

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AGENDA

and

PARTICPANTS

AGENDA

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

Friday, April	12
8:00-8:15	Arrival, welcome
8:15-8:30	Overview of project progress, plans for meeting
	- Progress to date
	- Priorities for the coming year
	- Priorities and strategy for the future
Session I – Ge	enomic Tools
Objective I: D	evelop genomic approaches and tools for cucurbit species
- Develop gen analy	omic and bioinformatic platforms for: genotyping by sequencing (GBS); sequence data processing and rsis; and genotype, phenotype and OTL databases
- Perform GBS	S analysis of PI collections and core populations of the four species to provide a
comm	nunity resource for genome wide association studies (GWAS) for current and future traits of interest
- [E/O] Provid	e access to cucurbit genomics tools and databases via the International Cucurbit Genome
Initia	tive (ICuGI) website and genomics and bioinformatics workshops

8:30-8:50	Overview of progress: bioinformatics platforms and website, GBS data and analysis, publications (Fei, Reddy)
8:50- 9:30	Status of core panels (seed stocks for first generation; resequencing) and timeline to finish watermelon (Levi) melon (McCreight) cucumber (Weng) squash (Mazourek)
9:30-10:30	Proposed genomics tools objectives for CucCAP 2 – Discussion and feedback from industry
9:30	Pan-genomic analysis, identify sources of individual variation, multiple reference genomes, nanopore resequencing representatives, cucurbit marker sets across genomes; genomics website and bioinformatics capacities (Fei)
9:50	Core populations seed supplies – can we partner with seed companies? Partner with NGS to distribute core populations (Yiqun, Amnon)

10:10-10:30 Break

Session II – Breeding for disease resistance

Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.

- Utilize genomic approaches to map resistance loci for key cucurbit diseases

- Develop and verify molecular markers for efficient trait selection and gene pyramiding

- Introgress resistances into advanced breeding lines

- [E/O] Provide web-based and face to face information via field trials, extension venues, and scientific meetings regarding breeding materials, markers, and breeding progress

10:30-11:30	Watermelon: Status for each disease
	- where were we at the outset? Where are we now? Priorities to finish CucCAP1?
	breeding lines, mapping/QTL, markers, introgression
	Present by disease:
	Fusarium, gummy stem blight, Phytophthora, powdery mildew, GCMMV, PRSV-W
	(Levi, Kousik, Ling, McGregor, Wechter, Wehner)
11:30-12:00	Feedback/priorities from industry (include teleconference if needed with commodity reps)

12:00-1:00	Lunch
1:00-1:40	<u>Melon</u> : Status for each disease – where were we at the outset? Where are we now? Priorities to finish CucCAP1? breeding lines, mapping/QTL, markers, introgression Present by disease: powdery mildew, CMV, CYSDV, Fusarium (McCreight Kousik Weetter Wintermentel)
1:40-2:00	Feedback/priorities from industry (include teleconference if needed with commodity reps)
2:00-2:25	<u>Cucumber</u> : Status for each disease – where were we at the outset? Where are we now? Priorities to finish CucCAP1? breeding lines, mapping/QTL, markers, introgression Present by disease: downy mildew, Phytophthora (Wang, Counst, Webser)
2:25-2:45	(weng, Grumet, wenner) Feedback/priorities from industry (include teleconference if needed with commodity reps)
2:45-3:00	Break
3:00-3:30	<u>Squash</u> : Status for each disease – where were we at the outset? Where are we now? Priorities to finish CucCAP1? breeding lines, mapping/QTL, markers, introgression Present by disease: PRSV-W, ZYMV, Phytophthora, powdery mildew? (Magaural: Wessel Basyor Street Howheels?)
3:30-3:45	Feedback/priorities from industry (include teleconference if needed with commodity reps)

Session III – Disease control information and Economic impact

Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

- Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets

- Use a risk-based simulation model to analyze economic potential of the disease resistant cucurbit cultivars

- [E/O] Develop a centralized cucurbit disease website, including content in English and Spanish, providing information about diagnostics and links to disease clinics; disease control recommendations; disease alerts and links to forecasting tools

3:45-4:15	Extension team: Progress to date, priorities to finish CucCAP1 (Schulthies, Hausbeck, Linares, Quesada, Smart, Lorscheider)
4:15-5:15	Socioeconomics team: Progress to date, priorities to finish CucCAP1 (Palma, Rivera)
5:15-5:45	Discussion and feedback from industry (include teleconference if needed with commodity reps)

6:00 CucCAP Networking Dinner

Saturday, April 13

8:00-8:15	Arrive
8:15-8:30	USDA-SCRI landscape and implications for the future

Session IV – Planning Sessions

8:30-9:15	Working session I: commodity group goals
	Watermelon, Melon, Cucumber, Squash
	How do we build on our accomplishments?
	 Utilize genomic approaches to map resistance loci/develop markers/introgress resistance <u>Possible future steps</u>: Refine list of diseases based on industry priorities, emerging diseases, progress to date? Diversify sources of resistance (how? GWAS? Does other genetic analysis of PIs help?) Identify syntenic regions across species? Continue development of markers/develop tools for pyramiding (what would those be?) Test performance of identified QTL when incorporated into advanced breeding lines – coordinate with extension/pathologists/industry? coordinated resistance trials? Phenotype core populations/ GWAS – which traits? multiple locations? Develop coordinated approach for phenotyping across locations/traits?(Michael?) New approaches to consider/highlight?
9:15-10:00	Working session II A. Genomics tools goals (Fei, lead) Priorities, strategies, partnering with industry
	B. Extension and Economics goals (Schultheis, Palma) Priorities, strategies, partnering with industry
10:00-10:15	Break
10:15-11:15	Priorities and strategies including matches Report out from each team (5 min each) Feedback from industry; including new industry partners?
11:15-12:00	Wrap up discussions, feedback from external reviewers

CucCAP Team

Project Director

Rebecca Grumet, Professor, Department of Horticulture 1066 Bogue Street, Michigan St Univ., East Lansing MI 48824 (grumet@msu.edu)

Team Leaders

<u>Watermelon (Citrullus lanatus)</u> Amnon Levi, Research Geneticist, Vegetable Research Laboratory 2700 Savannah Highway, USDA-ARS, Charleston SC 29414 (Amnon.levi@ars.usda.gov)

Melon (Cucumis melo)

Jim McCreight, Research Leader, US Agricultural Research Station 1636 E Alisal St, USDA-ARS, Salinas, CA 93905 (Jim.McCreight@ars.usda.gov)

Cucumber (Cucumis sativus)

Yiqun Weng, Research Geneticist, USDA-ARS and Associate Professor, Dept. Horticulture 1575 Linden Drive, Univ. Wisconsin, Madison, WI 53706 (Yiqun.weng@ars.usda.gov)

Squash (*Cucurbita spp.*)

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Genomics and Bioinformatics

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Socioeconomics

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Extension/Outreach

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<u>Co-PDs</u>

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Stakeholder Advisory Board				
Organization	Representative	Position		
Commodity Groups - Growers, Shippe	ers, Processors, Marketing			
National Watermelon Promotion	Mark Arney	Executive Director, National Watermelon		
Board		Promotion Board		
National Watermelon Association	Robert Morrissey	Executive Director, National Watermelon		
		Association		
California Melon Research Board	Milas Russell	Chair Elect, California Melon Research Board		
		President, Sandstone Melon Company		
California Melon Research Board	Steve Smith	Chair, California Melon Research Board		
		Co-Owner Turlock Fruit Company		
Pickle Packers International	Brian Bursiek	Executive Vice President, Pickle Packers		
		International		
Swanson Pickles and Pickle Packers	John Swanson	President Swanson Pickle Company;		
International		Research Board, Pickle Packers International		
Martin Farms (squash grower,	Mitch Beyler	Partner, John B. Martin and Sons Farms, Inc.		
shipper)				
Stony Brook Wholehearted Foods	Greg Woodworth	Founder, Stony Brook Wholehearted Foods		
(squash processor)				
Seed Industry	-			
Bayer Crop Science	Jovan Djordjevic/	Global R&D Lead, Melons and Watermelons,		
	Suren Baliji/	Bayer Crop Science		
	Peter Kraan			
HM Clause	Kishor Bhattarai	Phytopathology Project Manager, HM		
		Clause, Vegetable Seeds Division, Limagrain		
Hollar Seed Company	Bruce Carle	Plant Breeder, Hollar Seed Company		
Johnny's Selected Seeds	Rob Johnston/	Chairman, Johnny's Selected Seeds		
	Lindsay Wyatt			
Monsanto	Nischit Shetty	NAFTA Cucurbit Lead for Monsanto		
		Vegetable Seeds		
Sakata Seeds	Jeff Zischke/	Director of Research, Vegetables, Sakata		
	Nihat Guner	Seed		
Syngenta Seeds Inc.	Matt Kinkade/	Global Cucurbits Co-Lead, Syngenta Seeds		
	Sandhu Ajay			
United Genetics Seeds Co.	Xuemei Zhang	Melon Breeder, United Genetic Seeds		

Cucurbit Crop Curators

Robert Jarret - Citrullus spp.

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Kathy Reitsma - *Cucumis (C. melo* and *C. sativus)* USDA-ARS, North Central Regional Plant Introduction Station Iowa State University, Ames IA 50011 Kathleen.Reitsma@ARS.USDA.GOV

External Evaluators

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Nurit Katzir

Professor and Head of the Agricultural Research Organization Newe Ya'ar Research Center (recently retired) Newe Ya'ar Research Center, P.O.Box 1021, Ramat Yishay 30095, Israel katzirn@volcani.agri.gov.il

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CucCAP PROJECT OBJECTIVES,

TIMELINES and METRICS

CucCAP PROJECT OBJECTIVES

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

Obj. 1. Develop genomic approaches and tools for cucurbit species.

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

Obj. 2. Perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars.

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

Obj. 3 Perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control.

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

Table 1. Major disease threats to cucurbit crop production as identified by cucurbit industry stakeholders.				
Disease Identified as commodity funding priority ^a Also affects:		Also affects:		
Downy mildew	cucumber	melon, watermelon, squash/pumpkin		
<i>Fusarium</i> wilt	watermelon	melon, cucumber		
Gummy stem blight	watermelon	melon, cucumber, squash/pumpkin		
Phytophthora rot	cucumber, watermelon, squash/pumpkin	melon		
Powdery mildew	melon, watermelon, squash/pumpkin	cucumber		
Viruses (CMV ^b , CYSDV ^c ,	melon ^{b,c} , watermelon ^{d,e}	cucumber ^{c,e} , squash/pumpkin ^{b,d}		
PRSV-W ^d , CGMMV ^e				

Disease priorities identified by the cucurbit industries:

Project Structure – Team Organization

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

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

TIMELINE CHART

TIMELINE CHART						
Objective	Personnel/Institution Year					
	(initials as in Table 3)	1	2	3	4	
(a) Obj. 1. Develop common genomic approaches and tools for						
cucurbits						
1.1. Develop genomic and bioinformatics platforms						
1.1.1. Genotyping by sequencing	ZF (BTI)	X	X	X		
1.1.2. Sequence data processing/analysis	ZF (BTI)	X	X	Х	X	
1.1.3. ICuGI database development	ZF (BTI)	Х	X	Х	Х	
1.1.4 Community standardized nomenclature	YW (ARS-WI), AL (ARS-SC)		Х	Х		
	JM (ARS-CA). MM (CU)		v	v	v	
1.1.5. Genomic, bioinformatics workshops	ZF (B11), UR (WVSU), members		Х	Х	А	
1.2 Parform GRS analysis of PL collections establish core	of crop teams					
populations, provide community resource for genome wide association studies (GWAS)						
1.2.1. GBS of cucurbit species, establish molecular-informed core	ZF (BTI), RG (MSU)	Х	Х			
populations						
- watermelon	AL (ARS-SC)	Х	Х			
- melon	JM (ARS-CA)	Х	Х			
- cucumber	YW (ARS-WI),	Х	Х			
- squash	MM (CU)	Х	X			
1.2.2. Population genetics and GWAS analyses	UR (WVSU), ZF (BTI)		X	X	X	
- watermelon	AL (ARS-SC)		X	X	X	
- melon	JM (ARS-CA)		X	X	X	
- cucumber	Y W (ARS-WI), RG (MSU)		X	X	X	
- squasn	MM (CU)		Λ	Λ	Λ	
(b) Obj. 2. Genomic assisted breeding for disease resistance						
(*) * *,] = = = = = = = = = = = = = = = = = = =	Screen for resistance (Sc), develop	populatio	ons (P), pł	nenotype (F),	
2.1 QTL map resistances:	sequence (S), QTL map (Q)		· //1	51		
2.1.1. Watermelon						
- CGMMV	KSL (ARS-SC), AL (ARS-SC)	Sc	Sc,P	P,F,S	S,Q	
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	FSQ	Q			
race 2	PW (ARS-SC), AL (ARS-SC)	PFS	PFSQ	FSQ		
- gummy stem blight	CM (UGA), TW (NCSU)	Р	PFSQ	FQ		
- Phytophthora	SK (ARS-SC)	PFS	SQ			
- powdery mildew	SK (ARS-SC)	PFS	SQ	-		
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)	PF	FSQ	FSQ		
2.1.2. Melon		DE	DEC	FO	FO	
- powdery mildew	SK, PW (ARS-SC), JM (ARS-CA)	PF	PFS	FQ	FQ	
- Fusanum	PW(ARS-SC)	PFS E	PFS ES	FEO		
CMV	IM (ARS-CA), WW (ARS-CA) IM (ARS-CA), MM (CU)	Г D	гS F	15Q 80		
213 Cucumber	JWI (ARS-CA), WWI (CO)	1	T	JQ.		
- downy mildew	YW (ARS-WD TW (NCSID	PFS	SO	SO		
- Phytophthora	RG (MSU)	PF	PESO	so		
2.1.4 Squash			1152	~ ~		
- Phytophthora	MM (CU), CS (CU)	PF	PF	0		
- PRSV-W	MM	PFQ	Q	Ì		
- CMV	MM	PFQ	Q			
	Refine map (R) develop marker (M), verify (V)					
2.2 Marker development and verification:		1	1	1		
2.2.1. Watermelon		-				
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	R	RV	V	V	
race 2	PW (ARS-SC), AL (ARS-SC)			RM	RM	
- gummy stem blight	CM (UGA), IW (NCSU)			RMV	V	
- Phytophthora	SK (ARS-SC)			RM	V	
- powdery mildew	AL (ARS-SC) KSL (ADS SC)			RMV	v	
2.2.2 Melon	AL (ARS-SU), KOL (ARS-SU)	+	<u> </u>	IVI V	v	
2.2.2. INCION	SK (APS SC)			рм	V	
- Fusarium	PW (ARS-SC)	м	RM	RM	v	
- CVSDV	$WW(ARS_CA) W(ARS_CA)$	141	17141	RM	v	
- CMV	IM (ARS-CA) MM (CU)			RM	v	

	1	-			-
2.2.3. Cucumber					
- downy mildew	YW (ARS-WI), TW (NCSU)	RM	RM	V	V
- Phytophthora	RG (MSU)			RM	V
2.2.4 Squash					
- powdery mildew	MM(CU), LWB(UPR)	RM	V		
- Phytophthora	MM (CU)			RM	V
- PRSV-W	MM(CU), LWB(UPR)		RM	V	
- CMV	MM(CU), LWB(UPR)		RM	V	
	Develop breeding lines (B), introgr	ess into o	cultivated	(I),	
2.3. Introgress resistance into advanced breeding lines:	advanced lines (A), release to breed	ders (R)			
2.3.1. Watermelon					
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	В	Ι	IA	AR
race 2	PW (ARS-SC), AL (ARS-SC)	В	В	I	I
- gummy stem blight	CM (UGA), TW (NCSU)	B	B	Ī	Ī
- Phytophthora	SK (ARS-SC)	B	Ī	Ī	A
- powdery mildew	SK (ARS-SC)	B	T	T	A
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)	D	B	Ť	I
2.3.2 Melon			5	-	
- nowdery mildew	SK (ARS-SC) IM (ARS-CA)	в	T	T	IA
- Fusarium	PW (ARS-SC)	B	B	T	IA
- CYSDV	IM (ARS-CA) WW (ARS-CA)	Ĩ	Ī	IA	IAR
- CMV	IM (ARS-CA)	T	T	I	IA
2 3 3 Cucumber		-	-	1	
- downy mildew	YW (ARS-WD TW (NCSID	в	T	T	R
- Phytophthora	RG (MSID	B	B	I	I
2.3.4 Squash		D	D	1	1
2.3.4 Squash	Already exists				
Phytophthore	MM (CLD, CS (CLD)	т	т	٨D	٨D
	Almadu aviata	1	1	AK	AK
CMV	Already exists				
	Alleady exists				
(b) Obi 2 Economic impact analyzes disease control information					
(b) Obj. 5. Economic impact analyses, disease control information					
2.1. Derforment of the state of					
3.1 Perform economic analysis, cost of production/disease control		v	v		
3.1.1. Define, parameterize, simulate, validate production variables	LR (TAMU), MP (TAMU)	λ	Λ	37	N/
3.1.2. Estimate the potential economic impacts to the cucurbit industry	LR (IAMU), MP (IAMU)			Х	Х
3.2 Provide readily accessible information to facilitate disease			1		
control					
3.2.1. Develop a centralized cucurbit disease website	LQ (NCSU), JS (NCSU)	Х	Х		
3.2.2. Develop and post diagnostic resources and disease control	LO (NCSU) MH (MSU)	Х	Х	Х	Х
information in English and Spanish; prepare diagnostic poster	CS(CI) AIR(IPR)		Х	Х	
3.2.3 Provide disease alerts and forecasting tools		Х	Х	Х	Х
3.2.4. Field days and demonstration plots	Crop and extension teams	Х	Х	Х	Х

Crop and disease	Sources of resistance	Elite germplasm for introgression	Field testing locations	Resistant parental line	Phenotypic data for GWAS	Segregating populations	Analysis of inheritance	QTL analysis segregating populations	Marker development	Introgression into cultivated types	Advanced breeding lines for release	Cultivars for release to farmer
Watermelon												
Fusarium race2 (Fus)	PI 482246-USVL246 ^{FR2} ; PI 482252-		SC									
	USVL252 ^{FR2}			Х	х	Х				х		
Fusarium race 1	Calhoun Gray		SC	х	х	Х	Х	X		Х		
Gummy stem blight	PI 482276-UGA1081;	NC, Standard: Charleston Gray	NC, GA			v				v		
(GSB) Dhutanhthann (Dhut)	PI 526223-UGA157			X	x	X				X		
Phytophthord (Phyt)	PI 494531-USVL531MDR;	icebox: Sugar Baby	SC, NC	х	х	X				X		
(DM)	PI 560003- USVL003MDR		SC, NC	v		v	v			v		
(FW) CGMMV	Currently evaluating		GHÞ	~		~	~			~		
PRSV-W	PI 595203		SC	х	x	x	х					
Melon			50	~	~	~	Λ					
Powdery (PM)	MR-1		CA1.2. AZ	х		х	Х					
Fusarium (Fus)	MR-1	Cantaloupe: TopMark, Impac	CA1	х		Х	Х			х	х	
CYSDV	PI 313970; TGR1551	Honeydew: Green Flesh	CA1, AZ	Х	х	х	х					
CMV	PI 161375; Freeman cucumber	Honeydew or PMR Honeydew	CA1,2, AZ				Х					
Cucumber												
Downy mildew (DM)	PI 197088; PI 330628	Slicer: Poinsett 76	WI <i>,</i> NC	Х		Х	Х	Х		Х	Х	
Phytophthora (Phyt)	PI 109483	Pickling: NC-25, GY14	MI, NY		х	Х						
Squash												
Phytophthora (Phyt)	PI 211996; PI 483347; PI 634693	Butternut: Burpee Butterbush	NY	Х			Х					
Powdery (PM)	C. martenezii	— Tropical pumpkin: Soler Taina	PR	х			Х	х	х	х	х	х
PRSV-W	Menina, Nigerian Local	— Dorada	PR	Х			Х			х	Х	Х
CMV	Menina, Nigerian Local		PR	Х			Х			Х	Х	Х

Status of resistance breeding for the priority cucurbit diseases at project outset.

PROJECT METRICS

Metrics to be used in CucCAP project evaluation

Short term metrics (1-2 years)

- 1. State of the art, genotyping by sequencing (GBS) and data analysis platforms are developed for cucurbit species.
- 2. GBS sequence data are obtained for 1000-1600 PIs for each of the four cucurbit crops.
- 3. Community-standardized cucurbit gene/trait descriptors and nomenclature are established.
- 4. Germplasm lines with resistance to *Fusarium* r.1,2, *Phytophthora*, powdery mildew, and PRSV are established for watermelon; for CYSDV in melon, and *Phytophthora* in cucumber.
- 5. Markers developed for KASP-based assay for downy mildew in cucumber and powdery mildew and ZYMV in squash.
- 6. Field trials and field days are held to test and demonstrate disease resistant materials (average 1/yr/crop).
- 7. Representative farms are developed for economic analyses for three locations for each of the four commodities.
- 8. The CucCAP Cucurbit Disease Extension Website is established.
- 9. Participation in outreach to 15-20 stakeholder groups per year via industry events and field days.
- 10. A Cucurbit Genomics and Bioinformatics workshop is delivered at PAG 2017 attended by members from at least 20 cucurbit research laboratories.

Medium term metrics (3-4 years)

- 1. Population structure analysis is performed and molecular-directed core populations are established for the four cucurbit crops.
- 2. Breeder-friendly databases to store and distribute genomic, phenotypic, and genotypic information and development of associated data analysis tools are implemented (www.icugi.org). Based on current traffic, at least 1000 unique visitors are expected per week.
- 3. GWAS analyses are performed for CGMMV, *Fusarium*, gummy stem blight, *Phytophthora* and PRSV-W in watermelon; CYSDV in melon and *Phytophthora* in cucumber.
- 4. QTL associated with CGMMV, *Fusarium* r.2, gummy stem blight, *Phytophthora*, powdery mildew, and PRSV in watermelon; CMV, CYSDV, *Fusarium* and powdery mildew in melon; downy mildew, *Phytophthora* in cucumber; and CMV, PRSV and powdery mildew in squash have been identified.
- 5. Molecular markers have been developed for *Fusarium* r.1 in watermelon; CMV, CYSDV, *Fusarium* and powdery mildew in melon; downy mildew in cucumber; and CMV, PRSV and powdery mildew in squash.
- 6. Breeding lines with resistance to *Fusarium* r.1,2 and PRSV in watermelon; CMV, CYSDV, *Fusarium* and powdery mildew in melon; downy mildew in cucumber; and *Phytophthora* in butternut squash are available to researchers and seed companies.
- 7. Cucumber lines carrying multiple disease resistances (downy mildew/powdery mildew/ZYMV) developed by marker assisted selection.
- 8. Field trials and field days are held to test and demonstrate disease resistant materials (average 2/yr/crop).
- 9. Cucurbit disease informational materials in English and Spanish are developed and posted on the CucCAP disease website for each of the priority diseases.
- 10. Stakeholders use website and social media tools to obtain information about disease outbreaks, diagnosis and control. Based on prior experience with cucurbit disease tracking and informational websites, 1000-2000 hits per week are expected during peak growing season.
- 11. Participation in outreach to 15-20 stakeholder groups each year via industry events and field days.
- 12. Representative farms are compared to information available through USDA agencies and Extension service budgets for accuracy, and inputs are used to develop stochastic economic feasibility model by region.
- 13. Scenarios developed using project findings are run through economic feasibility models.
- 14. The Cucurbitaceae 2018 conference is hosted, expected attendance of 200-250 international cucurbit scientists from public and private sector.
- 15. Cucurbit genomics workshops are delivered at PAG 2018, 2019 and Cucurbitaceae 2018; expected attendance at Cucurbitaceae 2018, 100-200 people.
- 16. 15 graduate students and 3 post-docs are trained in cucurbit genetics, genomics, disease and economic analysis.
- 17. 4-5 refereed articles are published by each crop group

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

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TEAM PROGRESS REPORTS

and

PLANS FOR THE COMING YEAR

Genomics and Bioinformatics Team

Team members:

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

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

Work in progress and plans

1.1. Develop genomic and bioinformatic platforms for cucurbit crops

1.1.1. Genotyping by sequencing

In closely working with Cornell Genomic Diversity Facilty, we have set up the genotyping-by-sequencing (GBS) platform for the cucurbit species.

1.1.2. Sequence data processing/analysis

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

1.1.3. ICuGI database development

We have re-implemented the ICuGI database (now named Cucurbit Genomics Database (CuGenDB), and the new URL: <u>http://cucurbitgenomics.org/</u>) using the GMOD Tripal system (<u>http://gmod.org/wiki/Tripal</u>) and the Chado database schema (<u>http://gmod.org/wiki/Chado</u>). The newly designed and developed database was released in May 2017. Currently the database contains genome sequences of melon, watermelon (97103 and Charleston Gray), cucumber (Chinese Long and Gy14), wild cucumber (*Cucumis sativus* var. *hardwickii* PI 183967), four *Cucurbita* species (*C. pepo, C. maxima, C. moschata and C.*

argyrosperma) and bottle gourd. Genome syntenies between any two of the sequenced cucurbits have been identified and a synteny viewer have been implemented in the database. An "expression" module has been developed in the database using RNA-Seq datasets publicly available for cucurbit species, mainly collected from NCBI Sequence Read Archive (SRA). A set of tools to mine and analyze the RNA-Seq datasets, such as heatmap view of expression profiles and differential gene expression analysis, were implemented. The synteny viewer and the expression module have been packed as Tripal extension modules which can be implemented in other genomic databases developed using the Tripal system. Development of tools and interfaces to analyze and integrate genotype and phenotype data is ongoing. A manuscript describing the database has been published (Zheng et al., 2019, Nucleic Acids Research, 47:D1128).

1.1.4 Community standardized nomenclature. This is in progress.

1.1.5. Genomic, bioinformatics workshops

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

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

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

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

Batch	DNA plate No.	Multi-plex Level	Сгор	DNA Submission Date	Data Release Date
1	8	96	cucumber	4/13/2016	7/12/2016
2	9	96	cucumber	5/2/2016	7/12/2016
3	11,12,13,14	384	cucumber	8/24/2016	10/18/2016
4	2,5,6,16	384	cucumber	9/23/2016	11/21/2016
5	1,4,7,15	384	cucumber	10/3/2016	11/21/2016
6	31,34,35,36	384	watermelon	10/19/2016	11/21/2016
7	37,38,39,40	384	watermelon	10/31/2016	1/3/2017
8	41,42,43,44	384	watermelon	11/4/2016	2/15/2017

Table 1 Summary of cucurbit GBS

9	49	96	melon	12/8/2016	3/16/2017
10	3,10,17,46	384	cucumber	1/20/2017 & 2/2/2017	5/31/2017
11	50,51,52,53	384	melon	2/14/2017	5/5/2017
12	54,55,56,57	384	melon	2/22/2017	5/5/2017
13	58,59,60,61	384	melon	3/2/2017	5/5/2017
14	62,63,64,65	384	melon	3/16/2017	5/5/2017
15	66,67,68,69	384	melon	3/23/2017	5/5/2017
16	21,32,33,70	384	melon & watermelon	3/23/2017	5/31/2017
17	71,72,73,74	384	1melon&3squash	4/19/2017	6/13/2017
18	75,76,77,78	384	squash	5/31/2017	7/11/2017
19	22,23,79,80	384	squash	8/18/2017	9/25/2017
20	18,19,28,29	384	cucumber	1/24/2018	3/5/2018
21	90	96	watermelon	2/8/2018	3/5/2018
22	91	96	watermelon	2/8/2018	3/5/2018
23	92	96	watermelon	2/8/2018	3/5/2018
24	93	96	watermelon	2/8/2018	3/5/2018
25	94	96	watermelon	2/8/2018	3/5/2018
26	81,82	192	C. maxima	3/1/2018	3/22/2018
27	83,84	192	C. maxima	3/1/2018	3/22/2018
29	27	96	C. maxima	3/20/2018	10/9/2018

Note: Those in yellow background are samples from mapping populations.

T	ab	ole	2	S	ummarv	of	cucurbit	accessions	genotyr	oed	using	GBS
_			_	~					B			

	melon	cucumber	watermelon	С. реро	C. moschata	C. maxima
Total No. of plants genotyped	2090	1604	1377	854	318	463
No. accessions with low reads	5	3	11	0	0	0
No. accessions genotyped more than once	8	36	1	1	4	0
Final No. accessions genotyped	2077	1564	1365	852	314	463

Table 3 Summary of GBS sequencing and called SNPs

	melon	cucumber	watermelon
Total good barcoded reads	1,712,021,164	1,565,948,724	884,151,231
Total reads covering tags (>=10)	1,606,338,621	1,441,002,859	828,388,082
Mapped reads	1,260,870,721	1,054,491,682	551,465,448
Unmapped reads	345,467,900	386,511,177	276,922,634
Total tags	54,192,089	76,860,960	34,621,369
Tags with >= 10 reads	743,545	593,678	388,298

Mapped tags	373,133	351,594	246,506
Unmapped tags	370,412	242,084	141,792
No. raw SNPs	89,377	114,338	62,258
No. SNPs at missing rate < 0.5	62,789	91,092	41,601
No. SNPs at missing rate < 0.5 and MAF > 0.01	27,846	23,828	25,930

A core collection selection strategy has been developed. Briefly, a total of ~400 accessions will be selected for each species. Around 300 accessions which represent the majority of the genetic diversity of the germplasm, based on the core collection analysis using GenoCore (Jeong et al., 2017, PLoS ONE 12:e0181420), will be selected. Another ~100 accessions with interesting traits and/or parents of mapping/breed populations will be selected. In the final core collection, if a selected line is known to be derived from a PI accession that is also in the final core collection, then the corresponding PI should be replaced with the most closely related one on the phylogenetic tree. Accessions in the final core collection whose genomes have already been resequenced should also be replaced by the most closely related ones on the phylogenetic tree, unless they harbor very interesting/important traits.



Figure 1. Principal component analysis of the melon core collection (red) and the entire collection (gray)

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

1.2.2. Population genomics and GWAS analyses

Using SNPs called from the GBS data, we have performed population genomic analyses for cucumber, watermelon and melon accessions. Phylogenetic, PCA and population structure analyses have been done for accessions of cucumber, watermelon and melon. The results from these analyses for watermelon accessions are shown in **Figure 2** as an example. Linkage disequilibrium (LD) decay patterns and population differentiation have also been investigated for these species.



Figure 2. Phylogenetic relationship and population structure of Citrullus spp. accessions. (a) Maximumlikelihood tree of 1,367 Citrullus spp. accessions. (b) Modelbased clustering analysis with K from 2 to 5. Each accession is represented by a vertical bar. Each color represents one ancestral population, and the length of each colored segment in each vertical bar represents the proportion contributed by ancestral populations. (c) Principal component analysis of 1,367 watermelon accessions with PC1 and PC2 explaining 63.7%, and 2.1% of variance. (d) Principal component analysis of C. lanatus and C. mucuosospermus accessions with PC1 and PC2 explaining 4.6% and 2.3% of variance.

We have collected historical phenotype data from the USDA National Plant Germplasm System for cucumber, watermelon and melon accessions. GWAS have been performed to identify SNPs and regions that are significantly associated with important agronomic traits. GWAS for watermelon resistance to powdery mildew race 2 is shown in **Figure 3** as an example.

Manuscripts reporting the results from population genomics and GWAS analyses as well as core collection development for cucumber has been published (Wang et al., 2018, Horticulture Research 5:64), for watermelon has been submitted, and for melon is under preparation. Analysis of the GBS data for *Cucurbita* species is underway.



Figure 3. Genome-wide association studies (GWAS) of resistance to powdery mildew race 2 in stem (left) and leaf (right) of watermelon.

1.2.3 Genomic resequencing of core collections

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

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



Figure 4. Sequencing depth (based on the final cleaned data) of 96 cucumber accessions (left) and 21 C. pepo accessions (right).

Watermelon Team

Team members: Amnon Levi (USDA, ARS) Shaker Kousik (USDA, ARS) Kai-shu Ling (USDA, ARS)

Cecilia McGregor (Univ. Georgia) Pat Wechter (USDA, ARS) Todd Wehner (North Carolina St. Univ.)

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

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

Objective	Personnel/Institution	Year			
	(initials as in Table 3)	1	2	3	4
(a) Obj. 1. Develop common genomic approaches and tools for					
cucurbits					
1.2. Perform GBS analysis of PI collections, establish core					
populations, provide community resource for genome wide					
association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core	ZF (BTI), RG (MSU)	Х	Х		
populations					
- watermelon	AL (ARS-SC), TW (NCSU)	Х	Х		
1.2.2 Population genetics and GWAS analysis	UR (WSVU), ZF (BTI)		х	х	х
- watermelon	AL (ARS-SC)				
(b) Obj. 2. Genomic assisted breeding for disease resistance					
	Screen for resistance (Sc), develop	populatio	ons (P), pł	nenotype ((F),
2.1 QTL map resistances:	sequence (S), QTL map (Q)				
2.1.1. Watermelon					
- CGMMV	KSL (ARS-SC), AL (ARS-SC)	Sc	Sc,P	P,F,S	S,Q
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	FSQ	Q		
race 2	PW (ARS-SC), AL (ARS-SC)	PFS	PFSQ	FSQ	
- gummy stem blight	CM (UGA), TW (NCSU)	Р	PFSQ	FQ	
- Phytophthora	SK (ARS-SC)	PFS	SQ		
- powdery mildew	SK (ARS-SC)	PFS	SQ		
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)	PF	FSQ	FSQ	
2.2 Marker development and verification:	Refine map (R) develop marker (M), verify	(V)		
2.2.1. Watermelon					
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	R	RV	V	V
	Develop breeding lines (B), introgr	ess into c	ultivated	(I),	
2.3. Introgress resistance into advanced breeding lines:	advanced lines (A), release to breed	lers (R)			
2.3.1. Watermelon					
- Fusarium race 1	AL (ARS-SC), PW (ARS-SC)	В	Ι	IA	AR
race 2	PW (ARS-SC), AL (ARS-SC)	В	В	Ι	Ι
- gummy stem blight	CM (UGA), TW (NCSU)	В	В	Ι	Ι
- Phytophthora	SK (ARS-SC)	В	Ι	Ι	Α
- powdery mildew	SK (ARS-SC)	В	Ι	Ι	Α
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)		В	Ι	Ι

Work in progress and plans

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

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

Core populations.

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

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

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

Selfing of PIs to form core population is in progress.

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

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

Genetic mapping of QTL associated with resistance to Fusarium wilt race 2- Two-hundred and twenty $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R(s₃) were constructed in collaboration with Dr. Nihat Guner and team at Sakata Seeds. The 220 families were evaluated for resistance to Fusarium wilt (FW) race 2 resistance (Figure 1) in two separate experiments at the U.S. Vegetable Laboratory. The distribution of FW race 2 resistance in the population indicates polygenic inheritance (Figure 2). Genotyping-by-sequencing (GBS) of the $F_{2:3}$ population identified a major QTL on Chromosome 1 of USVL-252^{FR2} associated with resistance to FW race 2. KASP markers are being developed and will be validated for utility of incorporating the resistance into the genetic background of watermelon cultivars. We conducted a genetic mapping study to identify quantitative trait loci (QTLs) associated with resistance to *Fon* race 1 in segregating populations ($F_{2:3}$ and recombinant inbred lines) of *Citrullus amarus* (citron melon) derived from the *Fon* race 1 resistant and susceptible parents, USVL246-FR2 and USVL114, respectively. A major QTL (*qFon1-9*) associated with resistance to *Fon* race 1 was identified on chromosome 9 of USVL246-FR2. This discovery provides an additional host-resistance source of resistance to *Fon* race 1 in watermelon and as it co-locates with the QTL for *Fon* race 2 resistance in the same population, may provide non-race specific resistance (Branham et al. 2017, 2019).



Figure 1. Two-hundred and twenty $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R(_{S3}) being evaluated for Fusarium wilt race 2 in a greenhouse at the U.S. Vegetable Laboratory (Summer, 2017).



Figure 2. Distribution of $F_{2:3}$ families derived from the cross USVL-252^{FR} x PI 244019-PRSV-R(_{S3}) for resistance to Fusarium wilt (FW) race 2 (Left). A major QTL associated with FW race 2 resistance on Chromosome 1 of USVL-252^{FR} (right).

Converting QTL to Kompetitive Allele Specific PCR **KASP markers tightly linked to Fusarium wilt race 1 resistance**- DNA of the resistant and susceptible parents (*C. lanatus*) and the F_2 parental plants of the most resistant versus the most susceptible $F_{2:3}$ families (Lambel et al. 2014) were used for a QTL-seq analysis. QTL-seq narrowed the *Fon* race 1 QTL interval on chromosome 1 of watermelon (Lambel et al. 2014) by 500 kb (Branham et al. 2018). SNPs from the interval were converted to KASP primers. The KASP primers were used in genetic mapping of the same population used for the initial mapping of QTL associated with FW race 1 resistance (Lambel et al. 2014). QTL mapping yielded several KASP markers tightly linked to race 1 resistance and narrowed the QTL interval further from 1.56 Mb to 315 kb (Figure 3). In collaboration with the HM.Clause team in Davis, California we conducted QTL-seq and developed KASP markers tightly linked to FW race 1 resistance (Figure 3; Branham et al. 2018). We have developed KASP markers for *Fon* races 1 and 2 QTL in *C. amarus*. The FW races 1 and 2 resistant lines USVL246-FR2 and USVL252-FR2 were crossed with Charleston Gray, Calhoun Gray and Sugar Baby to generate F_1 , F_2 , BC₁ and BC₂ F_2 . The KASP markers will be used to incorporate resistance to *FW* races 1 and 2 into the genome background of watermelon cultivars.



Figure 3. KASP markers tightly linked to Fusarium wilt race 1 resistance (qFon1-1) on chromosome 1 of watermelon (Branham et al. 2018).

What do you plan to do during the next reporting period to accomplish the goals?

-Complete development and validation of KASP markers and incorporate FW races 1 and 2 resistance from USVL246-FR2^{FR} and USVL-252^{FR} into genomic background of watermelon cultivars (Wechter, Branham and Levi).

Papaya ringspot virus (PRSV) resistance (Amnon Levi, Kai-shu Ling, and Sandra Branham, USDA, ARS, U.S. vegetable Laboratory (USVL), Charleston, SC)

Identifying QTL associated with PRSV resistance

Several F_2 and BC_1 populations derived from the cross USVL-252^{FR} x PI 244019-PRSV-R(_{S3}) were constructed in collaboration with Dr. Nihat Guner and team at Sakata Seeds. The genetic populations were evaluated for resistance to PRSV-resistance at the U.S. Vegetable Laboratory. The distribution of PRSV-resistance in the population confirmed inheritance by a single homozygous recessive gene in PI 244019 (Guner, 2004; Guner and Wehner, 2008). Genotyping-by-sequencing (GBS) of an F_2 population identified a major QTL on Chromosome 3 of PI 244019 associated with PRSV-resistance (Figure 4). The major QTL interval comprises several ribosomal genes, among them the eukaryotic elongation factor eIF4E known to be associated with resistance to potyviruses in cucurbit crops (Ling et al. 2009). KASP markers are being developed and will be used for incorporating the resistance into the genomic background of watermelon cultivars.

What do you plan to do during the next reporting period to accomplish the goals?

-Complete development of KASP markers tightly linked to PRSV-resistance in PI 244019-PRSV-R(_{S3}) and use them to incorporate resistance into genome background of watermelon cultivars (Levi, Ling, Branham).



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

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

Inheritance of powdery mildew resistance, identification of QTL and RNAseq

USVL608-PMR (S₆), a red fleshed watermelon line with high levels of resistance to PM was used as the female parent (P₁) and crossed with USVL677-PMS which is highly susceptible (P₂). The parents, F₁, backcrosses to both parents (BC₁, BC₂) and a large F₂ population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. All susceptible parent (USVL677-PMS) plants were rated >7 [mean disease severity (DS) = 94%], whereas most resistant parent (USVL608-PMR) plants were rated as 1 (DS=2.5%). Majority of the BC₁ plants were rated ≤2 and considered as resistant. Of the 466 F₂ plants, 221 were rated ≤2 (DS=3.1%). Of the 76 BC₂ plants, 23 were rated ≤2 (DS=2.9%). Chi-square analyses of the observed segregation of phenotypes for the F₂ plants indicated that two genes control PM resistance with a good fit for a 7:9 resistance to susceptibility ratio. The proposed model for this ratio is two genes with one recessive for high resistance and one dominant for high resistance. This is supported by a backcrossing segregation ratio of 1:3. We have observed some highly and moderately resistant plants in the F₂ indicating the cumulative effect of the two
genes. QTL-seq analysis on the most resistant and most susceptible DNA bulks from the F_2 populations identified a major QTL in chromosome 2.

We have also completed RNA-seq analysis of the parents during PM infection. Plants of the resistant line USVL608-PMR and the susceptible line USVL677-PMS were with inoculated with 10⁵ conidia^{-ml} of *P.xanthii*. Symptom development was observed every day. In addition, leaf samples were collected for microscopy and for RNA extraction. RNA-seq profiling was done on leaf samples collected at 0, 1, 4, and 9 days post inoculation (DAI). Powdery mildew symptoms were visible on USVL677-PMS 4 DAI whereas leaves of USVL608-PMR were clean. We have completed RNA-seq on all these samples. Data analysis is in progress. A quick analysis of the differentially expressed genes (DEG) indicated several resistance genes in chromosome 2.

We also completed inheritance studies on the egusi type watermelon (*C. mucosospermus*) line USVL531-MDR. This line was found to be resistant to 11 PM isolates from across the U.S.A. and was released by USDA ARS in 2018. This line was used as the female parent (P₁) and crossed with USVL677-PMS which is highly susceptible (P₂). The parents, F₁, backcrosses to both parents (BC₁, BC₂) and a large F₂ population were inoculated with a local isolate of PM and assessed for disease severity on a 0-10 scale of increasing disease severity. The susceptible parent (USVL677-PMS) had mean disease severity of 8.14 on the 0-10 scale, whereas it was 1.17 for the resistant parent. Segregation patterns point to single gene inheritance, but also indicated another gene is inherited maternally. Chi-square analyses of observed segregation of phenotypes for the F₂ populations fit models for these gene models and were further supported by segregation patterns in the backcross populations. QTL-seq analysis on the extremes from the F₂ populations and RNA-seq analysis of the parents during PM infection are being conducted to identify the chromosomal regions involved in resistance. USVL531-MDR will serve as a useful source to incorporate PM resistance into commercial cultivars. We have developed several red fleshed resistant lines (at F₇) using USVL531-MDR as the source of resistance.

Advancing Powdery mildew resistant inbred lines

Fruit from F_2 plants from a cross of USVL531-MDR and USVL677-PMS with powdery mildew resistance, uniform red flesh and decent brix (>7) were collected and have been advanced till F_6 and further advancement to F_7 is in progress. We are currently evaluating 10 red fleshed F_7 lines that were homozygous for resistance. We completed assessment of fruit quality from F_5 and F_6 progenies that were homozygous for resistance to PM and had red flesh and brix >7 in 2018.

Identifying and developing multiple disease resistant lines from accessions

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

In 2018 we released four red fleshed lines with high levels of resistance to powdery mildew. We have completed making crosses with these powdery mildew resistant lines (USVL608-PMR, USVL313-PMR, USVL585-PMR and USVL225-PMR) with USVL677-PMS and 'Dixie Lee' to develop populations for conducting inheritance studies in 2019 and developing resistant inbred lines with high fruit quality. A paper documenting the release of these four PM resistant watermelon lines was published in HortScience in 2018.

Phytophthora fruit rot of watermelon (Shaker Kousik; USDA, ARS, U.S. Vegetable Laboratory,

Charleston, SC)

Inheritance of resistance, identification of QTL and RNAseq

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

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

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

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

Advancing resistant inbred lines

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

Project metrics (time line) for research on Phytophthora fruit rot and powdery mildew of watermelon

- Develop germplasm lines with resistance to Phytophthora fruit rot and powdery mildew for watermelon: **Completed.**
- Develop populations for phenotyping resistance to Phytophthora fruit rot and powdery mildew of watermelon: **Completed**
- Sequence and map Phytophthora fruit rot and powdery mildew QTL in watermelon: In progress.
- Introgress Phytophthora and powdery mildew resistance into cultivated type watermelon: In progress
- Participation in outreach to stakeholder groups per year via industry events and field days. **Completed**

<u>Watermelon gummy stem blight resistance</u> (Luis Rivera and Todd C. Wehner; NC State Univ.; Cecilia McGregor, University of Georgia, Athens, GA)

Inheritance of resistance, identification of QTL

Wehner

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

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



Figure 3. Field test for resistance to gummy stem blight

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

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

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



Figure 4. Gummy stem blight spore mass production and identification through PCR and electrophoresis



Inheritance of resistance, identification of QTL

McGregor

Population Development:

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

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

Phenotyping:

- WPop GSB1: PI 482276 x Crimson Sweet population of 178 $F_{2:3}$ lines (15 plants x 178 lines = 2670 plants) was phenotyped in a growth chamber using *C. citrilli* isolate 12178A (GA). Disease symptoms for each seedling were scored on a 0-5 scale and BLUPs calculated. Parents and F_1 and 4 other control genotypes were also included. **Complete**
- WPop GSB2: PI 189225 x Sugar Baby. This population is currently being phenotyped. In progress
- ZxD population (ZXRM x PI PI 244019). This population is currently being phenotyped. **In progress**

Genotyping and QTL mapping:

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

Goals for 2018-2019

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

<u>Cucumber green mottle mosaic virus</u> (Kai-shu Ling and Amnon Levi USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC)

- We have completed the initial screening of USDA watermelon germplasm (~1,400 accessions). In the repeated test, several selected lines showed promising level of tolerance to CGMMV (without visible symptom). However none of them was immune to CGMMV, the virus titer were detectable in the tolerant plants using ELISA tests.
- We made single plant selection of the promising lines and are developing segregating populations through crossing. S2 seeds have been generated from one of the most promising *Citrullus colocynthis* line.

- Seeds from seven PI lines with potential for resistance (tolerance) to CGMMV have been sent to the collaborator to generate plant tissue for support the re-sequencing efforts under the CucCAP project.
- We submitted a release notice 'Virus-resistant desert watermelon (*Citrullus colocynthis*) germplasm line 'USVL18-157VR' useful for enhancing CGMMV-resistance in watermelon cultivars. The release notice is currently in the process of review and approval by USDA, ARS, National Program Leaders (NPL).

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

Test Item Number Taxon		Seed
138	Citrullus colocynthis	30
145	Citrullus colocynthis	30
151	Citrullus colocynthis	30
157	Citrullus colocynthis	30
565	Citrullus lanatus	30
570	Citrullus lanatus	30
714	Citrullus lanatus	30

What do you plan to do during the next reporting period to accomplish the goals?

We are advancing through single plant selection of the most promising *Citrullus colocynthis* line to S3. Those seeds will be provided with the sponsoring seed companies to make crosses to your elite materials. In addition, once the F2, BC1 seeds are generated, materials from segregating populations will be used for Genotyping-by-sequencing or similar study to identify SNPs in association with the tolerance to CGMMV.

Melon Team

Team members:

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

Table 4. TIMELINE CHART							
Objective	Personnel/Institution	Year					
	(initials as in Table 3)	1 2 3			4		
(a) Obj. 1. Develop common genomic approaches and tools for cucurbits							
1.2. Perform GBS analysis of PI collections, establish core populations,							
provide community resource for genome wide association studies (GWAS)							
1.2.1. GBS of cucurbit species, establish molecular-informed core populations							
- melon	JM (ARS-CA)	Х	Х				
1.2.2 Population genetics and GWAS analyses	UR (WVSU), ZF (BTI)		х	х	х		
- melon	JM (ARS-CA)						

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

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

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

(b) Obj. 2. Genomic assisted breeding for disease resistance		Y1	Y2	Y3	Y4
2.1 QTL map resistances:	Screen for resistance (Sc), develop populations (P), phenotype (F				F),
	sequence (S), QTL map (Q)				
2.1.2. Melon					
- powdery mildew	SK,PW (ARS-SC), JM (ARS-CA)	PF	PFS	FQ	FQ
- Fusarium	PW (ARS-SC)	PFS	PFS	PFSQ	
- CYSDV	JM (ARS-CA), WW (ARS-CA)	F	FS	FSQ	
- CMV	JM (ARS-CA), MM (CU)	Р	F	SQ	

2.1.2. Melon

Powdery Mildew (Podosphaera xanthii) resistance in MR1xAY RIL

- Awaiting for growth chamber space availability for Race 2 test.
- Powdery Mildew (Podosphaera xanthii) resistance in Top Mark x PI 313970 F2:3
- Growth chamber test for resistance to race S is underway.
- Growth chamber test for resistance to race 1 scheduled to follow the race S test.
- Powdery Mildew (Podosphaera xanthii) California Field Tests

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

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

Downy mildew

• Initiated phenotyping resistance in the MR1 x AY RIL.

CYSDV

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

CMV

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

• Greenhouse evaluation–Controlled-inoculation tests at Salinas, Spring 2018: nine lines exhibited resistance: six lines were asymptomatic, with limited or no virus accumulation (Figure 1, Left), three lines exhibited only local lesions against a subgroup 1 CMV isolate from melon; the other 16 lines were susceptible with 15 lines exhibiting mosaic reactions.



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

• Field evaluation–Central Valley, University of California, Westside (Westside Research and Extension Center, Five Points; Planted 25 June 2018 for disease reactions to natural CMV-infection, adaptation, and fruit quality. Field was infested with melon aphid that was controlled with insecticide application. CMV was not present in the field; sampled plants were negative for the virus. The lines appeared to be poorly adapted to the Central Valley, CA, as indicated by plant size and condition. None of the lines exhibiting resistant reactions in the greenhouse were U.S. western shipper (USWS) type melons (Figure 2). Additional backcrossing is needed to combine CMV resistance with USWS type melon.



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

2.2 Marker development and verification

2.2 Marker development and verification:	Refine map (R) develop marker (M), verify (V)				
2.2.2. Melon					
- powdery mildew	SK (ARS-SC)			RM	V
- Fusarium	PW (ARS-SC)	М	RM	RM	V
- CYSDV	WW (ARS-CA), JM (ARS-CA)			RM	V
- CMV	JM (ARS-CA), MM (CU)			RM	V

Powdery Mildew

- Identified QTL on Chromosome 5 and 12 for resistance to Powdery mildew race 1.
- KASP markers for both Powdery mildew QTL have been developed and are in the process of being validated.
- KASP markers for sulfur resistance have been developed and are in the process of being validated.

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Fusarium wilt

• KASP markers have been developed for resistance to Fusarium wilt race 1 and Fusarium wilt race 2, and are being validated

CYSDV

• No progress to date.*

CMV

• No progress to date.

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

will be necessary to evaluate epidemiology of CCYV in the southwestern US desert production region, and to determine its impact on melon production and development of crinivirus-resistant cultivars. (See Wintermantel et al. 2019)

2.3 Introgress resistance into advanced breeding lines

2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines (B), introgress into cultivated (I), advanced lines (A), release to breeders (R)				
2.3.2. Melon					
- powdery mildew	SK (ARS-SC), JM (ARS-CA)	В	Ι	Ι	IA
- Fusarium	PW (ARS-SC)	В	В	Ι	IA
- CYSDV	JM (ARS-CA), WW (ARS-CA)	Ι	Ι	IA	IAR
- CMV	JM (ARS-CA)	Ι	Ι	Ι	IA

Melon USVL206 (derived from an MR-1 x AY cross) with resistance to Fusarium wilt race 1 and 2, sulfur resistance, powdery mildew race 1 resistance, and has orange sweet flesh has been crossed into Top Mark, Charentais and backcrossed into AY.

Cucumber Team

Team members:

Yiqun Weng (USDA, ARS)

Rebecca Grumet (Michigan St. Univ.)

Todd Wehner (North Carolina St. Univ.)

Objectives	Personnel/Institution	2016	2017	2018	2019
1. Develop genomic approaches and tools					
1.2. GBS PI lines; establish GWAS core	ZF (BTI), RG (MSU)	Х	Х		
	YW (ARS-WI)	Х	Х		
1.2.1. GBS of cucurbit species, establish molecular-	ZF (BTI), RG (MSU)	Х	Х		
informed core populations					
- cucumber	YW (ARS-WI)	Х	Х		
1.2.2 Population genetics and GWAS analysis	UR (WSVU), ZF (BTI)		Х	Х	х
- cucumber	YW (ARS-WI), RG (MSU)				
2. Genomic assisted breeding					
2.1 QTL map resistances	Sc=Screening, P=populations, F=phenotyping, S=sequence (S), Q=QTL				
2.1.3. Cucumber					
- downy mildew	YW (ARS-WI), TW (NCSU)	PFS	SQ	SQ	
- Phytophthora	RG (MSU)	PF	PFSQ	SQ	
2.2 Marker development and verification	R=Refining map, M=develop marker, V=	verificatio	n		
2.2.3. Cucumber					
- downy mildew	YW (ARS-WI), TW (NCSU)	RM	RM	V	V
- Phytophthora	RG (MSU)			RM	V
2.3. Advanced breeding line development	B=breeding line, I=introgression, A=advanced, R=release				
2.3.3 Cucumber					
- DM	YW (ARS-WI), TW (NCSU)	В	Ι	Ι	R
- PFR	RG (MSU)	В	В	I	Ι

1.2 GBS of PI collection, establish GWAS core

Personnel: Weng (Wang Y, Tan J, Dymerski R), Grumet (Grumet R, Hammar S.) and Wehner (Wehner T., Silverman EJ) Labs

GBS of PI lines and GWAS panel selection

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

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

Phenotyping of morphological traits and DM resistance in cucumber natural populations

Three hundred cucumber lines were grown in the University of Wisconsin Hancock Agricultural Research Station (HARS) for collection of morphological data. Meanwhile, 300 cucumber lines (2 reps, 6 plants per rep) were planted in North Carolina State University experimental field in summer 2018. Data for responses to DM natural infestation were collected. Unfortunately, only data form one rep were collected due to unforeseen natural disaster.

2019 work plan

- 1. Continue selfing and seed increase of the GWAS panel lines.
- 2. Seed increase of cucumber lines by self-pollination.
- 3. Prepare GWAS panel lines for re-sequencing (partial).
- 4. Approximately 250 lines in the GWAS panel will be planted in 2019 summer season in North Carolina State University fields for collecting data for responses to natural DM infestation.

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

Downy mildew (DM) (Weng and Wehner Labs)

2018 progress

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

 F_2 and RIL plants carrying respective QTL regions were selected to backcross with the susceptible cucumber line 9930. Near isogenic lines (NILs) for each QTL were developed in the susceptible 9930 genetic background. We have completed marker-assisted development of NILs for *dm4.1* and *dm5.2*. Secondary F_2 populations from crosses between resistant and susceptible NILs were developed, which were genotyped for DM inoculation responses in both field and controlled environments. The development of NILs for *dm5.3* has been advanced to BC₂.

In 2018, through QTL analysis in the secondary F_2 populations, the *dm4.1* and *dm5.3* loci have been delimited to ~60-80 kb intervals on chromosomes 4 and 5, respectively.

2019 work plan

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

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

Young fruit resistance to P. capsici

2018/19 progress:

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

i. An F2 population (n=397) of Gy14 X PI109483-53B from field grown plants, in summer 2017. ii. An F2 population (n=222) of Gy14 X doubled haploid (DH) line A4-3 grown in the greenhouse in spring 2018. The DH lines were generously produced by Rijk Zwaan from three resistant breeding lines derived from PI109483. Based on tests in summer 2017, DH A4-3, was chosen for further population development.

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

Three sets of harvests were performed for each experiment to provide replication in sampling dates, and at least 10 fruit per F_2 individual. The populations exhibited a normal distributions for disease scores, consistent with a quantitative trait. Individuals from each end of the distribution, representing the

most resistant and most susceptible plants were selected for bulk segregant QTL-seq analysis. Cleaned reads were aligned to the cucumber Gy14 version 2 reference genome (Weng et al., http://cucurbitgenomics.org/) and the Genome Analysis Toolkit pipeline (GATK; v3.6) (McKenna et al., 2010; https://software.broadinstitute.org/gatk/) used for identification of SNPs and indel variations. SNP-indices were calculated as described in Takagi et al. (2013) using QTL-seqr (Mansfeld and Grumet, 2018) and mapped across the cucumber genome. There was good correspondence between peaks observed on chromosomes 5 and 6 in the two field seasons. The greenhouse trial gave different peaks, suggesting possible environmental effects on response.



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

2019/20 work plan

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

Age-related resistance (ARR) to P. capsici

2018/19 progress:

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

<u>QTL mapping of ARR.</u> Doubled haploid (DH) lines derived from F_1 progeny of 'Gy 14' (ARR-) X 'Poinsett 76' (ARR+) were kindly produced by Rijk Zwan and used for QTL-seq analysis. Seed from 79 lines were planted in the greenhouse in 5 replicated blocks along with the two parental lines and F_1 progeny. Flowers were hand pollinated, and a single fruit per plant was harvested at 17 days post pollination. Fruits were inoculated with 12 equally spaced 30 µL droplets (10⁵ zoospores/ml) and scored at 7 days post inoculation (dpi) using a 0-5 point disease score (0 – no symptoms, 5 – extensive sporulation). A total of 424 fruit were phenotyped with a mean disease rating of 2.0 ± 0.1 . The parental lines were consistently either ranked resistant or susceptible with mean disease ratings of 0.3 and 3.0 for 'Poinsett 76' and 'Gy 14', respectively. Consistent with our prior studies suggesting a dominant major factor, the RIL population was bimodally distributed and F₁ fruit were largely resistant (1.1). High within-line variability of disease rating was observed in lines showing intermediate susceptibility, highlighting the need for reproducibility made available by using a fixed DH population. Fifteen of most resistant and susceptible lines were selected for a second trial in the greenhouse. The disease rating distributions of the two groups separated (Welch's T test, P = 0.003), with means of 2.1 (Resistant) and 4.0 (Susceptible). Eight resistant and susceptible lines which were consistently ranked in both experiments were selected for QTL-seq analysis.

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



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

2019/20 work plan:

Prepare publication describing infection response to *P. capsici* and early expression of defense on ARR-expressing fruit. Begin to examine QTL region on chromosome 3.

2.3 Advanced line development for downy mildew resistance <u>Marker-assisted QTL pyramiding</u> (Weng and Wehner Labs)

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

In 2019, we will:

- Continue marker-assisted backcrossing in Gy14 genetic background for pyramiding of *dm4.1* and *dm5.2* QTL from WI7120/PI 197088. Combine *dm3* into Gy14+*dm4.1*+*dm5.2* genetic background through marker assisted selection.
- 2) Conduct field and greenhouse screening tests to evaluate DM resistance and performance of horticulture traits.
- 3) Prepare public release of the introgression lines carrying *dm*4.1, *dm*5.2, and *dm*4.1+*dm*5.2 (in Gy14 background).

Breeding line development for DM resistance

(Wehner lab: T Wehner, EJ Silverman)

RIL development and evaluation of DM resistance.

The RILs population was developed in 2007 by a cross PI 197088 (HR) × Coolgreen (S). A total of 200 F_2 lines were generated and self pollinated in the greenhouse in 2009. The RILs have been tested in 7 years of field evaluations under high disease intensity. The 2017 population contains 146 lines; 71 at S12 generation, 35 at S11 generation, 32 at S10 generation, and 8 at the S9 generation. Several lines are being recovered and advanced for use in genetic studies.

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

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

In 2018, we evaluated the 127 sublines in S_8 to S_{13} for high resistance to the new downy mildew in the field in North Carolina. The design was a randomized complete block with 3 replications and 4 disease ratings. Sublines were rated for fruit traits such as skin type (smooth, cracked, netted) and spine color (black, white). The RILs ranged from 2.0 to 7.7 for best rating (0-9 scale) for DM resistance. The RILs were tested for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

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

Inbreds with resistance and quality

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

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

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

In 2018, we evaluated sublines for high resistance to the new downy mildew as well as fruit quality in the field in North Carolina. Lines were evaluated in a randomized complete block with 3

replications and 4 disease ratings. The RILs were selected for traits that make them suitable for use in crossing with elite breeding lines to develop resistant cultivars.

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

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

In 2017, 54 lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. Of those, 4 lines from Gy14, 3 lines from NC-25, and 2 lines from Poinsett 76 were advanced since they had resistance of 3 to 5 and quality of 5 to 7.

In 2018, lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. The most resistant lines with high fruit quality were advanced.

In 2019, lines (including checks) from the three populations (PI 197088 x Gy14, NC-25, or Poinsett 76) were tested for DM resistance (0-9 scale) and fruit quality (9-1 scale). The design was a randomized complete block with 3 replications and 4 disease ratings. The most resistant lines with high fruit quality and high yield were advanced. Those were 2 lines of Gy14, 4 lines of NC-25, and 2 lines of Poinsett 76.

Identify new sources of resistance.

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

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

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

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

Squash Team

Team members:

Michael Mazourek (Cornell Univ.) Linda Beaver (Univ. Puerto Rico)

Angel Linares (Univ. Puerto Rico) Chris Smart (Cornell Univ.)

Objective	Personnel/Institution	Year			
	(initials as in Table 3)	1	2	3	4
(a) Obj. 1. Develop common genomic approaches and tools for					
cucurbits					
1.2. Perform GBS analysis of PI collections, establish core					
populations, provide community resource for genome wide					
association studies (GWAS)					
1.2.1. GBS of cucurbit species, establish molecular-informed core	ZF (BTI), RG (MSU) X		Х		
populations					
- squash	MM (CU)	Х	Х		
1.2.2. Population genetics and GWAS analysis	UR (WVSU), ZF (BTI)		Х	Х	Х
- squash	MM (CU)				
(b) Obj. 2. Genomic assisted breeding for disease resistance					
	Screen for resistance (Sc), develop populations (P), phenotype (F)				F),
2.1 QTL map resistances:	sequence (S), QTL map (Q)				
2.1.4 Squash					
- Phytophthora	MM (CU), CS (CU)	PF	PF	Q	
- PRSV-W	MM	PFQ	Q		
- CMV	MM	PFQ	Q		
2.2 Marker development and verification:	Refine map (R) develop marker (M), verify	(V)		
2.2.4 Squash					
- powdery mildew	MM(CU), LWB(UPR)	RM	V		
- Phytophthora	MM (CU)			RM	V
- PRSV-W	MM(CU), LWB(UPR)		RM	V	
- CMV	MM(CU), LWB(UPR)		RM	V	
	Develop breeding lines (B), introgra	ess into c	ultivated	(I),	
2.3. Introgress resistance into advanced breeding lines:	advanced lines (A), release to breeders (R)				
2.3.4 Squash					
- powdery mildew	Already exists				
- Phytophthora	MM (CU), CS (CU)	Ι	Ι	AR	AR
- PRSV-W	Already exists				
- CMV	Already exists				

Establish core GWAS populations

<u>1.2.1.</u>, <u>1.2.2 GBS of cucurbit species, establish molecular-informed core populations and 1.2.2.</u> <u>Population genetics and GWAS analysis</u>

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

Three projects are already taking advantage of the GWAS population. For CucCAP, given the lack of phenotypic data, we have phenotyped the collection for qualitative traits of bush growth habit and hull-less seeds. Markers were created with this material validated in breeding populations to include as part of

the MS. A separate study is mapping cotyledon cucurbitacin content to support results from biparental populations.

Powdery mildew resistance in squash

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

Virus resistance in squash

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

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

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

Inheritance of resistance to PRSV:

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

Resistant to susceptible segregations in F_2 populations were variable, depending on how severity scores were grouped into the resistant versus susceptible classes. The most consistent results over similar types of crosses occurred when we grouped plants with an overall severity rating of ≤ 4 as resistant and grouped plants with an overall severity rating of ≥ 5 as susceptible. This grouping system also made biological sense since plants with ratings of ≥ 5 had high individual leaf severity scores, especially in leaves 4 and 5. Both F_2 crosses made with 'Nigerian Local' fit a 7:9 (R:S) genetic model while all three crosses using 'Menina' fit a 13:3 model (Table 1). The resistant x resistant cross (Nigerian Local x Menina) fit a 15:1 model. These segregations suggest that at least two genes are involved in the inheritance of resistance to PRSV for both 'Nigerian Local' and 'Menina'. The data clearly indicate that at least some of the genes for resistance in 'Nigerian Local' and 'Menina' are different. The resistance conferred by 'Menina' seems to be superior to that of 'Nigerian Local'.

An important consideration when evaluating disease resistance in the greenhouse is the association between greenhouse readings and readings taken in the field. In breeding for PRSV resistance both symptom severity and ELISA readings can be used as a way to evaluate resistance. In a previous CucCAP report we reported correlations between greenhouse and field ELISA readings to be poor. However, we have since looked at this issue from a different point of view. A high correlation *per se* is not important as long as plants judged resistant (or susceptible) in the greenhouse are also judged as

resistant (or susceptible) in the field. Figure 2 presents greenhouse and field data from 2017. Data from 2016 showed a similar trend. All plants of genotypes known to be susceptible (Mos166, Waltham Butternut and Taína Dorada) fell into the upper right-hand quadrant, meaning they were classified as susceptible in both the greenhouse and field according to their ELISA reading. The results for genotypes known to be resistant (Nigerian Local and Menina) were not as clear. For these genotypes, ELISA readings in the greenhouse were often expectantly high (positioned in the lower right-hand quadrant), while readings in the field were low. However, it should be noted that in this trial greenhouse readings were taken at 18 days post-inoculation (dpi) on the 3rd leaf. Since carrying out this study we have found that greenhouse ELISA readings for PRSV are best taken on the 4th leaf at about 21 dpi (PRSV ELISA readings tend to be high for all genotypes in the first few leaves).



Figure 1. Distributions of severity ratings in F_2 populations of tropical pumpkin (*Cucurbita moschata*) inoculated with *Papaya ringspot* virus (PRSV). Populations developed with resistant parent 'Nigerian Local' are shown on the left; populations developed with resistant parent 'Menina' are shown on the right. For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.



Figure 2. Distribution of combined severity ratings of plants (n=238) from the Nigerian Local x Menina F_2 population inoculated with *Papaya ringspot* virus (PRSV). For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12.

		Obs	erved			
		segre	gation	Tested ratio		
Genotype	Population	\mathbb{R}^1	\mathbf{S}^1	(R:S)	χ2	Prob.
Nigerian Local	Res. parent	34	0			
Menina	Res. parent	50	0			
Taina Dorada	Sus. parent	2	18			
Verde Luz	Sus. parent	4	16			
TP411	Sus. parent	0	9			
Resistant x susceptible crosses with Nig	erian Local as res	sistant par	<u>ent:</u>			
Nigerian Local x Taína Dorada	\mathbf{F}_1	8	2			
Verde Luz x Nigerian Local	\mathbf{F}_1	10	0			
Nigerian Local x Taína Dorada	F_2	47	64	7:9	0.0894	0.7950
Verde Luz x Nigerian Local	F_2	42	68	7:9	1.3859	0.2391
Resistant x susceptible crosses with Mer	iina as resistant p	oarent:				
Menina x Taína Dorada	\mathbf{F}_1	10	0			
Verde Luz x Menina	\mathbf{F}_1	10	0			
TP411 x Menina	\mathbf{F}_1	10	0			
Menina x Taína Dorada	F_2	91	29	13:3	2.3110	0.1285
Verde Luz x Menina	F_2	101	17	13:3	1.4611	0.2268
TP411 x Menina	F_2	91	20	13:3	0.0390	0.8434
Cross between two resistant parents:						
Nigerian Local x Menina	\mathbf{F}_1	20	0	n/a	n/a	n/a
Nigerian Local x Menina	F_2	224	14	15:1	0.0549	0.8147

Table 1. Number of plants evaluated and observed segregations in parental, F1 and F2 populations. 'Nigerian Local' was the resistant parent in the F_1 and F_2 crosses. Goodness-of-fit in F_2 populations was tested with chi-square.

¹For each plant, disease severity in leaf position 3, 4 and 5 was evaluated on a 0 to 4 scale (0 = no symptoms). Values were summed to produce an overall severity index of 0 to 12. Plants were then categorized R for resistant (overall severity rating of \leq 4) or S for susceptible (overall severity rating of \geq 5).



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

Phytophthora blight resistance in butternut squash

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

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

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

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

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



twelve days post inoculation

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

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



41 days post inoculation



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

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

Economic Team

Team members:

Marco Palma (Texas A&M Univ.)

Luis Ribera (Texas A&M Univ.)

(b) Obj. 3. Economic impact analyses, disease control information					
3.1 Perform economic analysis, cost of production/disease control					
3.1.1. Define, parameterize, simulate, validate production variables	LR (TAMU), MP (TAMU)	Х	Х		
3.1.2. Estimate the potential economic impacts to the cucurbit industry	LR (TAMU), MP (TAMU)			Х	Х

3.1 Perform economic analysis, cost of production/disease control

3.1.1. Define, parameterize, simulate, and validate production variables based on cucurbit production crop budgets

Completed:

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

In progress:

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

Publications

- Economic Impacts of Diseases on Cantaloupes. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-04. September 2018.
- Economic Impacts of Diseases on Fresh Cucumbers. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-01. September 2018.
- Economic Impacts of Diseases on Pickles. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-02. September 2018.
- Economic Impacts of Diseases on Squash. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-03. September 2018.
- Economic Impacts of Diseases on Watermelons. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Issue Brief 2018-05. September 2018.

• Economic Impacts of Diseases on Selected Cucurbit Products. Center for North American Studies, Department of Agricultural Economics, Texas A&M University. Report 2018-01. September 2018.



Extension/Outreach Team

Team members:

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

(b) Obj. 3. Economic impact analyses, disease control information					
3.2 Provide readily accessible information to facilitate disease					
control					
3.2.1. Develop a centralized cucurbit disease website	JS (NCSU)	Х	Х		
3.2.2. Develop and post diagnostic resources and disease control	LO (NCSID MILMSID	Х	Х	Х	Х
information in English and Spanish; prepare diagnostic poster	LQ (NCSU), MII (MISU), CS (CU) ALP (UPP)		Х	Х	
3.2.3 Provide disease alerts and forecasting tools	CS(CU), ALR (UPR)	Х	Х	Х	Х
3.2.4 Provide diagnostic and disease management assistance.	LQ (NCSU), MH (MSU), CS (CU)	X	Х	Х	Х
3.2.5. Field days and demonstration plots	Crop and extension teams	X	Χ	Χ	Х

3.2. Provide readily accessible information to facilitate disease control

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

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

3.2.1 Develop a centralized cucurbit disease website.

The CucCAP website was first presented at the CucCAP Annual meeting in March 2017. News about cucurbit disease management including disease outbreaks, current CucCAP research activities, announcements of new publications, upcoming presentations by CucCAP researchers at scientific meetings and cucurbit commodity events is posted on the website throughout the year. An email newsletter called the CucCAP Chronicle was sent monthly since June 2017. The newsletter reports recent news and events posted on the CucCAP website. The newsletter is also shared on the CucCAP Facebook and Twitter sites. A monthly post featuring important CucCAP team accomplishments was added to the website and newsletter in October 2018. The number of subscribers to the CucCAP Chronicle has grown from the initial 21 members of the CucCAP team to 93 subscribers in March 2019. A link to previous installments of the CucCAP Chronicle is available at:

https://us15.campaign-archive.com/home/?u=925e5a7bece071d0c087e746f&id=e0b5619a11

Google Analytics was set up for the website on September 1, 2017 and 1 $\frac{1}{2}$ years of website visitor data has been collected.



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



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

3.2.2. Develop and post diagnostic resources and disease control information

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

3.2.3. Provide disease alerts and forecasting tools

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

3.2.4 Provide diagnostic and disease management assistance.

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

3.2.5. Field days and demonstration plots

<u>Hausbeck</u> hosted a series of Phytophthora and Downy Mildew workshops for growers in Michigan. <u>Smart</u> and <u>Quesada</u> were guest speakers at two of the Michigan workshops.

<u>McCreight</u> Hosted a Cucurbit Field Day at the University of California Desert Research and Extension Center, Holtville, CA. The event focused on cucurbit powdery mildew differentials and the proposed melon core collection.

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

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

<u>Schultheis</u> conducted Variety trials on watermelon, melon, squash, and pumpkins in North Carolina in 2018.

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

<u>Smart</u> has yearly demonstration plots at the Phytophthora blight farm with variety trials for squash (winter squash and summer squash) and other vegetables.

2018 – 2019 Production guides

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2018 – 2019 Web content

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- Hausbeck, M.K. Project GREEEN 20th Anniversary MSU AgBioResearch Video about Downy Mildew on Pickling Cucumbers Published on Sep 20, 2018 https://cuccap.org/2018/09/20/project-greeen-20th-anniversary-msu-agbioresearch/
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2018 – 2019 Publications from demonstration plots

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- 2. Noël N. A. and <u>Quesada-Ocampo L. M</u>. (2018) Tolerance of watermelon cultivars to cucurbit anthracnose, 2017. Plant Disease Management Reports 12: V135.
- Adams M. L. Collins H., and <u>Quesada-Ocampo L. M</u>. (2018) Evaluations of fungicides for control of powdery mildew on winter squash, Kinston 2017. Plant Disease Management Reports 12: V147.
- Schultheis, J.R., and K.D. Starke. (2018). 2017 Yellow & zucchini squash cultigen evaluations. Hort Res. Series 223. 33 pp. <u>https://cucurbits.ces.ncsu.edu/wp-content/uploads/2019/03/2017-Yellow-Summer-and-Zucchini-squash-cultigen-evaluations-Horticulture-Series-223.pdf</u>
- 5. Schultheis, J.R., and K.D. Starke. (2019). 2018 zucchini squash cultigen evaluations. Hort Res.

Series 224. 24 pp.

https://cucurbits.ces.ncsu.edu/wp-content/uploads/2019/03/2018-Zucchini-Squash-Cultigen-Evaluations-Horticulture-Series-224.pdf

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- Schultheis, J.R. and K.D. Starke. 2019. 2018 triploid standard and mini watermelon cultigen evaluation studies. Hort. Res. Series 226. 49 pp. . <u>https://cucurbits.ces.ncsu.edu/wpcontent/uploads/2019/08/2018-Triploid-Standard-and-Mini-Watermelon-Cultigen-Evaluation-Studies-Horticulture-Series-226-updated-8-6-19.pdf</u>

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Cumulative CucCAP

PUBLICATIONS,

RESOURCE MATERIALS

and

PRESENTATIONS

PUBLICATIONS

REFEREED PUBLICATIONS, BOOK CHAPTERS and CONFERENCE PROCEEDINGS

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

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- Bertucci, M.B., K.M. Jenning, D.W. Monks, J.R. Schultheis, P. Perkins-Veazie, F.J. Louws, and D.L. Jordan. 2018. Early season growth, yield and fruit quality of standard and mini watermelon grafted onto several commercially available cucurbit rootstocks. HortTechnology 28(4):459-469.
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Crane, M., T. C. Wehner and R. P. Naegele. 2018. Cucumber cultivars for container gardening and the value of field trials for predicting cucumber performance in containers. *HortScience 53: 16-22 Crandall S. G., Rahman A., Quesada-Ocampo L. M., Martin F. N., Bilodeau G. J., and Miles T. D. (2018)*

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EXTENSION and OUTREACH RESOURCES

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Web Content

Anthracnose of cucurbits: <u>http://content.ces.ncsu.edu/anthracnose-of-cucurbits</u> Cucurbit downy mildew: <u>http://content.ces.ncsu.edu/cucurbit-downy-mildew</u> Cucurbit powdery mildew: <u>http://content.ces.ncsu.edu/cucurbit-powdery-mildew</u> Fusarium wilt of watermelon: <u>http://content.ces.ncsu.edu/fusarium-wilt-of-watermelon</u> Gummy stem blight of cucurbits: <u>https://content.ces.ncsu.edu/gummy-stem-blight-and-phoma-</u>

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- Quesada-Ocampo, L. and N. Miller. 2015. Fusarium Wilt of Watermelon. <u>Spanish translation by Linares Ramírez, A.M. 2017.</u> Marchitez de Fusarium en Sandia. [Factsheet]. <u>https://content.ces.ncsu.edu/marchitez-de-fusarium-en-sandia</u>
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 <u>Spanish translation by Linares Ramírez, A.M. and R. McPhail.</u> Infección de Phytophthora.
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<u>Webinars</u>

Smart, C.D. Vegetable Diseases (for beginning growers), March 15, 2017. This was a 1.5 hour webinar.

- Smart C.D. Managing cucurbit downy mildew in organic systems in the northeast. December 6, 2016. 1.5 hour webinar with 135 participants.
- Smart, C. D. Vegetable Diseases (for beginning growers), March 16, 2016. 1 hour webinar.

PRESENTATIONS

Scientific Conferences and University Presentations (149)

Alzohairy, S., and Hausbeck, M. 2015. Transcriptomic profiling of *Cucurbita* species to characterize the age-related resistance against *Phytophthora capsici*. Page 19 in: Proceedings of the 1st International Soilborne Oomycete Conference, Duck Key, FL, 8-10 Dec. Abstract.

 Alzohairy, S.A., Hammerschmidt, R., and Hausbeck, M.K. 2017. Characterization of the structural basis of winter squash fruit age-related resistance to *Phytophthora capsici*.
 American Phytopathological Society Annual Meeting, San Antonio, TX, 5-9 Aug. Poster

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

Ando, K., X. Wang, U. Reddy, Z. Fei, and J. McCreight 2018. Exploring Genetic Diversity in The U.S. National Melon Collection. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.

Ando, K., X. Wang, Z. Fei, W. Wintermantel, and J. McCreight. 2018. Where in The New Melon Classification Schemes Does Cucumis melo ssp. agrestis var. texanus Belong? Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.

Ando, K., X. Wang, U. Reddy, Z. Fei, and J. McCreight[.] 2018. Exploring Genetic Diversity in The U.S. National Melon Collection. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.

Ando, K., W.M. Wintermantel, and J.D. McCreight. 2018. Phylogenetic analyses confirm the unique status of the wild new world melon, Cucumis melo ssp. agrestis var. texanus, and suggest it be tentatively designated Group Texanus in the recent revision of melon nomenclature, American Society for Horticultural Science 2018 Annual Conference, Washington, D.C., July 30–August 3,.

Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2017.
 Critical period for weed control in grafted vs nongrafted watermelon. Southern Weed
 Science Society Annual meeting. Birmingham, AL. January 24.

Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R., Smith S.C., Basinger N.A., Waldschmidt M.D. 2017. Influence of grafting on the critical period for weed control in watermelon. Southern Region American Society for Horticultural Science Annual meeting. Mobile, AL. February 4.

Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2017. Influence of grafting on the critical period for weed control in watermelon. Weed Science Society of America Annual meeting. Tuscon, AZ. February 7.

 Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2017.
 Influence of grafting on the critical period for weed control in watermelon. Weed Science Society of North Carolina Annual meeting. Raleigh, NC. March 6.

 Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2018. Palmer amaranth Interference and Seed Production in Grafted and Nongrafted Watermelon.
 Southern Weed Science Society Annual meeting. Atlanta, GA. January 23.

Branham S, Levi A, Farnham M, Wechter P. 2017. Quantitative Trait Loci Mapping of Resistance to Fusarium oxysporum f. sp. niveum race 2 in Citrullus lanatus var. Citroides using Genotyping-by-Sequencing (GBS). PAG. https://pag.confex.com/pag/xxv/meetingapp.cgi/Paper/25554

Branham SE, Levi A, Katawczik M, Fei Z, Wechter WP. 2018. Construction of a High-Density Genome-Anchored Genetic Map for Melon (Cucumis melo L.) and Identification of Fusarium oxysporum f. sp. melonis Race 1 Resistance QTL. Plant and Animal Genome Conference (poster).

- Branham SE, Levi A, Wechter WP. 2018. Genetics of Resistance to Fusarium Wilt Races 1 and 2 in Watermelon. Southern Region American Society for Horticultural Science Annual Meeting (Oral presentation).
- Branham, S.E., Levi, A., Katawczik, M.L., Fei, Zhangjun and Wechter, W.P. 2018. Keynote address. Genomics-enabled genetic mapping and marker development of disease resistance loci in melon and watermelon. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.
- Branham, S., Patrick, W.W., Mandal, M.K., Ikerd, J.L. and Kousik, C.S. 2018. QTL Mapping of Resistance to Powdery Mildew in Cucumis melo MR-1 Using a Recombinant Inbred Line Population. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 44.
- Colle M, Mansfeld B, Grumet R. 2017. Genome-wide SNP discovery and identification of age-related resistance loci in cucumber by QTL-seq. PAG XXV.
- Daley, J., S. Branham, A. Levi, R. Hassell, and P. Wechter. 2017. Mapping resistance to Alternaria cucumerina in muskmelon. Plant & Animal Genome XXV Conference. https://pag.confex.com/pag/xxv/meetingapp.cgi/Paper/25467
- Daley, J. and T. Wehner. 2017. Screening for bacterial fruit blotch resistance in watermelon fruit. Abstract and Poster. Crop Science Society of America, Tampa, FL.
- D'Arcangelo K., Miles T., and <u>Quesada-Ocampo L. M. (2018)</u> Pseudoperonospora cubensis populations infecting wild and commercial cucurbit hosts display host-specific sensitivity to fungicides. Phytopathology
- D'Arcangelo K., Miles T., and <u>Quesada-Ocampo L. M.</u> (2017) Occurrence of fungicide resistance in Pseudoperonospora cubensis populations causing cucurbit downy mildew in commercial and wild hosts. Phytopathology 107: S5.63.
- Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Dept. of Plant Biology, Cornell University. March
- Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. College of Horticulture, Shandong Agric. Univ. April
- Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. College of Food Science and Engineering, Hefei University of Technology. May
- Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Texas A&M University. September, 2016
- Fei, Z. 2016. Genome sequencing of sweetpotato wild progenitors. Institute of Vegetables and Flowers, CAAS. April, 2016
- Fei, Z. 2016. Genome sequencing provide insights into watermelon evolution, domestication and important agronomic traits. Nanjing Agricultural University. July, 2016
- Fei, Z. 2017. Genome variation elucidates evolution and domestication of fruit ripening and quality traits in watermelon. PAG. January, 2017
- Fei Z. 2017. Cucurbit Genomics Database Workshop. SolCuc. Valencia, Spain. September 2017 https://pag.confex.com/pag/xxv/meetingapp.cgi/Paper/25600
- Fei, Z. 2018. Cucurbit Genomics Database for cucurbit genomics, genetics and breeding. PAG. January.
- *Fei Z. 2018. Application of bioinformatics and genomics to crop improvement. Chinese Academy of Agricultural Science. November*

- *Fei Z. 2018. CucCAP: Leveraging Applied Genomics to Increase Disease Resistance in Cucurbit Crops. Northeast Agricultural University. August*
- *Fei Z. 2018. CucCAP: Leveraging Applied Genomics to Increase Disease Resistance in Cucurbit Crops. Agricultural Genomics Institute of Shenzhen. May*
- Fei Z. 2018. Cucurbit genome database. CUCURBITACEAE 2018. November
- *Fei Z. 2019. CucCAP: Leveraging Applied Genomics to Increase Disease Resistance in Cucurbit Crops. Syngenta. March*
- Fei, Z., K. Ando, K. Bao, J. Labate, A. Levi, M. Mazourek, J. McCreight, T. Patel, A. Ramirez-Madera, U. Reddy, P. Reeves, X. Wang, T. Wehner, Y. Weng, S. Wu and <u>R. Grumet</u>. 2018. Characterization of the USDA germplasm collections for watermelon, melon, cucumber and squash using genotyping-bysequencing. https://ashs.confex.com/ashs/2018/meetingapp.cgi/session/9120
- Fei Z, Wu S. 2017. Cucurbit Genomics Database Workshop. The XIV Solanaceae and III Cucurbitaceae Genomics Joint Conference. September.
- Gimode, W.R., J. Clevenger and C.E. McGregor. 2018. Effort to Identify Quantitative Trait Locus Associated with Gummy Stem Blight Resistance in Watermelon. IPBGG Retreat 2018, Pine Mountain, GA.
- Gimode, W.R., Y. Xu, Z. Fei and C.E. McGregor. 2019. Identification of Quantitative Trait Loci Associated with Gummy Stem Blight Resistance in Watermelon. Southern Region American Society for Horticultural Science, Birmingham, AL.
- Grumet, R. 2016. Introduction to CucCAP developing genomic resources for the cucurbit community. Plant and Animal Genome Conference. San Diego, CA. https://pag.confex.com/pag/xxiv/webprogram/Paper18951.html
- Grumet R. 2017. The USDA-SCRI CucCAP project: Leveraging applied genomics to increase disease resistance in cucurbit crops. SCRI Advisory Board Meeting, Aug. 17, Traverse City MI
- Grumet R. 2018. The CucCAP project: leveraging applied genomics to increase disease resistance in cucurbits. Fifth International Research Congress, Beijing China
- *Grumet R. 2018. Cucumber fruit development and resistance to Phytophthora capsici. Nanjing Agricultural University, Nanjing China*
- *Grumet R. 2018. Cucumber fruit development and resistance to Phytophthora capsici. Beijing Vegetable Research Institute, Beijing China*
- Grumet R. et al., 2018. Genomic analysis of cucurbit PI collections. American Society for Horticultural Science, Washington DC
- Grumet R. 2018. Genomic analysis of cucurbit PI collections. NC-7 Meeting, Regional Plant Introduction Station, Ames IA
- Grumet R. 2018. Cucumbers the CucCAP project, genetic diversity, and resistance to Phytophthora capsici. University of Illinois, Champaign IL
- Grumet, R., Z. Fei, Y. Weng, X. Wang, K. Bao, Y. Zheng, T. Wehner, U. Reddy, A. Levi, J. McCreight, M. Mazourek, S. Kousik, K-S Ling, C. McGregor, P. Wechter, L. Wessel-Beaver, W. Wintermantel, M. Hausbeck, A. Linares-Ramirez, L. Quesada-Ocampo, and C. Smart. 2018. The CucCAP project: Genomic Tools and Resources to Facilitate Breeding for Disease Resistance in Cucurbits. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.
- Guo, Y., Krasnow, C., and Hausbeck, M.K. 2018. Population structure, virulence and resistance to mefenoxam of Phytophthora capsici in Michigan. American Phytopathological Society Annual Meeting, Boston, MA, 29 Jul-3 Aug. Poster presentation.

- Guo, S. 2018. Comparative population genomics reveals the evolution of fruit quality traits during watermelon domestication. PAG. January.
- Hartman, J. and T. C. Wehner. 2017. Inheritance of citrulline and lycopene content in two watermelon populations. Watermelon Research Development Group, TX.
- Indermaur, E., J. Schultheis, and K. Starke. 2018. Galia specialty melon opportunities and evaluations. SR-ASHS, Jacksonville, FL, February.
- Indermaur*, E. J. Schultheis, and K. Starke. 2019. Galia specialty melons evaluations and opportunities. HortScience 54 (abstr.)
- Kaur, N., Chen, W., Fei, Z. and Wintermantel, W.M. 2017. "Transcriptome changes occurred in the whitefly, B. tabaci MEAM1 in response to feeding on melon infected with the crinivirus, CYSDV," 3rd Hemipteran-Plant Interactions Symposium, Madrid. Spain. June 4-8, 2017.
- Kaur, N., Chen, W., Fei, Z. and Wintermantel, W.M. 2017. "Transcriptome changes occurred in the whitefly, B. tabaci MEAM1 in response to feeding on CYSDV-infected melon," American Phytopathological Society annual meeting, San Antonio, TX, August 5-9, 2017.
- Kousik, C.S. 2016. Breeding rootstocks of cucurbit vegetable crops for resistance to biotic and abiotic stress. (Invited presentation). Platinum Jubilee Celebrations, Indian Horticultural Congress. November 15, 2016. (>300 attendees at the talk).
- Kousik, C.S. 2017. Presented an invited seminar on "Progress and challenges in managing watermelon diseases". Department of Plant Pathology, University of Georgia Athens, GA, Aug. 2017. >50 attendees
- Kousik, C.S. 2018. Progress and Challenges in managing Phytophthora fruit rot of cucurbits. Keynote address presented at the 2nd International Soilborne Oomycete Conference, Keys, FL. December 2018. Proceedings of the 2nd International Soilborne Oomycete conference, Page 13.
- Kousik C.S., Egel D., Ji P., and Quesada-Ocampo L. M. 2016. Fungicide rotation schemes and Melcast for managing Phytophthora fruit rot of watermelon in Southeastern United States. Phytopathology. 106: S4.68.
- Kousik, C.S. and Ikerd, J.L. 2015. Reaction of Phytophthora fruit rot resistant germplasm lines to a broad range of *Phytophthora capsici* isolates from across United States of America. International soilborne Oomycete conference, Duck Key, FL. December
- Kousik, C.S., Ikerd, J.L., and Mandal, M.K. 2016. Breadth of resistance of Phytophthora fruit rot resistant watermelon germplasm to *Phytophthora capsici* isolates from across United States of America. Phytopathology S4.40 (Abstract)
- Kousik, C.S., Ikerd, J.L. and Mandal, M.K. 2017. Long term monitoring of cucurbit powdery mildew (*Podosphaera xanthii*) races in Charleston, South Carolina. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 503-P
- Kousik, C.S., Ikerd, J.L., and Mandal, M.K. 2018. Relative susceptibility of commercial watermelon varieties to powdery mildew. Presented at the Annual meeting of the Southern Division, American Phytopathological Society (SD-APS).Fayetteville, AR Feb 16-18, 2018.
- Kousik, C.S., Ikerd, J.L., and Mandal, M.K. 2018. Developing Sources of Resistance in Winter Squash (Cucurbita moschata) to Crown and Root Rot caused by Phytophthora capsici. Proceedings of the 2nd International Soilborne Oomycete conference, Page 39.
- Kousik, C.S., Ikerd, J.L., and Mandal, M.K. 2018. Relative Susceptibility of Commercial Watermelon Varieties to Powdery Mildew and Phytophthora Fruit Rot. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 51.

- Kousik, C.S., Ikerd, J.L., and Mandal, M.K. 2018. New Sources of Resistance to Phytophthora Crown and Root Rot in Cucurbita moschata. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 54.
- Kousik, C.S., Ikerd, J.L., Mandal, M.K. and Wadl, P. 2018. Genetics of Resistance to Powdery Mildew in Watermelon Line USVL608-PMR. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 55.
- Kousik, C.S., Mandal, M.K., Ikerd, J.L., Adkins, S., and Turechek, W. 2018. Powdery mildew resistant watermelon germplasm lines USVL608-PMR, USVL278-PMR, USVL313-PMR and USVL585-PMR. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.
- Kousik, C.S. Pingsheng Ji and Quesada-Ocampo, L.M. 2015. Fungicide rotation schemes for managing Phytophthora fruit rot of watermelon across Southeastern United States (NC, SC, GA). International soilborne Oomycete conference, Duck Key, FL. December
- Krasnow, C., and Hausbeck, M. 2015. Using directed fungicide applications to manage Phytophthora fruit rot of processing squash. Page 23 in: Proceedings of the 1st International Soilborne Oomycete Conference, Duck Key, FL, 8-10 Dec. Abstract.
- Krasnow, C.S., and Hausbeck, M.K. 2016. Age-related resistance of Cucurbita spp. fruit to *Phytophthora capsici*. Abstr. Phytopathology 106 (Suppl.):S1.5.
- LaPlant, K. and Mazourek M. 2018. Introgression mapping of wild species-derived resistance to viruses in Cucurbita. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.
- Lebeda, A., E. Kristkova, B. Sedlakova, J.D. McCreight, and E. Kosman. 2018. Application of a new approach for study of virulence variation in cucurbit powdery mildew populations. International Congress of Plant Pathology, Boston, 30 July-2 August 2018
- Lebeda, A., B. Sedlakova, E. Kristkova, J. McCreight, E. Kosman. 2018. Cucurbit powdery mildew population virulence variation–complex view from a global perspective. Cucurbitaceae 2018, Univ. California, Davis, 12-15 November 2018.
- *Lin YC, Grumet R. 2018. Genetic analysis of young fruit resistance to Phytophthora capsici in cucumber. American Society for Horticultural Sciences*
- Lin YC, Grumet R. 2018. Genetic analysis of young fruit resistance to Phytophthora capsici in cucumber. National Association of Plant Breeders, Guelph Ontario
- *Lin YC, Grumet R. 2018. QTL-seq of young fruit resistance to Phytophthora capsici in cucumber. Cucurbitaceae 2018, Davis CA*
- Linares-Ramirez, A.M. 2016. Cucurbits: From to the field to the lab. Agricultural Experimental Station, University of Puerto Rico.
- Ling, K.-S. 2017. Presented invited seminars on "Developing genome-guided strategies to manage viral

diseases of cucurbit crops" in four institutions throughout China, including: Zhengzhou Fruit Research Institute,

Beijing Vegetable Research Center (China, Israel, and the U.S. Workshop on Cucurbit Research), Zhejiang Academy of Agricultural Sciences

Fujian Agricultural and Forestry University.

Lonnee M., W.R. Gimode, C.E. McGregor. 2018. Evaluation of Gummy Stem Blight Resistance in Watermelon Using Stagonosporopsis spp. Isolates. CAES, Young Scholars Athens, GA.

- McGregor, C.E. 2019. Out of Africa: The Story of Watermelon Disease Resistance. Georgia Association og Plant Pathologists annual meeting. Savannah, GA.
- Mandal, M.K., Ikerd, J.L., Soorni, A. and Kousik, C.S. 2016. Molecular dissection of resistance signaling in watermelon fruit through transcriptomic approach. Phytopathology S4.153 (Abstract)
- Mandal, M.K., Ikerd, J.L., Wallace, E.C., Rebbeca, G., Turechek, W., Quesada-Ocampo, L.M. and Kousik, C.S. 2017. Population biology of the downy mildew pathogen on tolerant and susceptible cucumber in the southeastern United States. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 563-P
- Mandal, M.K., Suren, H. and Kousik, C.S. 2017. Transcriptomic profiling of watermelon-powdery mildew (Podosphaera xanthii) interactions. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 361-P
- Mandal, M.K., Suren, H., Ward, B., Boroujerdi, A., Kousik, C.S. 2018. Role of Antioxidant Molecule Melatonin in Plant-Host Resistance and Pathogen Suppression in Cucurbits. Presented at Cucurbitaceae 2018, Davis, CA. Cucurbitaceae 2018 conference abstracts, Page 11.
- Mansfeld B, Colle M, Grumet R. 2017. Genome-wide SNP discovery and identification of age-related resistance loci in cucumber by QTL-seq. PAG XXV, San Diego CA.
- Mansfeld B, Grumet R. 2018. QTLseqr: An R package for bulk segregant analysis with next generation sequencing. PAG XXVI, San Diego CA.
- Mansfeld B, Grumet R. 2018. Differential transcriptomic responses to infection associated with cucumber age-related resistance to Phytophthora capsici. American Society for Plant Biology
- Mansfeld B, Grumet R. 2018. QTLseqr: An R package for bulk segregant analysis with next generation sequencing. National Association for Plant Breeders, Guelph Ontario
- Mansfeld B, Grumet R. 2018. Inhibitory effects of cucumber fruit age-related resistance to Phytophthora capsici manifest within 24 hours of infection. Cucurbitaceae 2018, Davis CA
- Mansfeld B, Grumet R. 2019. Gene expression dynamics of age-related resistance of cucumber to Phytophthora capsici. PAG XXVII https://plan.core-apps.com/pag_2019/abstract/918bc1c1-89c4-4b70-acc5-21223f797605
- Mantooth, K.L., Ikerd, J.L., Mandal, M.K. and Kousik, C.S. 2017. Potential sources of resistance to Phytophthora crown rot in *Cucurbita maxima* and *Cucurbita moschata*. Presented at the Annual meeting of the American Phytopathological Society, San Antonio, TX August 2017. 291-P
- Mazourek M, Holdsworth WL, Hernandez C, LaPlant KE. 2016. Making up for lost time in Cucurbita molecular breeding. Plant and Animal Genome Conference. San Diego, CA.
- McCreight, J.D., D. Coffey Michael, K. Ando, and S. Kousik Chandrasekar. 2018. Cucurbit powdery mildew races on melon: current status in the U.S., American Society for Horticultural Science 2018 Annual Conference, Washington, D.C., July 30–August 3.
- McCreight, J.D., W.M. Wintermantel, and E.T. Natwick. 2015. Evaluations of melon germplasm reported to exhibit host plant resistance to sweetpotato whitefly. Entomological Society of America, Annual Meeting, Minneapolis, MN, Nov. abstract
- McCreight, J.D., W.M. Wintermantel, and E.T. Natwick. 2016. Expression of Host Plant Resistance in Melon to Sweetpotato Whitefly in the Desert Southwest United States. XXV International Congress of Entomology, Orlando, FL, Sep. abstract
- Miller N. F. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for management of Fusarium wilt of watermelon. Phytopathology. 106:S4.2

- Miranda-Vélez, M, L. Wessel-Beaver, Jose C. Verle-Rodrigues and W. Seda-Martínez. 2016. Effect of leaf position on the assessment of resistance to *Papaya ringspot virus* and *Zucchini yellow mosaic virus* in tropical pumpkin. Proceedings of the 41st meeting of the Sociedad Puertorriqueña de Ciencias Agrícolas, November 18, 2016, Corozal, Puerto Rico. p. 57. (abstract)
- Miranda-Vélez, M., L. Wessel-Beaver and J.C.V. Rodrigues. 2018. Disease assessment, inference on virus movement, and seedling development in tropical pumpkin (Cucurbita moschata) infected with Papaya ringspot virus and Zucchini yellow mosaic virus. Reunión Científica de Estudiantes Graduados, Sociedad Puertorriqueña de Ciencias Agrícolas. May 18, 2018. College of Agriculture, University of Puerto Rico, Mayaguez.
- Miranda-Vélez, M., L. Wessel-Beaver and J.C.V. Rodrigues. 2018. Disease assessment in seedlings of tropical pumpkin infected with PRSV and ZYMV. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.
- Natwick, E.T., W.M. Wintermantel, R.L. Gilbertson, S.G. Blanco, and J.D. McCreight. 2017. "Evaluation of potential new sources of melon host plant Resistance to the whitefly, *Bemisia tabaci*," 3rd Hemipteran-Plant Interactions Symposium, Madrid. Spain. June 4-8, 2017.
- Noel N. and Quesada-Ocampo L. M. 2016. Fungicide resistance and host susceptibility of *Colletotrichum orbiculare* infecting cucurbit crops in North Carolina. Phytopathology. 106:S4.36
- Noel N. and Quesada-Ocampo L. M. (2017) Characterizing Colletotrichum orbiculare, the causal agent of cucurbit anthracnose, for fungicide efficacy and host susceptibility in North Carolina. Phytopathology 107: S5.77.
- Parada-Rojas C. H. and Quesada-Ocampo L. M. (2017) Population structure of the oomycete soilborne pathogen Phytophthora capsici in North Carolina. Phytopathology 107: S5.21.
- Patel, T. and T. Wehner. 2017. Identification of new resistance sources and SNPs markers in watermelon for anthracnose (*Colletotrichum orbiculare*). Abstract and Poster. National Association of Plant Breeders, Davis, CA.
- Quesada-Ocampo LM. 2017. From the field to the lab and back: translational strategies to improve disease control in vegetable crops. VIB-PSB-NC State Plant Sciences Workshop, Ghent, Belgium
- *Quesada-Ocampo L. M. (2017) Next generation sequencing to develop molecular diagnostics for Pseudoperonospora cubensis. Phytopathology* 107: S5.150.
- Rahman A. and Quesada-Ocampo L. M. 2016. Early detection and quantification of *Pseudoperonospora cubensis* airborne sporangia using real-time PCR. Phytopathology. 106:S4.16
- Rahman A. and Quesada-Ocampo L. M. (2018) Biosurveillance for precision disease management of Pseudoperonospora cubensis, the cucurbit downy mildew pathogen. Phytopathology
- Rahman A., Martin F., Shands A., Miles T., and Quesada-Ocampo L. M. 2017. Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen Pseudoperonospora cubensis. Oomycete Molecular Genetics Network Meeting, Pacific Grove, CA.
- Rahman A., Wallace E., Crouch J., Martin F., and Quesada-Ocampo L. M. (2017) Unravelling historical shifts in Pseudoperonospora cubensis populations in the U.S. that resulted in the 2004 cucurbit downy mildew epidemic. Phytopathology 107: S5.22.
- Reyes, J., Villari, C., Brewer, M. and C.E McGregor. 2018. Detection of the Gummy Stem Blight-Causing Pathogens (Stagonosporopsis spp.) in Watermelon Using Species-Specific LAMP Assays. Cucurbitaceae 2018, University of California, Davis-CA.

- Rivera-Burgos L. and T. Wehner. 2017. Evaluation of gummy stem blight resistance in a recombinant inbred line watermelon population. Abstract and Poster. National Association of Plant Breeders, Davis, CA
- Schultheis, J.R. and K.D. Starke. 2018. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. HortScience 53(9):S502 (abstr.).
- Schultheis, J.R. and W.B. Thompson. 2016. Watermelon cultivar yield and quality trial results, North Carolina, 2015. HortScience. 51(9):S37
- Schultheis, J.R. and W.B. Thompson. 2016. Watermelon yield and fruit size response to grafted versus non-grafted transplants in plasticulture and bare ground production systems. HortScience. 51(9):S38
- Seda-Martínez, W., M. Miranda-Vélez, L. Wessel-Beaver and A. M. Linares-Ramirez. 2017. Approaches to Phenotyping PRSV and ZYMV Resistance in Tropical Pumpkin. HortScience 45(8):S234.
- Seda-Martínez, W., L. Wessel-Beaver and A. Linares-Ramírez. 2018. Effect of two Potyviruses on development and yield of tropical pumpkin. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.
- Smart, C.D. A tale of two *Phytophthora*: life with and without sex. Michigan State University, East Lansing, MI, March 2, 2017.
- Smart, C.D. Multiplex detection for vegetable diseases. National Plant Diagnostic Network National meeting. Crystal City, VA, March 10, 2016.
- Starke, K.D. and J.R. Schultheis. 2018. Mini watermelon cultivar yield and quality results, North Carolina, 2017. HortScience 53 (9):S502 (abstr.).
- Starke, K.D., B. Thompson, C. Jiang, and J. Schultheis. 2016. Planting density influences mini-watermelon yield and quality. VII International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops, Pretoria, South Africa, September 2016.
- Smart, C.D. SUNY Potsdam November 2017, Potsdam NY. Genomic approaches to understand and manage plant disease epidemics.
- Smart, C.D. James Hutton Institute, August 10, 2017, Dundee Scotland. Comparing sexual and asexual Phytophthora species.
- Sui, X., Li, R., Wu, Z., Ling K.-S. 2018. Seed Transmissibility of Cucumber Green Mottle Mosaic Virus in Cucurbits and Seed Health Assays. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.
- Thammapichai P, Pan JS, Koo D-H, Han YH, Jiang JM, Weng Y (2018) Genomics-aided development and characterization of Cucumis hystrix introgression lines in cucumber. Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)
- Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and non-grafted watermelon. SR-ASHS, Jacksonville, FL, February.
- Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and nongrafted watermelons. HortScience 53(9):S461 (abstr.).
- Trandel, M., P. Perkins-Veazie, and J. Schultheis. 2018. Tissue firmness and hollow heart development in 2012, 2013, and 2014 triploid watermelon variety trials.
- Trandel, M.A., P. Perkins-Veazie, J. Schultheis, C. Gunter, and E. Johannes. 2018. Hollow heart formation in grafted and nongrafted watermelons. HortScience 53(9):S503 (abstr.).Cucurbitaceae 2018. Production and Quality session. Davis, CA (abstr.).

- Vogel, G., LaPlant, K., Reeves, E., Mazourek, M., Gore, M. and Smart, C.D.<u>2017</u>. Evaluation of *Cucurbita pepo* breeding lines with reduced susceptibility to root and crown rot caused by *Phytophthora capsici*. American Phytopathological Society. San Antonio TX August 2017
- Vogel, G., LaPlant, K., Mazourek, M., Gore, M., and Smart, C.D. 2018. Bulked segregant analysis with whole-genome resequencing to map QTL involved in Phytophthora crown and root rot resistance in Cucurbita pepo. (Abstr.) Phytopathology 108:S1.1. <u>https://doi.org/10.1094/PHYTO-108-10-</u> <u>S1.1</u>.
- Vogel, G., LaPlant, K. Mazourek M., Gore M. and Smart, C. 2018. Next-Generation Sequencing Bulk Segregant Analysis Reveals Multiple Loci Involved in Phytophthora Root and Crown Rot Resistance in Squash. Cucurbitaceae 2018. November 12-15, 2018. Davis, California.
- Wallace E. C. and Quesada-Ocampo L. M. (2016) *Pseudoperonospora cubensis* on commercial and noncommercial cucurbits in North Carolina: population structure determine by simple sequence repeats (SSRs). Phytopathology. 106:S4.12
- Wallace E. C. and Quesada-Ocampo L. M. 2016. Genetic structure of *Pseudoperonospora cubensis* populations infecting commercial and non-commercial cucurbits in North Carolina. XIth Eucarpia Cucurbitaceae Proceedings
- Wallace E. C. and Quesada-Ocampo L. M. (2017) Examining the population structure of the cucurbit downy mildew pathogen, Pseudoperonospora cubensis, by host, location, and time. Phytopathology 107: S4.6.
- Wang YH, Tan JY, Wu ZM, VandenLangenberg K, Wehner TC, Wen CL, Zheng XY, Owens K, Thornton A, Bang HH, Hoeft E, Kraan PAG, Suelmann J, Pan JS, Weng Y (2018) A loss-of-susceptibility mutation in the STAYGREEN gene (CsSGR) provides durable, broad-spectrum disease resistances for US cucumber production. . Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)
- Wang YH, VandenLangenberg K, Wehner TC, Weng Y (2018) Genetic architecture of downy mildew resistance in cucumber. Abstract for Plant and Animal Genome Meeting XXVII (Jan 12-16, 2018, San Diego, CA)
- Wang X. 2018. The USDA Cucumber (Cucumis sativus L.) Collection: Genetic Diversity, Population Structure, Genome-Wide Association Studies and Core Collection Development. CUCURBITACEAE 2018. November
- Wechter, P. 2017. "Identification of quantitative trait loci associated with resistance to race 1 Fusarium wilt in *Cucumis melo*," American Phytopathological Society annual meeting, San Antonio, TX, August 5-9, 2017
- Wechter, P. 2017. "Mapping Resistance to *Alternaria cucumerina* in Muskmelon," Plant and Animal Genome meeting, San Diego, CA, January 14-18, 2017.
- Wechter, W.P. 2019. Cucurbit and Brassica pathology research at the U.S. Vegetable Laboratory: Oldschool and New-school approaches to plant disease resistance. Invited seminar at the Department of Plant Pathology, University of California-Davis.
- Wechter P, Branham S, Levi A. 2017. 66-P: GBS-SNP-based linkage mapping and QTL associated with resistance to race 1 Fusarium wilt in Cucumis melo. American Phytopathological Society. 266-P
- Weng Y. 2017. Improve QTL detection power: cucumber downy mildew resistance. An invited talk at China Agricultural University (Beijing, China, July 111, 2017)
- Weng et al. 2018<u>.</u> Genetic architecture of downy mildew resistance in cucumber. Cucurbit workshop. Plant and Animal Genome Conference (Jan 9-13, 2018, San Diego, CA).

- Weng Y (2018) Genetic basis of downy mildew resistance in cucumber. Cucurbitaceae 2018 International Meeting Abstracts (November 12-15, 2018, Davis, California)
- Weng Y (2018) 'Molecular Breeding Infrastructure Development in the USDA-ARS/University of Wisconsin Cucumber Improvement Program'. Invited talk at First China National Conference on Molecular Breeding in Horticulture Crops (July 29, 2018, Harbin, China)
- Weng Y (2018) 'Cucumber Molecular Breeding- current status and perspectives'. Invited talk in the Institute of Vegetable Research, Sichuan Academy of Agricultural Sciences (May 7, 2018, Chengdu, China)
- Wintermantel WM, J.D. McCreight, and E.T. Natwick. 2016. Epidemiology of Cucurbit yellow stunting disorder virus (CYSDV) and associated whitefly-transmitted viruses in the US Southwest and development of CYSDV resistant melon. Paper presentation at 2nd International Whitefly Symposium, February 14-19, Arusha, Tanzania.
- Wintermantel WM, J.D. McCreight, and E.T. Natwick. 2016. Reservoir hosts of Cucurbit yellow stunting disorder virus and development of resistant melon. 13th International Plant Virus Epidemiology Symposium. Avignon, France, June 6-10, 2016.
- Wu S, Zhong Y, Grumet R, Levi A, Weng Y, Mazourek M, McCreight J, Katzir N, Garcia-Mas J, Fei Z. 2017. Cucurbit genomics database. Sol/Cuc Conference, Valencia, Spain
- Wu S. 2018. Cucurbita genome sequences provide insights into polyploid genome evolution and heterosis in interspecific hybrid. PAG. January.
- Wu S. 2018. Pan-genomes of the Citrullus Species. CUCURBITACEAE 2018. Davis CA
- Zhang C, Mansfeld BM, Grumet R. 2018. Development of a real-time fluorescence based microplate assay for pathogen growth on plant tissue: Phytophthora capsici infection of cucumber fruit. Cucurbitaceae 2018, Davis CA
- Zheng Y. 2018. Cucurbit Genomics Database: Integration genetic and genomics resources for cucurbit breeding. PAG. January

Extension and Outreach Presentations (145)

- Adams M. L. and Quesada-Ocampo L. M. 2016. Managing fungal diseases in cucurbits. NC Watermelon Convention. Wrightsville Beach, SC, Mar.
- Adams M. L. and Quesada-Ocampo L. M. 2015. Managing fungal foliar diseases in cucurbits. 30th Annual
- Ando, K. and J.D. McCreight. 2017. "Potential for Production of Turkmen Melons in California," National Association of Plant Breeders, Davis, CA, August 2017. Southeast Vegetable and Fruit Expo.
 Myrtle Beach, NC, Dec.
- Arteman, L. T.C. Wehner, and J.R. Schultheis. 2015. Evaluation of parthenocarpic pickling cucumbers for North Carolina production. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. Dec.
- Bertucci, M., K. Jennings, D. Monks, D. Jordan, F. Louws, and J. Schultheis. 2015. Competitiveness of grafted watermelon plants versus nongrafted watermelon plants at various times of weedy and weed-free intervals. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. Dec.
- Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2015. Critical period for weed control in grafted and nongrafted triploid watermelon (Poster). North Carolina Crop Protection School. December 2, Cary, NC.
- Bertucci M.B., Jennings K.M., Monks D.W., Louws F.J., Jordan D.L., Schultheis J.R. 2016. Effect of grafting on the critical period of weed control of triploid watermelon (Poster). North Carolina Crop Protection School. December 6, Cary, NC.
- Bertucci, M.B., K.M. Jennings, D.W. Monks, J.R. Schultheis, W.B. Thompson, F.W. Louws, D.L. Jordan, N.A.
 Basinger, S.C. Smith, M.D. and Waldschmidt. 2017. Early season crop development, yield, and fruit quality of standard and mini watermelons grafted to several cucurbit rootstocks. Watermelon Research Group, Mobile, AL. February 2017.
- Birdsell, T., J. Schultheis, and P. Perkins-Veazie. 2017. Butternut squash cultivar, production, harvest, and enterprise budget considerations. Annual North Carolina Vegetable Growers Association. Myrtle Beach, SC, November.
- Chacko, N. J.-B. Mou, and M.D. Coffey. 2016. Powdery mildew race variation in California. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
- Grumet, R. 2015. Update on resistance to *Phytophthora capsici* in cucumber. PPI Annual Meeting October 30, 2015, Fort Worth, TX
- Grumet, R. 2015. Introduction to CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- Grumet, R. and M. Colle. 2015. Development of genetic stocks for cucumber fruit resistance to *Phytophthora capsici*. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- Grumet, R. 2016. Introduction to CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
- Grumet R, Mansfeld B, Lin Y-C. 2016. Genetic characterization and development of breeding materials for resistance of young cucumber fruit to infection by *Phythophthora capsici*. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- Grumet R. 2017. CucCAP: Leveraging applied genomics to increase disease resistance in cucurbit crops PPI Spring Meeting, April 19, Milwaukee WI
- Grumet R, Lin YC, Mansfeld B. 2017. Resistance of cucumber fruit to Phytophthora capsici. PCIC/PPI, Nov. 1, Chicago IL

- Grumet R, Lin YC. 2017. Resistance to Phytophthora fruit rot in cucumber. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- *Grumet R, Lin YC. 2018. Resistance to Phytophthora fruit rot in cucumber. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.*
- Harlan, B., and Hausbeck, M. 2017. Vegetable diseases and control strategies. Michigan Agribusiness Association Meeting, Lansing, MI, 11 Jan. 60 attendees.
- Hausbeck, M. 2015. The downy mildew report. Pickling Cucumber Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. 144 attendees.
- Hausbeck, M. 2015. Downy mildew research. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec. 30 attendees.
- Hausbeck, M.K. 2015. Ten years of downy mildew in Michigan. Pickle Packers International Inc Annual Meeting, Fort Worth TX, Oct. 30 attendees.
- Hausbeck, M. 2016. The downy mildew report. Syngenta Meeting, Lansing, MI, Feb. 120 attendees.
- Hausbeck, M. 2016. Soilborne *Phytophthora capsici* on vine crops: Update and implications, Extension Specialist Breakfast Meeting via Zoom videoconference, East Lansing, 16 Jun. 15 attendees
- Hausbeck, M. 2016. *Phytophthora capsici*: Pathogen biology. *Phytophthora capsici* Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, Dec. 25 attendees.
- Hausbeck, M. 2017. A smorgasbord of vegetable diseases is on today's menu. MSU Extension and AgBioResearch State Council Meeting, Lansing, MI, Mar. 30 attendees.
- Hausbeck, M. 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Vegetable Growers' Meeting, East Aurora, NY, 15 Feb. 40 attendees.
- Hausbeck, M. 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Syngenta Meeting, East Lansing, MI, 9 Feb. 75 attendees.
- Hausbeck M 2017. Managing Phytophthora crown and fruit rot in cucurbit crops. Wisconsin Fresh Fruit and Vegetable Conference, Wisconsin Dells, WI, 23 Jan. 40 attendees.
- Hausbeck, M. 2018. Phytophthora management for winter squash, cucumber and pepper. Grower Meeting, Hudsonville, MI, 21 Feb. 30 attendees.
- Hausbeck M. 2018. Fungicides & Diseases: Managing Diseases for Higher Profitability and a Safer Environment at MSU Agriculture Innovation Day: Focus on Fruit and Vegetable Technologies. June 28.
- Hausbeck M. 2018. Managing Cucumber Diseases in 2019 at the Great Lakes Expo and Michigan Greenhouse Growers Expo. Dec. 4
- Hausbeck M. 2019. Phytophthora and Downy Mildew Workshop at the Southwest Michigan Research and Education Center. Jan. 23
- Hausbeck M. 2019. Real-world management strategies for Phytophthora-infested ground. Oceana County MSU Extension, Hart, MI. Jan 31.
- Hausbeck M. 2019. Biology and control of Cucurbit Downy Mildew and Phytophthora capsici in Pickling Cucumbers. Saginaw Valley Research and Extension Center in Frankenmuth, MI. Feb. 14
- Hausbeck M. 2019. Managing Phytophthora Crown and Fruit Rot. Ontario Fruit and Vegetable Convention. Feb 20-21.
- Hausbeck M. 2019. Disease Management. SE Michigan Winter Vegetable Meeting. March 12.

- Hausbeck, M.K., and Cook, A. 2015. The downy mildew report. Pages 9-14 in: Pickling Cucumber
 Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec.
 Online.
- Hausbeck MK, Goldenhar K. 2016. Downy mildew prevention and control. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- Hausbeck MK, Golenhar K, Bello JR. 2016. Downy mildew: What's next? Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.
- Indermaur, E., K. Starke, and J. Schultheis. 2017. Galia and canary melon cultivar evaluation. Annual North Carolina Vegetable Growers Association. Myrtle Beach, SC, November.
- Kousik, C.S. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. Indiana Horticultural Congress, Indianapolis, IN. January. 45 attendees (at the talk)
- Kousik C.S. 2016. Managing Phytophthora fruit rot of watermelon. Georgia Watermelon Association, St. Simmons, GA. January. Over 100 attendees
- Kousik, C.S. 2016. Breadth of resistance of USVL developed Phytophthora fruit rot resistant germplasm lines to *Phytophthora capsici* isolates from across USA. Watermelon Research and Development Group meeting. San Antonio, TX. Feb >50 attendees
- Kousik, C.S. 2016. Chaired and organized Watermelon Research and Development Group meeting. San Antonio, TX. Feb >50 attendees, 26 talks.
- Kousik, C.S. 2016. Progress and challenges in managing Phytophthora fruit rot of watermelon. U.S. Vegetable Laboratory Seminar. Charleston, SC. March
- Kousik, C.S. 2017. Chaired and organized Watermelon Research and Development Group meeting. Mobile, AL. February 2017. >65 attendees, 38 talks.
- Kousik, C.S. 2017. Presented information on Phytophthora fruit rot and powdery mildew of watermelon to the U.S. Secretary of Agriculture, Dr. Sonny Purdue and his team when they visited the U.S. Vegetable Laboratory, USDA, ARS in Charleston, SC. August 21, 2017.
- Kousik, C.S. 2017. Provided information to Sarah Mock, Washington D.C. Bureau Chief for RFD-TV on research being conducted on watermelon at the U.S. Vegetable Laboratory and details of the visit of Dr. Sonny Purdue to USVL. August, 21, 2017. The interview was aired by RFD-TV and is located at website: <u>https://youtu.be/R4tHGZSJqRI</u>
- Kousik, C.S. 2018. Best practices to reduce impact of *Phytophthora*. Southeast Regional Fruit and Vegetable Conference, Savannah, GA, January 2018. >100 attendees at the talk.
- Kristie, M., Ikerd, J.L., Mandal, M., Hassell, R., and Kousik, C.S. 2017. Development of Phytophthora crown rot (*Phytophthora capsici*) resistant rootstocks of Cucurbita maxima and *C. moschata* for watermelon grafting. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017.
- Krasnow, C., and Hausbeck, M. 2016. Progress in cucumber downy mildew control. Southwest Hort Days, Benton Harbor, MI, Feb. 35 attendees.
- Krasnow, C., and Hausbeck, M. 2016. *Phytophthora capsici*: Fungicide programs and crop resistance. *Phytophthora capsici* Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, Dec. 55 attendees.
- Krasnow, C., and Hausbeck, M. 2016. Orondis: a new tool for controlling Phytophthora blight on pepper and squash. Syngenta Meeting, Lansing, MI, Feb. 75 attendees.
- Krasnow, C., and Hausbeck, M. 2016. Phytophthora blight: management strategies for pepper and squash. Southwest Hort Days, Benton Harbor, MI, Feb. 35 attendees.

- Krasnow, C., and Hausbeck, M. 2016. Rots and blights of vegetables. Bay Area Growers Extension Meeting, Bay City, MI, Jan. 40 attendees.
- Levi, A. S. Steck, M. Horry, R.L. Jarret, P. Wechter, S. Kousik, B. Ward, G. Miller, R. Hassell, and A. Keinath. 2017. An overall small root system in watermelon cultivars indicates a need to improve their lateral fibrous root capacity. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017.
- Mandal, M.K., Kousik, C.S. and Ward, B. 2016. Molecular dissection of resistance signaling in watermelon fruit through metabolomics and transriptomic approach. Watermelon Research and Development Group meeting. San Antonio, TX. Feb >50 attendees
- Mandal, M.K., Ikerd, J.L., Shrestha, S. Battiste, A., Boroujerdi, A., Ward, B., Kousik, C.S. 2017. 1H NMR and HPLC-based metabolite profiling of watermelon varieties. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017.
- Mandal, M.K., Suren, H., Ikerd, J.L., and Kousik, C.S. 2018. Molecular Dissection of Resistance Signaling during Compatible and Incompatible Watermelon- Powdery Mildew (*Podosphaera xanthii*) Interactions using RNA-Seq Approach. Presented at the Watermelon Research and Development Group (WRDG) meeting. Southern Region ASHS, Jacksonville, FL. February 2018.
- Kousik, C.S. 2018. Best practices to reduce impact of Phytophthora. Southeast Regional Fruit and Vegetable Conference, Savannah, GA, January 2018. >100 attendees at the talk.
- Mandal, M.K. and Kousik, C.S. 2018. Multidimensional approaches to study host-resistance signaling in cucurbits against diseases: from epidemiology to omics. Invited talk to USVL USDA-ARS, Charleston SC. January, 2018. >40 attendees
- McCreight, J.D. 2015. Melon host plant resistance to powdery mildew and CYSDV. Fall Desert Crops workshop, sponsored by the University of California ANR Cooperative Extension, Imperial County, and University of Arizona Cooperative Extension, Yuma County. El Centro, CA, Oct.
- McCreight, J.D. and E.T. Natwick. 2016. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweetpotato whitefly biotype B. California Melon Research Board, Annual Meeting, San Diego, CA, Jan.
- McCreight, J.D. and E.T. Natwick. 2017. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweetpotato whitefly biotype B. California Melon Research Board, Annual Meeting, San Diego, CA, Jan. 4, 2017.
- McCreight, J.D. 2017. "Agricultural Research Technology Center," Western Regional Seed Physiology Research Group, University of California, Davis, January 24, 2017.
- McCreight, J.D. 2017. AgKnowledge class annual visit to U.S. Agricultural Research Station, Grower-Shipper Association of Central California, Salinas, CA, June 2017.
- McCreight, J.D. 2017. Assisted Seed Central (http://www.seedcentral.org) hosting 100 persons from ag related companies with research updates and provided laboratory and greenhouse tours, Salinas, CA, April.
- McCreight, J.D. 2017. U.S. Plant Breeding: Lettuce, Spinach, Melon, and Sugar beet. Seed Central (an initiative of the Seed Biotechnology Center at the University of California Davis, and Seed Quest), Salinas CA, April.
- McCreight, J.D. 2017. Melon powdery mildew race variation in California. University of California, Cooperative Extension, Imperial County, 28th Annual Fall Desert Crops Workshop, Imperial, CA, December.

- McCreight, J.D. and E. T. Natwick. 2018. Evaluation and breeding of new sources of host plant resistance to CYSDV and sweet potato whitefly biotype. California Melon Research Board, Annual Meeting, San Diego, 4 January 2018.
- McCreight, J.D. 2018. Lettuce and melon breeding for resistance to diseases and insects. University of California, Plant Breeding Retreat, Monterey, CA, 17-18 December 2018.
- McGregor, C.E. 2016. Advances in Watermelon Breeding. Southeast Regional Fruit & Vegetable Conference, 8-10 January 2016, Savannah, GA .
- Meadows I., Mauney C., and Quesada-Ocampo L. M. 2016. Agent training on disease diagnostics and management in vegetable crops. Extension Conference. Raleigh, NC, November 2016.
- Miller N. F. and Quesada-Ocampo L. M. 2015. New control options for Fusarium wilt in watermelon. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, Dec.
- Miller N. F. and Quesada-Ocampo L. M. 2016. Evaluation of a new fungicide for management of Fusarium wilt of watermelon. NCSU Masters Symposium, Raleigh, NC, November 2016.
- Miller, N., M. Adams, and L.M. Quesada-Ocampo. 2017. Evaluation of a new fungicide for management of Fusarium wilt of watermelon. Watermelon Research Group. Mobile, AL, February 2017.

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

Quesada-Ocampo L. M. 2015. Diagnostics and management of cucurbit downy mildew. Pickle Packers International Annual Meeting. Fort Worth, TX, Oct.

- Quesada-Ocampo L. M. 2016. Downy mildew and *Phytophthora* control in cucurbits. NC Watermelon Convention. Wrightsville Beach, NC, Mar.
- Quesada-Ocampo L. M. 2016. Cucurbit downy mildew management, diagnostics, and pathogen populations. Pickle Packers International Spring Meeting. Raleigh, NC, Apr.
- Quesada-Ocampo L. M. 2016. Downy mildew updates for cucurbits. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, Jan.
- Quesada-Ocampo L. M. 2016. Disease identification on vegetables. Certified Crop Advisor Training. Smithfield, NC, December 2016.
- Quesada-Ocampo L. M. 2016. Fungicides and host resistance for cucurbit downy mildew management. 31st Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2016.
- Quesada-Ocampo L. M. 2016. Management of cucumber downy mildew using fungicides and host resistance. Pickle Packers International Annual Meeting. Charleston, SC, October 2016.
- Quesada-Ocampo L. M. 2017. Cucurbit disease management. Commercial vegetable grower symposium. Henderson, NC, February 2017.
- Quesada-Ocampo LM. 2019. Biology and control of Cucurbit Downy Mildew and Phytophthora capsici in Pickling Cucumbers. Saginaw Valley Research and Extension Center in Frankenmuth, MI. Feb. 14

Ribera, Luis A. Trade impact talks:

APHIS Project Kick-Off, Raleigh, North Carolina, January 31, 2017.

C-FARE, Washington, DC, April 6, 2017.

Viva Fresh 2017, Austin, Texas, April 21, 2017.

Texas A&M AgriLife Program Planning Meeting, Rosenberg, Texas, May 9, 2017.

Texas International Produce Association, Mission, Texas, May 30, 2017.

Moosejaw, Canada, June 27, 2017.

Imperial Valley EDC, Calexico, California, August 15, 2017

Ag. Economics Extension Tailgate Workshop, College Station, Texas, September 30, **2017**. Extension Outlook Conference, Stillwater, Oklahoma, October 20, **2017** Del Rio Economic Development Council, Del Rio, Texas, November 2, **2017**. Imperial Valley EDC Annual Banquet, Calexico, California, November 16, **2017** 29th Annual Texas Plant Protection Conference, Bryan, Texas, December 5, **2017**.

Schultheis, J.R. 2016. Grafted vs. nongrafted watermelon studies. NC Watermelon Convention. Wrightsville Beach, NC, Mar.

- Schultheis, J.R. 2017. A perspective on melons; some North Carolina cultivar results and some "food" for thought. Eastern Cantaloupe Growers Association. Nashville, TN, 16 February 2017.
- Schultheis, J. 2017. Perspectives and opportunities for growing orange flesh and specialty melons. Vine crops Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI Dec. 2017
- Schultheis, J. 2017. The North Carolina pickling industry and use of parthocarpic fruiting types. Pickling Cucumber Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec.
- Schultheis, J. 2018. Melon varieties; orange flesh and various specialty melon opportunities. DelMar Vegetable meeting. City, DE, January 2018.

Schultheis, J. 2018. A melon mix of specialty and cantaloupe types. Ontario Fruit and Vegetable Convention. Niagara Falls, ON Canada. February 2018

Schultheis, J._2018. The North Carolina pickling industry and use of parthenocarpic fruiting types. . Regional Pickling Cucumber meeting, Wilson Co., NC, March

- Schultheis, J.R. and T. Birdsell. 2017. Butternut squash production. Winter Vegetable Conference and Trade Show. Asheville, NC, 8 February 2017.
- Schultheis, J.R. and S. Johnson. 2015. Grafted versus nongrafted watermelon studies using bare ground or plasticulture production methods. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC. Dec.

Schultheis, J.R. and K.D. Starke. 2017. Pollenizer placement considerations effects on watermelon (*Citrullus lanatus*) yield and quality. Watermelon Research Group, Mobile, AL. Feb. 2017

Schultheis, J. and K. Starke. 2017. Specialty melon opportunities. Annual North Carolina Vegetable Growers Association, Myrtle Beach, SC, November.

Schultheis, J. and K. Starke. 2017. Watermelon production considerations; pollenizer and grafting. Annual North Carolina Vegetable Growers Association, Myrtle Beach, SC, November.

- Schultheis, J. and K. Starke. Standard size watermelon cultivar and quality results, North Carolina, 2017. Georgia Watermelon Association. Saint Simons Island, GA, January 2018.
- Schultheis, J. and K. Starke. <u>2018</u>. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. 2018 Watermelon Research and Development Group Annual meeting, Jacksonville, FL, February.
- Schultheis, J.R. and K.D. Starke. 2018. Pollenizer placement considerations effects on watermelon (*Citrullus lanatus*) yield and quality over two growing seasons. Watermelon Research and Development Group Annual meeting, Jacksonville, FL, February.
- Schultheis, J. and K. Starke. 2018. Standard size watermelon cultivar yield and quality results, North Carolina, 2017. North Carolina Watermelon Association, Wrightsville, NC, March.

- Schultheis, J.R. and W.B. Thompson. 2016. Watermelon yield and fruit size response to grafted versus non-grafted transplants in plasticulture and bare ground production systems. Watermelon Research Group, San Antonio, TX, Feb.
- Schultheis, J.R. and W. B. Thompson. 2016, Watermelon cultivar yield and quality trial results, North Carolina, 2015. 2016. Watermelon Research Group, San Antonio, TX, Feb.
- Smart, C. 2015. Disease problems common during the 2015 growing season. Twilight meeting, Eden Valley, NY.
- Smart, C. 2016. Disease update. Western NY Vegetable Growers meeting. Lockport, NC, Mar.
- Smart, C. 2016. Managing cucurbit diseases. Empire State Producers Expo. Jan.
- Smart, C.D. 2016. Understanding and controlling diseases of cucurbits. Crop Consultant Meeting, December 1, Syracuse NY.
- Smart, C.D. 2016. Why is the *Phytopthora* blight from important? New York State Ag Experiment Station Task Force, October 10, 2016.
- Smart, C.D. 2016. Field walk and discussion of diseases of cucurbits and other crops. Western NY Field Days. Portland, NY, Aug 31, 2016.
- Smart, C.D. 2016. Vegetable disease management (1.5 hour discussion with growers and educators). Willsboro, NY, Aug 4, 20116
- Smart, C.D. 2016. Vegetable disease management (1.5 hour discussion with growers and educators). Canton, NY, Aug 3, 2016.
- Smart, C.D. 2016. How the NY Farm Bureau helped established the *Phytophthora* blight farm. Midwest Farm Bureau visit to NYSAES, June 24, 2016.
- Smart, C. and Lange, H. 2016. *Vine Crop Update 2015*. Proceedings of the 2016 Empire State Producers Expo, Syracuse, NY.
- Smart, C.D. 2017. Twilight discussion of cucurbit diseases. Western NY Field Days. Portland, NY, Aug
- Smart, C.D. 2017. On-farm discussion of methods to control Phytophthora blight in summer squash. Seneca Falls NY, July 2017
- Smart, C.D. 2017. Role of cover crops in Phytophthora blight control. Northeast Cover Crops Council. November 2017
- Smart, C.D. and Lange, H. 2018. Fungus, Water Mold or Bacteria: Which is Which in My Vine Crops? New York State Producers Expo. January
- *Smart, CD. 2019.* Real-world management strategies for Phytophthora-infested ground. Oceana County MSU Extension, Hart, MI. Jan 31.
- Starke, K.D. and J.R. Schultheis. 2016. Watermelon (*Citrullus lanatus*) yield and quality response to grafted versus non-grafted plants, 2016. Watermelon Research Group, Mobile, AL. February 2017.
- Starke, K. and J. Schultheis. 2017. Honeydew, crisp flesh and piel de sapo melon options. Annual North Carolina Vegetable Growers Association. Myrtle Beach, SC, November.
- Starke, K.D. and J.R. Schultheis. 2018. Mini-watermelon cultivar yield and quality evaluations in North Carolina, 2017. Watermelon Research and Development Group Annual meeting, Jacksonville, FL, February.
- VandenLangenberg K, Wehner T. 2015. High resistance over the production season to the new downy mildew in cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)
- Wallace E. C. and Quesada-Ocampo L. M. 2015. Controlling downy mildew in cucumber. 30th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, Dec.

- Weng, Y. 2015. QTL Mapping for downy mildew resistance in WI7120 cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)
- Weng Y. 2017. "Genetic architecture of downy mildew resistances in cucumber". 2017 PPI Annual Meeting (Chicago, IL, Nov 1 2017)
- Weng Y. 2017. Genetic resources for cucumber breeding a molecular perspective. PPI 2017 Spring Meeting (4-19-2017, Milwaukee, WI).
- Wang Y, Haider KR, Weng Y. 2016. Pyramiding Downy Mildew Resistance Genes into Elite US Processing Cucumber with Marker-assisted Selection. Pickling Cucumber Commodity Meeting, Grand Rapids
- Wechter, W. P., S.E. Branham, S. Lambel, N. Guner, and A. Levi. 2017. Towards the identification of quantitative trait loci and development of molecular markers linked to Fusarium wilt resistance in watermelon. Presented at the Watermelon Research and Development Group meeting. Mobile, AL. February 2017.
- Wessel-Beaver, L. and A.M. Linares Ramirez. 2017. Dos Virus Importantes en la Calabaza: Mosaico Amarillo del Calabacín (ZYMV) y Mancha Anular de la Papaya (PRSV). Expo Hort, Lajas, PR 4 Abril. 118 attendees