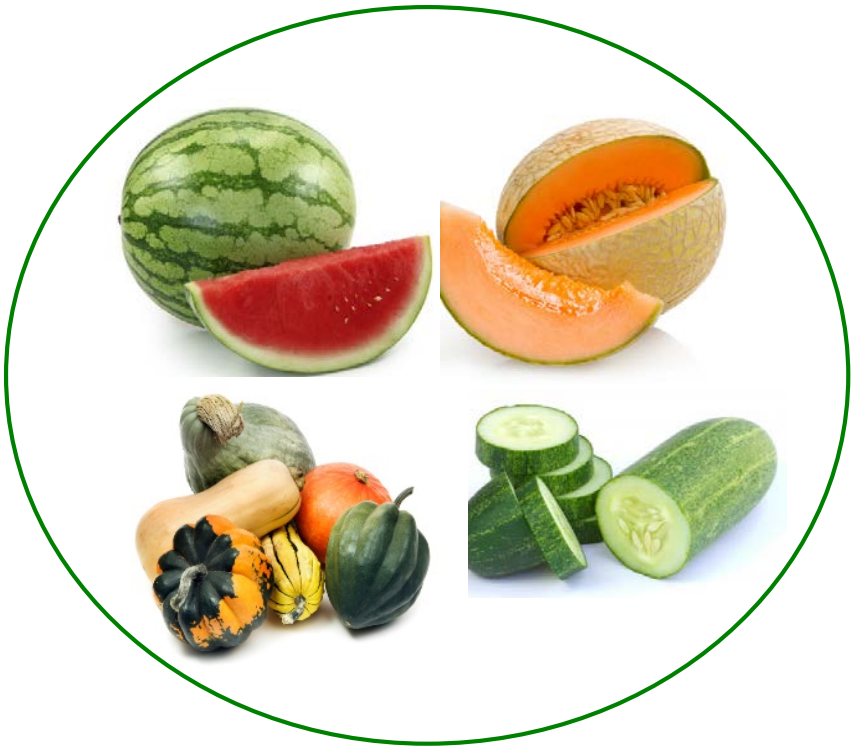
CucCAP:

Leveraging applied genomics to improve disease resistance in cucurbit crops



First Annual CucCAP Team Meeting

June 1-2, 2016

East Lansing MI

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AGENDA

and

PARTICPANTS

AGENDA

Wed. June 1

8:00-8:15	Arrival, welcome
8:15-8:45	Introductions (team members, industry advisory board, external reviewers, germplasm curators, guests)
8:45-9:15	Overview of project goals, questions, discussion (Grumet)
9:15-9:45	Bioinformatics team report, questions, discussion (Fei et al)
9:45-10:30	Watermelon team report, questions, discussion (Levi et al)
10:30-10:45	Break
10:45-11:15	Melon team report, questions, discussion (McCreight et al)
11:15-11:45	Cucumber team report, questions, discussion (Weng et al)
11:45-12:15	Squash team report, questions, discussion (Mazourek et al)
12:15-1:15	Lunch. Commodity team meetings.
1:15-1:45	Extension team report, questions, discussion (Schultheis et al)
1:45-2:15	Socioeconomics team report, questions, discussion (Palma and Ribera)
2:15-2:30	Break
2:30-3:00	Views from the cucurbit industries – needs and concerns
3:00-3:30	Views from the seed industry
3:30-5:00	Workshops I A. Core collections and germplasm management (Mazourek lead) (curators, seed company reps, members of crop teams) B. Planning for representative farms – economic analysis (Palma lead) (commodity reps, socioeconomic and extension teams)
7:00	Evening barbecue (Grumet and Smith home, 933 Lantern Hill Dr., East Lansing)

Thurs. June 2

8:00-8:15	Arrive
8:15-9:00	Workshops II A. Gene nomenclature (Weng lead) (members of crop teams, curators, seed company reps) B. Extension plans and industry needs (Schulthies lead) (commodity reps, extension, socioeconomic, crop team members)
9:00-10:30	Workshops III A. Web plans and needs – bioinformatics (Fei lead) B. Web plans and needs – cucurbit diseases (Schultheis, Quesada leads)
10:15-10:45	Break
10:45-12:00	Wrap up discussions, feedback from advisory board and external reviewers

CucCAP Team

Project Director

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Stakeholder Advisory Board		
Organization	**Representative**	**Position**
Commodity Groups - Growers, Shippers, Processors, Marketing		
National Watermelon Promotion Board	Mark Arney	Executive Director, National Watermelon 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 International	John Swanson	President Swanson Pickle Company; Research Board, Pickle Packers International
Martin Farms (squash grower, shipper)	Mitch Beyler	Partner, John B. Martin and Sons Farms, Inc.
Stony Brook Wholehearted Foods (squash processor)	Greg Woodworth	Founder, Stony Brook Wholehearted Foods
Seed Industry		
Bayer Crop Science	Jovan Djordjevic/ Suren Baliji	Global R&D Lead, Melons and Watermelons, Bayer Crop Science
HM Clause	Alyson Thornton	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/ Lindsay Wyatt	Chairman, Johnny's Selected Seeds
Magnum Seeds, Inc.	Ken Owens	President, Magnum Seeds
Monsanto	Nischit Shetty	NAFTA Cucurbit Lead for Monsanto Vegetable Seeds
Sakata Seeds	Jeff Zischke/ Benito Juarez	Director of Research, Vegetables, Sakata Seed
Syngenta Seeds Inc.	Jim Brusca	Global Cucurbits Co-Lead, Syngenta Seeds
United Genetics Seeds Co.	Xuemei Zhang	Melon Breeder, United Genetic Seeds

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Emillio Sarria Villada, Rijk Zwaan

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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

Disease priorities identified by the cucurbit industries:

Table 1. Major disease threats to cucurbit crop production as identified by cucurbit industry stakeholders.

Disease	Identified as commodity funding priority[a]	Also affects:
Downy mildew	cucumber	melon, watermelon, squash/pumpkin
Fusarium 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], PRSV-W[d], CGMMV[e]	melon[b,c], watermelon[d,e]	cucumber[c,e], squash/pumpkin[b,d]

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 Weng***	(YW)	ARS-WI
	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 Schultheis***	(JS)	NCSU
	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

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

TIMELINE CHART

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	X	X
1.1.3. ICuGI database development	ZF (BTI)	X	X	X	X
1.1.4 Community standardized nomenclature	YW (ARS-WI), AL (ARS-SC) JM (ARS-CA). MM (CU)		X	X	
1.1.5. Genomic, bioinformatics workshops	ZF (BTI), UR (WVSU), members of crop teams		X	X	X
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	ZF (BTI), RG (MSU)	X	X		
- watermelon	AL (ARS-SC)	X	X		
- melon	JM (ARS-CA)	X	X		
- cucumber	YW (ARS-WI),	X	X		
- squash	MM (CU)	X	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	YW (ARS-WI), RG (MSU)		X	X	X
- squash	MM (CU)		X	X	X
(b) Obj. 2. Genomic assisted breeding for disease resistance					
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), 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)	P	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					
- 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)	P	F	SQ	
2.1.3. Cucumber					
- downy mildew	YW (ARS-WI), TW (NCSU)	PFS	SQ	SQ	
- Phytophthora	RG (MSU)	PF	PFSQ	SQ	
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)**				
2.2.1. 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), TW (NCSU)			RMV	V
- Phytophthora	SK (ARS-SC)			RM	V
- powdery mildew	SK (ARS-SC)			RM	V
- PRSV-W	AL (ARS-SC), KSL (ARS-SC)			RMV	V
2.2.2. Melon					
- powdery mildew	SK (ARS-SC)			RM	V
- Fusarium	PW (ARS-SC)	M	RM	RM	V
- CYSDV	WW (ARS-CA), JM (ARS-CA)			RM	V
- CMV	JM (ARS-CA), MM (CU)			RM	V

2.2.3. Cucumber - downy mildew - Phytophthora	 YW (ARS-WI), TW (NCSU) RG (MSU)	 RM	 RM	 V RM	 V V
2.2.4 Squash - powdery mildew - Phytophthora - PRSV-W - CMV	 MM(CU), LWB(UPR) MM (CU) MM(CU), LWB(UPR) MM(CU), LWB(UPR)	 RM	 V RM RM	 RM V V	 V
2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
2.3.1. Watermelon - Fusarium race 1 race 2 - gummy stem blight - Phytophthora - powdery mildew - PRSV-W	 AL (ARS-SC), PW (ARS-SC) PW (ARS-SC), AL (ARS-SC) CM (UGA), TW (NCSU) SK (ARS-SC) SK (ARS-SC) AL (ARS-SC), KSL (ARS-SC)	 B B B B B	 I B B I I B	 IA I I I I I	 AR I I A A I
2.3.2. Melon - powdery mildew - Fusarium - CYSDV - CMV	 SK (ARS-SC), JM (ARS-CA) PW (ARS-SC) JM (ARS-CA), WW (ARS-CA) JM (ARS-CA)	 B B I I	 I B I I	 I I IA I	 IA IA IAR IA
2.3.3. Cucumber - downy mildew - Phytophthora	 YW (ARS-WI), TW (NCSU) RG (MSU)	 B B	 I B	 I I	 R I
2.3.4 Squash - powdery mildew - Phytophthora - PRSV-W - CMV	 Already exists MM (CU), CS (CU) Already exists Already exists	 I	 I	 AR	 AR
(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)	X	X		
3.1.2. Estimate the potential economic impacts to the cucurbit industry	LR (TAMU), MP (TAMU)			X	X
3.2 Provide readily accessible information to facilitate disease control					
3.2.1. Develop a centralized cucurbit disease website	LQ (NCSU), JS (NCSU)	X	X		
3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster	LQ (NCSU), MH (MSU), CS (CU), ALR (UPR)	X	X X	X X	X
3.2.3 Provide disease alerts and forecasting tools		X	X	X	X
3.2.4. Field days and demonstration plots	Crop and extension teams	X	X	X	X

Current status of resistance breeding for the priority cucurbit diseases.

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-USVL252^{FR2}	Standard: Charleston Gray Icebox: Sugar Baby	SC	X	x	X				x		
Fusarium race 1	Calhoun Gray		SC	x	x	X	**X**	**X**		X		
Gummy stem blight (GSB)	PI 482276-UGA1081; PI 526223-UGA157		NC, GA	x	x	X				X		
Phytophthora (Phyt)	PI 494531-USVL531MDR; PI 560003- USVL003MDR		SC, NC	x	x	X				X		
Powdery mildew (PM)			SC, NC	X		X	**X**			X		
CGMMV	Currently evaluating		GH[b]									
PRSV-W	PI 595203		SC	X	x	x	X					
Melon												
Powdery (PM)	MR-1	Cantaloupe: TopMark, Impac Honeydew: Green Flesh Honeydew or PMR Honeydew	CA1,2, AZ	x		X	X					
Fusarium (Fus)	MR-1		CA1	x		X	X			X	X	
CYSDV	PI 313970; TGR1551		CA1, AZ	X	x	X	**X**					
CMV	PI 161375; Freeman cucumber		CA1,2, AZ				X					
Cucumber												
Downy mildew (DM)	PI 197088; PI 330628	Slicer: Poinsett 76 Pickling: NC-25, GY14	WI, NC	X		X	**X**	**X**		X	X	
Phytophthora (Phyt)	PI 109483		MI, NY		**x**	X						
Squash												
Phytophthora (Phyt)	PI 211996; PI 483347; PI 634693	Butternut: Burpee Butterbush	NY	X			X					
Powdery (PM)	*C. martenezii*	Tropical pumpkin: Soler,Taina Dorada	PR	x			X	**x**	x	x	x	x
PRSV-W	Menina, Nigerian Local		PR	X			X			x	X	X
CMV	Menina, Nigerian Local		PR	x			x			x	X	X

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.

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)

Objectives Year 1

Develop common genomic approaches and tools for cucurbits

(a) Obj. 1. Develop common genomic approaches and tools for cucurbits		Year 1	Year 2	Year 3	Year 4
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	X	X
1.1.3. ICuGI database development	ZF (BTI)	X	X	X	X
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	ZF (BTI), RG (MSU)	X	X		
- watermelon	AL (ARS-SC)	X	X		
- melon	JM (ARS-CA)	X	X		
- cucumber	YW (ARS-WI),	X	X		
- squash	MM (CU)	X	X		

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 Facility, we have set up the genotyping-by-sequencing (GBS) platform for the four cucurbit species: watermelon, melon, cucumber and squash. Due to the recent patent issue of the GBS technology, Cornell Genomic Diversity Facility has stopped GBS service for samples that are currently not registered in their system. Fortunately, this will not affect our proposed GBS of 10,000 cucurbit samples.

Meantime, the Mazourek lab is currently testing an alternative genotyping technology for cucurbit species. This genotyping strategy is based on sequencing variable regions of moderately repetitive elements (a few thousand copies per genome) and much cheaper and easier to work than GBS, but generates much less markers. It has been shown to work well in maize which has a highly repetitive genome. It will provide a nice alternative if it works in cucurbits.

1.1.2. Sequence data processing/analysis

We have evaluated and compared the performance of TASSEL-GBS (http://www.maizegenetics.net/#!tassel/c17q9) and GATK (https://www.broadinstitute.org/gatk/) in SNP calling using GBS data we have generated for *C. maxima* and *C. moschata.* SNPs called from the deep genome resequencing data (>60x genome coverage) of two individual plants

(parents of the mapping populations) were used to serve as positive controls. Our results showed that GATK achieved higher accuracy in GBS SNP calling than TASSEL-GBS. More extensive and thorough evaluation of these two programs is underway. The obtained results will guide us to use more appropriate software and parameters in our future GBS data analysis.

1.1.3. ICuGI database development

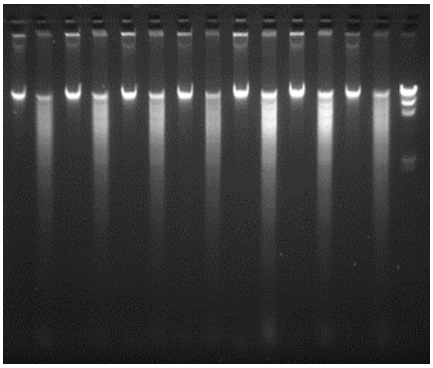
We are in the process of re-implementing the ICuGI database using the GMOD Tripal system (http://gmod.org/wiki/Tripal) and the Chado database schema (http://gmod.org/wiki/Chado). Melon genome sequence has been processed and will be included in the database. Genome syntenies between watermelon, melon and cucumber have been identified and a genome syntenty browser will be implemented in the database using GBrowse_syn (http://gmod.org/wiki/GBrowse_syn). The newly implemented database is expected to go public this fall.

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

(i) Establishment of high-throughput DNA isolation capacity

We have purchased equipment funded by Michigan State Univ. to establish a multiuser facility to facilitate high-throughput DNA isolation from samples in a 96 well format. This facility will serve as a resource for the full CucCAP project to prepare DNA for GBS on PI collections for the four cucurbit species. Protocols have been optimized to ensure high quality DNA preparation from cucurbit leaf samples, including both sample collection and preparation steps, and DNA isolation. Each crop team wishing to use the facility will grow the PI seedlings, sample leaf tissue in accordance with the optimized protocols and send the samples to the Grumet lab for processing. Given the extensive numbers of samples to be handled, it is important to ensure optimization, uniformity, and proper sample identity throughout the process. Quality of the DNA is verified according to the standards required by the Cornell Genomics Diversity Facility, including quantification, and gel analysis of uncut and HindIII digested DNA (300-500 ng/sample) as shown here for several cucumber samples.



(ii) Sample collection, preparation and genotyping

The four crop team leaders have been working (or will work) with the germplasm curators to assemble a panel of diverse genotypes that includes materials in the PI collection at the National Plant Germplasm System (NPGS) as well as important open-pollinated or pureline cultivars. DNA will be sampled from a total of 1000-1600 accessions of each crop.
We have initiated DNA preparation from the cucumber PI collection. The first two plates have been sent to Cornell for GBS and sequencing data will be expected in the coming month. Data will be analyzed to assess the efficiency of the established high-throughput tissue sampling and DNA isolation protocol in GBS analysis.

Watermelon Team

Team members:

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

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

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

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 - watermelon	ZF (BTI), RG (MSU) AL (ARS-SC), TW (NCSU)	X X	X X		
(b) Obj. 2. Genomic assisted breeding for disease resistance					
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), sequence **(S)**, QTL map **(Q)**				
2.1.1. Watermelon - CGMMV - Fusarium race 1 race 2 - gummy stem blight - Phytophthora - powdery mildew - PRSV-W	 KSL (ARS-SC), AL (ARS-SC) AL (ARS-SC), PW (ARS-SC) PW (ARS-SC), AL (ARS-SC) CM (UGA), TW (NCSU) SK (ARS-SC) SK (ARS-SC) AL (ARS-SC), KSL (ARS-SC)	 Sc FSQ PFS P PFS PFS PF	 Sc,P Q PFSQ PFSQ SQ SQ FSQ	 P,F,S FSQ FQ FSQ	 S,Q
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

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)

1.2.1. GBS of cucurbit species, establish molecular-informed core populations (T.Wehner, T. Patel)

i. Increasing watermelon PI accessions and preparing leaf samples for DNA isolation and genome wide association study (GWAS).

We will collect and increase *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 list of the PI accessions and gene type lines is being developed to use for the DNA sampling. The sampling protocol is from Michigan State University, and will involve 1000 PI's sampled at the seedling stage, with one plant per accession.

ii. 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*).
A list of the 42 type lines is included in **Appendix 3**.

2.1 QTL map resistances

2.1.1. Watermelon

2.1.1.1 CGMMV

Evaluating the watermelon PI collection for resistance to *Cucumber green mottle mosaic virus* (CGMMC) and conducting genome-wide association mapping to identify SNPs associated with CGMMV resistance (KS Ling, A Levi)

CGMMV is a tobamovirus, similar to *Tobacco mosaic virus* (TMV). This virus was first discovered in Europe and has caused serious epidemics in several Asian countries, like China, Japan, and Korea, and more recently in Australia and the Middle East (Jordan and Israel). Due to its seed-borne nature and global seed trade CGMMV geographic distribution has been expanding rapidly and it became a major threat to all major cucurbit crops and the entire cucurbit industries in the U.S. and around world. The CGGMV has been identified as an emerging virus on cucumber, melon, watermelon and other cucurbit crops in Canada and the United States (Ling et al., 2014; Tian et al., 2014) and in Australia (http://www.nt.gov.au/d/cgmmv/).

- Commercial watermelon cultivars are highly susceptible to CGMMV infection. With a start-up fund support from a USDA germplasm evaluation grant and a watermelon-CGMMV consortium, we have planned to screen the entire collections of USDA watermelon germplasm (~1600 accessions).
- Under a special USDA-APHIS permit we have designated an isolated greenhouse at the USDA, ARS, U.S. vegetable Laboratory for working specifically with CGMMV.

- We completed the first screening of 800 accessions using mechanical inoculation with an Asian CGMMV isolate.
- Most of the watermelon PIs evaluated were susceptible CGMMV while several PI plants that may have some level of resistance or tolerance were selected and are under further evaluation.
- Screening of the second half of germplasm collection (800 PI accessions) are underway and should be completed over the 2016 summer.
- Once the primary screening has been completed, a repeat screening with selected promising resistance accessions will be carried out in the second half of the year 2016, as well as for fruit and seed production on the selected resistant plants.
- Once the watermelon germplasm screening is completed. Single plant selection will be conducted. Genetic populations will be developed to generate F1, F2, BC1S, and BC1R populations for the genetic of inheritance study.
- The phenotypic data obtained from this disease resistance screening will be further used in genome-wide association study (GWAS) to identify putative SNPs associated with CGMMV resistance, in collaboration with CucCAP collaborators.

2.1.1.2- Fusarium race 1,2

Genetic mapping of QTL associated with resistance to Fusarium oxysporum races 1 and 2 in *Citrullus lanatus* var. *citroides*. (P Wechter, A Levi)

Fusarium oxysporum f. sp. *niveum* which causes fusarium wilt of watermelon, is considered one of the most important diseases of watermelon production in the United States. There are currently no economical or even viable chemical control strategies or methods that can control this soil-borne pathogen. To date, only a few watermelon lines have been identified and reported as resistant or tolerant to this pathogen. Unfortunately, although some of these lines were reported more than twenty-five years ago, no commercial cultivar is available with resistance to the most serious of the races of this fungus, race 2.

- We developed two *Citrullus lanatus* var. *citroides* (*Clc*) germplasm lines derived from United States plant introductions (PIs) with high levels of resistance to Fon race 1 and 2, USVL246-FR2 and USVL252-FR2. Genetic populations F_1, F_2, F_3 BC_1F_2 are being generated by crossing each of these resistant lines with susceptible parents.
- Genetic populations F_2:F_3 are being generated from USVL246-FR2 crossed with the susceptible *Clc* parent PI 542114 (Pop 46-14); and for USVL252-FR2 crossed with the susceptible *Clc* parent PI 244017 (Pop 52-17) or PI 244019 (Pop 52-19).
- Inheritance studies are being performed using the populations generated from USVL246-FR2 and the susceptible *Clc* parent PI 542114 (Pop 46-14), while inheritance study and GBS analyses will be performed for Pop 52-17 and Pop 52-19 in early 2017.
- The first round of Fusarium race 2 assays have been performed on the F_3 individuals of Pop 46-14.
- Additional rounds of phenotyping for tolerance to Fon race 2 will be performed during the next 4 months, with Fon race 1 assays beginning at that time.

- DNA has been isolated from 200 F_2 plants and each of the F2 plants is being self-pollinated to generate an F_3 families of Pop 46-14.
- Genotype-by-sequencing (GBS) will be performed during the next 4 months for Pop 46-14.

2.2.1.1.Converting a Fusarium wilt race 1-resistance QTL to a DNA marker (A Levi, P Wechter)

Fusarium wilt (*FW*) race 1 is a major disease of watermelon throughout the United States. In a recent study (Lambel et al. 2014), we identified on chromosome 1 of watermelon a major quantitative trait locus (QTL) associated with resistance to *FW* race 1. There is a need to develop a SNP marker(s) useful in marker assisted selection (MAS) to precisely predict the presence of resistance in large genetic populations.

We have been developing genetic populations F1, F2, BC1R, BCS [Calhoun Gray (R) x Sugar Baby (S); or Calhoun Gray (R) x Black Diamond (S)] segregating for resistance to *FW* race 1. Also, the bioinformatics team led by Dr. Zhangjun Fei at Boyce Thompson Institute developed for us a data set to compare SNP sequences within this specific QTL on Charleston Gray, Calhoun Gray, Sugar Baby, and Black Diamond.

To identify a tightly linked DNA marker, we will screen candidate SNPs using the DNA of the original F2 plants used to identify the QTL based on the phenotyping of their F3 families (Lambel et al. 2014). Also, we will validate the marker using 500 F2 plants of [Calhoun Gray (R) x Sugar Baby (S); or Calhoun Gray (R) x Black Diamond (S)] segregating for FW race 1 resistance.

2.1.1.3. Gummy stem blight

Develop molecular markers for high resistance to gummy stem blight (GSB) using genome-wide association studies (GWAS) approach in the USDA watermelon germplasm collection, and introgress GSB resistance into watermelon cultivars. (T Wehner, Luis Rivera)

Phenotyping: The WmGsb 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 296 lines will be screened in the MAF greenhouse and the field at Clinton NC. Resistance will be rated several times on each plot, in an experiment having 2 years, 4 replications, and 2 locations (greenhouse and field).

Genotyping: A group of 384 watermelon accessions are being selected to develop a core watermelon populations. The core watermelon population will be used to develop sequence-based molecular markers (SNPs) using the genotyping by sequencing (GBS) method at Cornell University. We expect to get several thousand of SNPs for the association analysis.

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

QTL mapping, marker validation and trait introgression of Gummy Stem Blight resistance in watermelon. (C McGregor)

Our goal for this year was to develop two mapping populations for gummy stem blight resistance.

WPop GSB 1: PI 482276 x Crimson Sweet population of at least 100 seeds per line for 184 $F_{2:3}$ lines. The resistant source for this population is a selection from PI 482276 (*Citrullus amarus*; previously *C. lanatus* var. *citroides*). *C. amarus* is a crop wild relative (CWR) of the sweet, edible watermelon types (*C. lanatus*; previousely *C. lanatus* var. *lanatus*). Severe segregation distortion and pollen fertility issues have been observed in crosses between *C. amarus* and *C. lanatus*. These issues as well as possible chromosomal re-arrangements make population development and QTL mapping challenging in this population.

WPop GSB 2: PI 526233 x Sugar Baby population of at least 100 seeds per line for 92 $F_{2:3}$ lines. The resistance source is a selection from PI 526233. PI 526233 is *C. lanatus* which should simplify mapping and trait introgression. However, the level of resistance in PI 526233 is not a high as PI 482276.

Progress Year 1:

For WPop GSB 1, 149 $F_{2:3}$ lines are complete (100 seeds per line). An additional 53 plants have fruit set and fruit will be harvested once mature.

For WPop GSB 2, 50 $F_{2:3}$ lines are complete (minimum 100 seeds per line). An additional 50 plants will be transplanted in June.

Leaf samples of all parental, F_1 and F_2 plants were collected and are currently stored at -80°C.

A Ph.D. Student, Winnie Gimode has been appointed to carry out research on this project.

Conclusion and Future Work: We are on course to complete these 2 populations by the end of 2016. After discussion with the project leader, Amnon Levi, an additional population was initiated. WPop GSB 3 will be developed from a cross between PI 482276 and a susceptible *C. amarus* accession. This will be a backup population that can be used if the segregation distortion and chromosomal rearrangements in the inter-specific cross (WPop GSB 1) hampers its use. Phenotyping will be the biggest challenge of this project. Syngenta has agreed to supply us with control lines with known, confirmed (field and greenhouse) phenotypes for Gummy Stem Blight resistance. The inclusion of such controls will ensure consistent results across experiments.

2.1.1.4. Phytophthora fruit rot of watermelon (S. Kousik)

Inheritance of resistance.

We conducted a study to determine inheritance of resistance to Phytophthora fruit rot using the the segregating population derived from the cross of USVL531-MDR x PI 269677. Fruit from parents, F1, F2 and back cross populations were harvested when mature and placed on wire shelves in a walk-in-humid chamber. Each fruit was inoculated with a 7-mm agar plug from an actively growing colony of *Phytophthora capsici* as described (Kousik et al., 2014). Data on fruit rot was recorded five days after inoculation. We are currently compiling and analyzing the data from this study.

2.1.1.5 Powdery mildew (S Kousik)

Inheritance of resistance.

Inheritance of resistance to powdery mildew of watermelon caused by *Podosphaera xanthii* was conducted on the segregating population derived from the cross of USVL531-MDR x PI 269677. A total of 713 plants were evaluated. Of these 66 plants were of the resistant parent (USVL531-MDR derived from PI 494531) and 81 plants of susceptible parent (PI 269677). Of the segregating population, 112 were F_1, 311 F_2, 64 BCF_{1S} and 80 BCF_{1R}. All the plants were inoculated using a suspension (10^5 conidia^{-ml}) of powdery mildew conidia in sterile water plus 0.02% tween 20 as described before (Kousik et al., 2011). Powdery mildew ratings on a 0-10 scale of increasing disease severity was recorded for hypocotyl, cotyledons and true leaves. Resistance to powdery mildew in cotyledons and true leaves appears to be a dominant trait in USVL531-MDR. Leaf samples from the segregating population and parents were collected for DNA extraction and further analysis by GBS. Of the F_2 plants we self-pollinated 186 plants kept in a net house to generate $F_{2:3}$ populations for further evaluation. Fruit from F_2 plants with powdery mildew resistance, uniform red flesh and decent brix (>7) were also collected for further advancement.

2.1.1.6 PRSV-W (A Levi, K-S Ling)

Identification of QTL associated with papaya ringspot virus (PRSV) in watermelon

Genetic populations F2:F3 are being generated using PRSV-susceptible *Clc* parent USVL252-FR2 crossed with the PRSV-resistant *Clc* parents PI 244017 (Pop 52-17) or PI 244019 (Pop 52-19) mentioned above. In early 2017 these genetic populations will be evaluated for PRSV-resistance (as described by Ling et al. 2009) and GBS will be conducted for identification of SNPs and QTL associated with the resistance.

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)	X	X		

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 accessions held by North Central Plant Germplasm Resources Center, Ames, Iowa will be grown in a greenhouse at Salinas and sampled for GBS beginning late in year 1 and continuing through year 2.

Completing production of 200 RILs of MR-1 x Ananas Yokneam (MR1xAY). Seed of each RIL will be produced for remaining studies, including GBS on ~190 RIL for identification of high-quality SNPs for use in QTL analysis and mapping. Finished generation of one hundred and seventy-five F_7 or greater MR1xAY RIL. Will have completed 200 F_7 or greater lines by the end of 2016. Seed has been distributed for powdery mildew screens and fruit quality assessment. Seed being increased for 2017 screens.

Performed GBS of 89 MR1xAY RILs and have identified 2200, high-quality SNPs that have been used to identify 2 QTLs linked to Alternaria leaf blight resistance and to generate a high-density map of MR-1 x AY.

Will perform GBS with another 95 RILs and identify high-quality SNPs from the sequencing data. Will begin the Fusarium assays for Race 1 and 2. Will repeat powdery mildew tests and begin QTL analysis and mapping of Powdery mildew resistance.

(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**), 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)	P	F	SQ	

2.1.2.1 Powdery Mildew (Podosphaera xanthii) resistance in MR1xAY RIL

Charleston, South Carolina Race 1

- greenhouse
- 166 RIL lines
- Plants of Powdery mildew melon race differential lines were included
- 3 rep of 4 plants
- Inoculated with powdery mildew pathogen on May 16, 2016.
    - Conidial suspension (10^5 conidia^{-ml} in distilled water with 0.02% tween 20)
    - Uniformly sprayed on the plants.
- Disease reaction will be recorded 14 and 21 days post-inoculation (May 30th and June 7th).
- All the lines will be planted in the field for further observation, space permitting.

California and Arizona

- Field tests
    - Imperial Valley (DREC)
        - watered March 4
        - Disease reaction evaluated mid-June
        - Variable plant stands
    - Yuma (YARC),
        - watered March 31
        - Disease reaction evaluated mid- to late June
        - Stands fairly uniform
        - CMV present in late April
    - Westside (WREC),
        - Planting scheduled for early to mid-June
        - Evaluation in early to mid-September
- Natural infection
- 138 MR1xAY RIL
- Race identities unknown
    - Determined by CPM Differentials
    - Likely will not be race 1 or 2
- QTL identification and mapping of genetic regions involved in resistance.

2.1.2.2. Fusarium wilt resistance in MR1xAY RIL

- Perform Fusarium race 1 and 2 assays
- QTL identification and mapping of genetic regions involved in resistance

2.1.2.3. CYSDV

- Backcrossed resistant field selections and selfed for testing in Fall 2016.

2.1.2.4. CMV

- Increase advanced CMV-resistant lines (western U.S. shipping type cantaloupe, and honeydew) developed by M. Kyle-Jahn and H.M. Munger for assessment of CMV resistance in controlled-inoculation greenhouse tests and adaptation and fruit quality in field tests at three locations in Arizona and California.

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)	M	RM	RM	V
- CYSDV	WW (ARS-CA), JM (ARS-CA)			RM	V
- CMV	JM (ARS-CA), MM (CU)			RM	V

Powdery Mildew

- No planned research for this period

Fusarium wilt

- See Objective 1.

CYSDV

- No planned research for this period

CMV

- No planned research for this period

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)	B	I	I	IA
- Fusarium	PW (ARS-SC)	B	B	I	IA
- CYSDV	JM (ARS-CA), WW (ARS-CA)	I	I	IA	IAR
- CMV	JM (ARS-CA)	I	I	I	IA

Powdery Mildew

- Testing MR1xAY RILs at Charleston, Imperial Valley, Yuma, and Westside

Fusarium wilt

-

CYSDV

- Backcrossed resistant field selections from Fall 2015 for selfing in the greenhouse for testing in Fall 2016.

CMV

- Increasing advanced CMV-resistant lines for testing in Arizona and California

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) YW (ARS-WI)	X X	X X		
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 - Phytophthora	YW (ARS-WI), TW (NCSU) RG (MSU)	PFS PF	SQ PFSQ	SQ SQ	
2.2 Marker development and verification	R=Refining map, M=develop marker, V= verification				
2.2.3. Cucumber - downy mildew - Phytophthora	YW (ARS-WI), TW (NCSU) RG (MSU)	RM	RM	V RM	V V
2.3. Advanced breeding line development	B=breeding line, I=introgression, A=advanced, R=release				
2.3.3 Cucumber - DM - PFR	YW (ARS-WI), TW (NCSU) RG (MSU)	B B	I B	I I	R I

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
(Y Weng lab - YH Wang, AR Madera, KR Haider; R Grumet lab - S Hammar, M Colle)

2015-2016 progress:

At present, 1,429 plant introduction (PI) lines of cultivated cucumber (*Cucumis sativus* var. *sativus*) are available in the USDA-GRIN collection. Seeds of all these PI seeds were requested from Ames, Iowa. Leaf samples were collected from seedlings of these PI lines for genomics DNA collection. DNA isolation, quantification, and quality check has been performed for approximately 1000 samples. Two plates have been sent to Cornell University Genomics Core Facility for Illumina sequencing.

2016-2017 work plan:
1. More cucumber lines (~300) representing different market classes and historical cultivars will be included for GBS.
2. Core collections (384) will be selected pending GBS data analysis from Bioinformatics team.
3. Seed increase of the 384 PI lines
4. The 384 lines will be planted in 2017 field season in both Wisconsin (Hancock) and North Carolina (NCSU) for data collection focusing on DM resistance.

2.1 QTL map resistances

2.1.3. Cucumber

2.1.3.1 QTL mapping of DM resistance (Weng lab)

Fine mapping of major-effect QTL of DM resistance

2015-2016 progress:

We aim to conduct QTL mapping of DM resistance from two resistant sources: PI 330628 (WI7120) and PI 197088. Two mapping populations were developed for QTL mapping including 243 $F_{2:3}$ families from the cross between WI7120 (PI 330628 resistant) and 9930 (susceptible), and 150 RILs from PI 197088 × Coolgreen. From the WI7120 × 9930 population, we have identified 4 QTL, *dm2.1*, *dm4.1*, *dm5.1* and *dm6.1* for DM resistance which together could explain 62-76% phenotypic variations. Among them, *dm4.1* and *dm5.1* were major-effect QTL. We also identified 6 QTL using the PI 197088 × Coolgreen RIL population with the major-effect QTL located in chromosome 5. Three QTL were found to be shared between PI 197088 and WI 7120. The data provide the start point for fine mapping of major-effect QTL as proposed in the present project.

2016-2017 work plan:

1. Continue marker-assisted backcrossing in Gy14 genetic background to develop NILs carrying different combinations of *dm2.1*, *dm4.1* and *dm5.1* QTLs from WI7120.
2. Narrow down the QTL region (1.5 LOD interval) of target QTL regions through fine genetic mapping and GWAS.

2.3. Advanced breeding line development

2.3.3.1. Breeding line development for DM resistance (Wehner and Weng labs)

Research efforts are focused on developing downy mildew resistant populations of cucumber with high fruit quality, high yield, and good agronomic traits. The populations are derived from biparental crosses with susceptible and resistant parents to generate genetic diversity from which to evaluate and select the top 10% with the best attributes.

2015-2016 progress:

Introgression of major-effect QTL (Y Weng)

We plan to introgress two major-effect QTL of DM resistance from WI7120 into the elite pickling cucumber inbred line Gy14. Initial crosses have been made between Gy14 and plants carrying *dm2.1*, *dm4.1* and *dm5.1* QTL from WI7120. BC1 progeny will be selected based on marker information.

RILs (T Wehner lab; EJ Silverman)

The RILs population was developed in 2007 by a cross PI 197088 (HR) x Coolgreen (S). A total of 200 F2 lines were generated and self pollinated in the greenhouse in 2009. The RILs have been tested in 7 years of field evaluations under high disease intensity. The population contains 130 lines; 93 at S10 generation, 43 at S9 generation, 13 at S8 generation, and 7 at the S7 generation. Several lines are being recovered and advanced for use in genetic studies.

Inbreds with resistance and quality (T Wehner lab)

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

Phenotyping of DM resistance in cucumber natural populations (Wehner and Weng Labs)

As a test case, 100 cucumber lines were planted in North Carolina State University experimental fields to collect data for natural infection of the DM pathogen.

2016-2017 work plan:

1. Develop inbred cucumber populations. Three populations (PI 197088 x 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.
2. Identify new sources of resistance. A new population derived from PI 605996 (HR) x '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.
3. Start pyramid major-effect QTL for DM resistance from both WI7120 and PI 197088 resistance sources.
4. Growth chamber and field evaluation of DM resistance of the NILs (Wisconsin and North Carolina).

2.1.3.2 QTL mapping of Phytophthora capsici resistance in cucumber (R Grumet lab - B Mansfeld, M Colle; in collaboration with C. Smart)

Young fruit resistance to *P. capsici*.

2015-2016 progress:

Screening 1076 accessions from the cucumber USDA PI collection led to identification of three PI accessions with potential young fruit resistance to *P. capsici:* PI109483, PI178884, and PI214049. Selected individuals within the accessions were self-pollinated to obtain true-breeding resistant lines suitable for use in breeding programs. This led to development of three resistant S_4 families derived from PI 104983 from Turkey. Seed was produced from these families in the greenhouse this spring (2016). Crosses also were made between resistant S_4 generation, PI 104983-derived plants and the susceptible, sequenced pickling cucumber breeding line, GY14.

2016-2017 work plan:
1. Test promising PI 104983-derived families with multiple *P. capsici* isolates.
2. Replicated trial of PI 104983-derived families in *P. capsici* infested field. Screening to date has been performed by direct inoculation of fruit under laboratory conditions to ensure strong pathogen growth and minimize escape from infection. We will assess performance when exposed to the pathogen under field conditions in collaboration with Dr. Chris Smart who maintains a heavily *P. capsici*-infested field in NY.
3. Produce F2 progeny from PI-derived resistant lines x GY14, phenotyping for response to *P. capsici* and initiate QTL analysis.
4. Initiate GWAS analysis for resistance to *P. capsici*. Using GBS data for cucumber PI accessions and data from prior *P. capsici* screening of the cucumber PI collection.

<u>Age-related resistance (ARR) to *P. capsici*</u>
2015-2016 progress:

Reciprocal crosses were made between cultivars that do, and do not, express ARR; fruit from F_2 progeny were phenotyped for response to *P. capsisi* and young leaf tissue collected from each plant at seedling stage. Sets if individuals expressing the most resistant and most susceptible responses from each experiment were selected for DNA extraction. Quantified and normalized DNA from each phenotype group was pooled and has been sent for Illumina Hi-Seq paired-end sequencing.

2016-2017 work plan:
1. Perform QTL seq analysis for ARR from F2 populations of GY14 X Poinsette and GY14 X Vlaspik.
2. Transcriptomic and metabolomic analysis of peels from ARR+ and ARR- cultivars. Results of transcriptome analysis will be compared with QTL seq analysis to help identify genomic regions of greater interest.

Squash Team

Team members:

Michael Mazourek (Cornell Univ.) Angel Linares (Univ. Puerto Rico)
Linda Beaver (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 populations	ZF (BTI), RG (MSU)	X	X		
- squash	MM (CU)	X	X		
(b) Obj. 2. Genomic assisted breeding for disease resistance					
2.1 QTL map resistances:	Screen for resistance (**Sc**), develop populations **(P)**, phenotype (**F**), 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	
2.3. Introgress resistance into advanced breeding lines:	Develop breeding lines **(B),** introgress into cultivated **(I)**, advanced lines **(A)**, release to breeders **(R)**				
2.3.4 Squash					
- powdery mildew	Already exists				
- Phytophthora	MM (CU), CS (CU)	I	I	AR	AR
- PRSV-W	Already exists				
- CMV	Already exists				

Powdery mildew resistance in squash

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

Powdery mildew is a major fungal disease on squash and pumpkins (*Cucurbita spp*) in the USA and throughout the world. Genetic resistance to the disease is not known to occur naturally within *Cucurbita pepo* and only infrequently in *Cucurbita moschata*, but has been achieved in both species through the introgression of a major resistance gene from the wild species *Cucurbita okeechobeensis* subsp. *martinezii*. Today, this gene, *Pm-0,* is used extensively in breeding, and is found in nearly all powdery mildew-resistant *C. pepo* and *C. moschata* commercial cultivars. In this study, we mapped *C. okeechobeensis* subsp. *martinezii*-derived SNP marker alleles in a set of taxonomically and morphologically diverse and resistant *C. pepo* and *C. moschata* cultivars bred at Cornell University that, by common possession of *Pm-0*, form a shared trait introgression panel. High marker density was achieved using genotyping-by-sequencing, which yielded 266,913 *de novo* SNP markers in the three *Cucurbita* species genotyped. A single 516.4 kb, wild-derived introgression was present in all of the resistant cultivars and absent in an equally diverse set of heirlooms that predated the *Pm-0* introgression (See Figure 1). The contribution of this interval to powdery mildew resistance was confirmed by

association mapping in a *C. pepo* cultivar panel that included the Cornell lines, heirlooms, and 68 additional *C. pepo* cultivars. The region containing the resistance allele was refined to a final candidate interval of 76.4 kb. Studies are currently underway to validate markers in this region and explore gene candidates.

Virus resistance in squash

2.1.4.3, 2.1.4.4. Mapping resistance (M. Mazourek lab)

We are using an association mapping approach similar to that for powdery mildew resistance, above, to identify introgressions from the sources of resistance: 'Nigerian Local' and 'Menina'. This goal will be accomplished by utilizing a genome-wide association study (GWAS), with a focus on *C. pepo*, for mapping the resistance genes to *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). We selected 95 cultivars as the basis of the GWAS panel. The market classes represented in the panel include zucchini, summer squash, crookneck, acorn squash, and pumpkin. The cultivars in the panel include 43 cultivars with reported resistance to ZYMV, 27 cultivars with reported resistance to WMV, 13 with reported resistance to PRSV, and 10 with reported resistance to CMV. Genotyping-by-sequencing (GBS) was used to genotype the panel. 96-plex GBS libraries were prepared using the restriction enzyme ApeK1 for digestion.

We grew the cultivars in the greenhouse for inoculation screens to verify the resistance phenotype for each virus. We performed an initial inoculation screen on a single replicate of the cultivar panel for each of the four viruses. Plants were inoculated with a 1:10 ratio of disease leaf tissue to phosphate inoculation buffer at the first true leaf stage, and then reinoculated a week later to prevent escapes. Viral symptom severity was recorded several times after the second inoculation. The viral titer in each plant, as indicated by concentration of viral coat protein, was determined using an enzyme-linked immunosorbent assay (ELISA). Young leaf tissue was collected from each plant, and samples were prepared using a 1:10 ratio of leaf tissue to extraction buffer. Analysis of the ELISA revealed that the majority of plants, regardless of symptom severity, accumulate the viruses. We are currently performing inoculation screening on additional replicates for each virus and evaluating the viral titer of each plant using ELISA, which confirms successful inoculation.

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

The PR portion of the project emphasizes developing resistance to potyviruses in tropical pumpkin (*Cucurbita moschata*), with primary emphasis on *Papaya ringspot virus (*PRSV), although work will be done on *Zucchini yellow mosaic virus* (ZYMV) as well. In Puerto Rico and other parts of the tropics, tropical pumpkin is used like a winter squash, that is, in its mature state. It is the most important local vegetable crop in Puerto Rico. Many people consume it daily as part of a traditional "rice and beans" dish, where pieces of pumpkin are added to the bean sauce. Summer squash (*C. pepo*) and other types of winter squash (butternut types of *C. moschata* and *C. maxima* winter squash) are very seldom grown (nor consumed) in Puerto Rico and most *Cucurbita* cultivars developed for temperate areas are not adapted to the humid tropics. The local market also expects tropical pumpkin ("calabaza") to have a certain appearance, and most mainland U.S. winter squash cultivars would not be acceptable in the Puerto Rican market. Thus, it is important to incorporate potyvirus and other resistances into germplasm adapted to local conditions.

The primary local tropical pumpkin cultivars in Puerto Rico are 'Taína Dorada', 'Soler' and 'Verde Luz'. We also would like to incorporate potyvirus resistance into an important breeding line,

TP411. These genotypes range from mildly to very susceptible to both PRSV and ZYMV. Two very good sources of resistance to PRSV are known to exist: 'Nigerian Local' (NL) and 'Menina'.

Development of Biparental Populations in Puerto Rico *C. moschata*:

We have developed F2 populations from crosses between the resistant parents ('Nigerian Local' and 'Menina') and two susceptible genotypes ('Taína Dorada' and 'Verde Luz'), and are in the process of developing F2 populations between the resistant parents and another two genotypes we will work with ('Soler' and 'TP411'). We are also developing F2 populations between the highly susceptible 'Waltham Butternut' and the two resistant genotypes. A previous study concerning the inheritance of resistance to PRSV was done using 'Waltham Butternut' x NL, and resistance was reported to be a single recessive gene. We are moving ahead on the assumption that there is a major gene for PRSV resistance. Considerable seed has been or will be produced of each F2 population (at least 400 seeds). Inheritance studies of PRSV will be carried out in at least some of the F2 populations (in all populations if time and resources permit). That work will be done over the next 12 months. Each plant will be phenotyped for symptom severity and tested with ELISAIf markers are identified from the association panel study, then the DNA samples and associated phenotype data could be used to begin validating the those markers in collaboration with Cornell. Further marker validation will be done by self-pollinating a subset of F2 plants (resistant and susceptible types with good horticultural characteristics) to the F4, and then testing those lines for the presence/absence of PRSV resistance markers while at the same time evaluating PRSV symptoms and line performance for horticultural traits.

Phenotyping potyvirus resistance

Our experience suggests that PRSV resistance may be controlled by more than a single recessive gene. Type of symptoms and symptom severity varies among susceptible genotypes. It is possible, even likely, that the two resistant genotypes do not carry the same alleles for resistance (populations for an allele test of 'Nigerian Local' and 'Menina' are also being developed). Therefore, one of the challenges to working with PRSV resistance will be to effectively phenotype plants in a segregating population. We are using both ELISA and symptom severity to phenotype plants. We have completed a preliminary study that looked at variability in ELISA readings of tissue taken from different parts of a plant. A paper on that topic has been submitted for the Cucurbitaceae 2016 meeting this summer in Poland. Currently we are working on developing a screening system that will likely have 3 to 5 phenotypic classes (not just susceptible vs. resistant) for both symptom severity and ELISA readings. We will test this system when we begin screening the first biparental population (Taína Dorada x Nigerian Local) this month (May 2016) using both of these phenotyping methods.

Field study of effect of PRSV and ZYMV on yield and other horticultural traits

In early April we initiated a field study using seven tropical pumpkin genotypes that are known to have a range of resistance from highly resistance (Nigerian Local, Menina) to intermediately susceptible (Taina Dorada, Soler, Verde Luz) to highly susceptible (Waltham, Mos166). Plants were inoculated in the greenhouse with PRSV or ZYMV, or not inoculated (control), evaluated in the greenhouse for symptom severity and ELISA. About 3 weeks post-inoculation the plants were transplanted to the field (single plant plots, 5 reps per genotype-inoculation type combination). We are documenting how symptoms continue to develop in the field (Do symptoms weaken? Strengthen? Does it depend on the genotype?). Tissue will be collected from each plant on two occasions for ELISA testing. Horticultural traits (flowering date, fruit size, number and weight, flesh thickness, color [*L, chroma, hue], °Brix, %dry matter). We know of no similar study documenting the impact of potyvirus infection in tropical pumpkin.

Mechanism of PRSV and ZYMV resistance

We have initiated a study (May 2016) to better understand the mechanism of resistance to PRSV in 'Menina' and Nigerian Local. ELISA and RT-PCR are being used to quantify virus concentration and movement as plants develop. We are working with a group of genotypes that vary from susceptible to intermediately resistant to resistant, sampling plants as each new leaf expands. We hope to determine what differentiates a susceptible plant from a resistant plant and when that difference happens. Questions we hope to answer include:

Do differences in virus concentration in resistant vs. susceptible genotypes occur immediately after inoculation of cotyledons, in the 1st or 2nd leaf, or do differences occur in later leaves? Is resistance a matter of less virus replication, a restriction in the movement of the virus, or a combination of these things?

Is there a difference between the two resistant parents (Nigerian Local and Menina) in terms of virus replication and movement?

Nigerian Local and 'Menina' are resistant to both PRSV and ZYMV. We hope that eventually we can test whether markers developed for PRSV resistance might also be useful for ZYMV resistance.

Possible change in future composition of Puerto Rico team:

Linda Wessel Beaver is currently the lead Puerto Rico PI, but will likely be passing the project to Angela Linares sometime in 2017 when she retires. At present, Angela Linares is working with the Extension Group. UPR-Mayagüez is currently in the process of recruiting a vegetable extension specialist, and it is likely that this person will come onto the project to carry on the extension aspects, while Angela Linares will be responsible for the breeding and testing aspects of the project.

Phytophthora blight resistance in butternut squash

Field facility for screening (C. Smart)

Smart runs a unique facility known as the 'blight farm'. In 2007, she established a nine acre farm used exclusively for studies on Phytophthora blight at Cornell University's New York State Agricultural Experiment Station in Geneva, NY. Since that time, pathologists, breeders and horticulturalists have worked together and used the farm to test thousands of new vegetable breeding lines for resistance to *Phytophthora capsici*, the pathogen that causes the disease. For these experiments, transplants of each breeding line are grown in the greenhouse, planted into the field at the blight farm, and inoculated with *P. capsici* 7-10 days after transplanting. Each plant is inoculated near the crown with 5 mL of a zoospore suspension. The zoospores germinate and cause disease on susceptible plants. Plants are rated for disease incidence twice per week for 4-6 weeks and results are statistically analyzed. In concert with screening for resistance, we also teste new management practices (both chemical and cultural) on the blight farm.

Based on work from the blight farm, the best management practices and most resistant vegetable varieties can then be tested on grower farms. Through demonstration trials, field days, farm visits, webinars and winter vegetable production meetings we are working with growers to develop management practices that will be successful on their farms. Importantly, we have also characterized pathogen isolates for genetic diversity and resistance to commonly used fungicides, so we are certain to test breeding lines against a diversity of pathogen isolates.

In addition to screening breeding lines at the blight farm, Smart also performs greenhouse screens on thousands of plants to identify resistant individuals that will be used as breeding programs move forward. Small plants, about 1 month old, are spray inoculated with *P. capsici* zoospores. Symptoms

begin to appear 5-7 days post inoculation and many plants are dead within 10-14 days. Those plants that have some resistance (are still alive at 14 days) are used as parents in the next generation.

2.1.4.2 Mapping resistance and breeding new butternut squash with resistance to Phytophthora blight and 2.3.4.2 Introgress resistance into advanced breeding lines (M. Mazourek)

Phytophthora capsici affects the vegetative portions of squash plants as well as the fruit. Fruit on vining cucurbits are of special significance not only because of their economic value to the grower but also because as they set on infested soil off raised beds, they are especially prone to disease and infected fruit serve to further multiply the pathogen. Fruit rot from Phytophthora blight has thus far only been effectively controlled through the development of squash with hard rinds that pose a challenge for kitchen preparation. We hope to address all these factors by developing butternut squash that combine bush habit with resistance in the vegetative portion of the plant. A bush habit will keep fruit on raised beds and from resting on pathogen infested soil.

We have previously evaluated *C. moschata* accessions PI 211996, PI 483347, and PI 634693 and found them to be asymptomatic when inoculated with *P. capsici* on the blight farm. We will create F2:3 populations for QTL analysis by crossing them with a powdery mildew resistant bush butternut developed by Mazourek. *Phytophthora* resistant, bush squash will be crossed to a high quality parent for further breeding and validation of QTL from the initial F2:3 populations.

Economics Team

Team members:
Marco Palma (Texas A&M Univ.)
Lius Rivera (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)	X	X		
3.1.2. Estimate the potential economic impacts to the cucurbit industry	LR (TAMU), MP (TAMU)			X	X

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.

In progress:

- Identify the number of representative farms to be developed depending on geographic location, common production practices, and marketing windows, among others variables. (Summer 2016)

- Identify facilitators to develop representative farms (Summer 2016)

- Develop and validate representative farms (Fall 2016-Summer 2017)

Extension/Outreach Team

Team members:

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

(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)	X	X		
3.2.2. Develop and post diagnostic resources and disease control information in English and Spanish; prepare diagnostic poster	LQ (NCSU), MH (MSU), CS (CU), ALR (UPR)	X	X X	X X	X
3.2.3 Provide disease alerts and forecasting tools		X	X	X	X
3.2.4 Provide diagnostic and disease management assistance.	LQ (NCSU), MH (MSU), CS (CU)	X	X	X	X
3.2.5. Field days and demonstration plots	Crop and extension teams	X	X	X	X

3.2. Provide readily accessible information to facilitate disease control

The extension component of this grant will be used to communicate the grant's goals, progress, results and its applications. The extension component will reach beyond those directly involved in the grant, such as breeders, seed company personnel, allied industry partners, growers, and other interested persons. The leadership for extension by commodity will be provided mainly by Mary Hausbeck (cucumber), Lina Quesada (watermelon), Chris Smart (squash), and Jim McCreight (melon). The focus will be on aspects related to disease. Linda Wessel-Beaver and Angela Lineares will be the lead persons providing translation of documents from English to Spanish. Jonathan Schultheis will complement these lead plant pathology PIs with pertinent cultural management information. He will also be involved with leading the efforts with respect to the Cucurbit CAP webpage.

Many of the extension activities will actively incorporate both stakeholders and extension personnel via field days, extension workshops, and commodity meetings at the local, state, national, and international levels. Specifically, the information which follows provides some of the plans, works in progress as well as the results to date for the specific objectives.

3.2.1 Develop a centralized cucurbit disease website.

The website was created and Quesada asked for it to be released (https://cucurbits.ces.ncsu.edu/). The website will need to be populated with information once the website manager is hired by Jonathan Schultheis. The Web Manager position has been posted and anticipated hire date is June 2016. Some information such as links to the Cucurbit Downy Mildew IPMpipe and NCSU disease alerts are already added by Quesada.

The website will be linked to the Cucurbit Genomics Database (http://www.icugi.org) housed at Cornell University to consolidate the genomics information across the project. As written in the proposal, the website will provide project information and events, diagnostic resources and disease control recommendations, disease alerts and forecasting tools.

Current posted information will be linked to the CucurbitCAP website as referenced below, while other activities have been ongoing prior to the creation and populating the website.

3.2.2. Develop and post diagnostic resources and disease control information

Fact Sheets

Quesada:

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

Publications

Hausbeck, M. 2016. Downy mildew spends a decade damaging cucumbers. Vegetable Grower News 50(5):16-17.

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.

Natwick, E.T., M.I. Lopez, W.M. Wintermantel, J.D. McCreight, O. Batuman, and R.G. Gilbertson. Watermelon whitefly insecticide efficacy trial, 2015. Arthropod Management Tests. March 2016 (submitted)

Smart, C. and Lange, H. 2016. *Vine Crop Update 2015.* Proceedings of the 2016 Empire State Producers Expo, Syracuse, NY.

3.2.3. Provide disease alerts and forecasting tools

Quesada-Ocampo. Keep an eye out for gummy stem blight in watermelons. Extension Plant Pathology Portal. May 5, 2016. http://buff.ly/1TBhsUi

Smart has an active facebook and twitter accounts where information can be posted about this project. When sharing with growers about the project, Smart explains that there will be advances in breeding that will increase the chances that resistant varieties will become available. Growers enthusiastically support the project.

3.2.4 Provide diagnostic and disease management assistance.

Since the project started, Quesada has provided diagnostics and disease management recommendations for 9 cucumber, 11 watermelon, 2 melon, 4 squash, and 4 pumpkin samples submitted to the NCSU Plant Disease and Insect Clinic. Quesada has also been involved in providing disease management recommendations through oral presentations and generating disease management resources such as the North Carolina Agricultural and Chemicals Manual and the Southeastern US 2016 Vegetable Crop Handbook.

Production guides

In subsequent years, updates will be provided annually with respect to disease management of cucurbit crops in the Vegetable Crop Handbook Publication. The information in these

publications contains cultural management and cultivar disease management recommendations, along with registered chemical control options.

Southeastern Vegetable Extension Workers (Queseda, Schultheis). Kemble J., Lewis Ivey M., Jennings K. M., and Walgenbach J. F., Eds. (2016) Southeastern US 2016 Vegetable Crop Handbook.

Quesada-Ocampo L. M., Ed, (2016) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.

To date, there have been numerous disease management presentations provided at various Research and Commodity meetings at state, national and international levels. Some presentations have been variety related with disease considerations. Please see list of presentations (p. 43)

Hausbeck has been involved in:

Weekly conference calls, MSU Vegetable Working Team**:** These calls began Wednesday, 27 April and will continue through 31 August. Dr. Hausbeck communicates directly with the extension educators in Michigan via these calls.

Weekly conference calls, Cucurbit ipmPIPE**:** These calls will be initiated in May and will include plant pathologists from the eastern US (Hausbeck, Quesada, Smart).

New Michigan Website**:** to be available mid-May. This new website will be easy to navigate to find the items of interest. It will have a map of confirmed occurrences of cucurbit downy mildew by county, a list of spore counts/day for sites with a spore trap, how downy mildew spores are monitored in Michigan, how spore counts are done and how to interpret them, how to submit samples, and symptoms of downy mildew on various crops. Recommendations to limit downy mildew disease will be highlighted and updated as needed.

3.2.5. Field days and demonstration plots.

Quesada recruited a part-time graduate student (Nicholas Noel) that will focus on evaluating commercial watermelon varieties for anthracnose resistance and that will have yearly demonstrations plots for field days with growers and industry. Quesada and Noel will collaborate with Wehner, also on the watermelon team, who has a graduate student focusing on the genetic basis of disease resistance to anthracnose in watermelons. Quesada also supported demonstration plots to evaluate fungicides for disease control and combinations of tolerant varieties and fungicide applications.

McCreight has planted three field planted for evaluation in June and July, and will plant another field trial in mid-June. No field days have been schedule to date; however, there is opportunity to have one.

Schultheis is involved in several cucurbit variety trials which includes summer squash, butternut, watermelon, melon, parthenocarpic pickling cucumber and pumpkin. These trials will be open to the industry and extension agents to evaluate for yield, quality and potential diseases. These projects are highly interactive with multiple seed companies. An agent in-service training focusing on butternut squash and watermelon will be scheduled for August in Salisbury, NC. Cultural and disease management will be featured at this in service training.

Publications from demonstration plots

Adams M. L., Noel N. A., and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2015. PDMR 10: V084.

Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of powdery mildew of winter squash, Cleveland 2015. PDMR 10: V076.

Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Cleveland 2015. PDMR 10: V085.

Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Kinston 2015. PDMR 10: V086.

Lange, H.W., Smart, C.D. and Seaman, A.J. 2016. Evaluation of fungicides allowed for organic production on downy mildew of cucumber, 2015. Plant Disease Management Report. Vol. 10

Oral and Poster Presentations

Extension and Industry Venues

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 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.

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. and M. Colle. 2015. Development of genetic stocks for cucumber fruit resistance to *Phytophthora capsici*. Pickling Cucumber Commodity Meeting, Grand Rapids, Dec.

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. 2015. Update on resistance to *Phytophthora capsici* in cucumber. PPI Annual Meeting (October 30, 2015, Fort Worth, TX)

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.

Hausbeck, M. 2016. The downy mildew report. Syngenta Meeting, Lansing, MI, Feb. 120 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.

Krasnow, C., and Hausbeck, M. 2016. Progress in cucumber downy mildew control. Southwest Hort Days, Benton Harbor, MI, Feb. 35 attendees.

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

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

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

Kousik, C.S. 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
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
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. 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.
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.
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. Downy mildew and *Phytophthora* control in cucurbits. 2016. NC Watermelon Convention. Wrightsville Beach, NC, Mar.
Quesada-Ocampo L. M. Downy mildew updates for cucurbits. 2016. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, Jan.
Quesada-Ocampo L. M. Diagnostics and management of cucurbit downy mildew. 2015. Pickle Packers International Annual Meeting. Fort Worth, TX, Oct.
Schultheis, J.R. 2016. Grafted vs. nongrafted watermelon studies. NC Watermelon Convention. Wrightsville Beach, NC, Mar.
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.
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 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.
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. 2015. Disease problems common during the 2015 growing season. Twilight meeting, Eden Valley, NY.
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)

Webinars

Smart, C. 2016. Vegetable diseases. Mar. (1 hr)

Scientific Conferences and Universities

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

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. 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

Grumet, R. 2016. Introduction to CucCAP - developing genomic resources for the cucurbit community. Plant and Animal Genome Conference. San Diego, CA. January 10.

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. January 10.

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.

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.

Wintermantel, W.M. and J.D. McCreight. 2016. Epidemiology of Cucurbit yellow stunting disorder virus (CYSDV) and associated whitefly-transmitted viruses in the US Southwest and development of CYSDV resistant melon. 2nd International Whitefly Symposium, Mount Meru Hotel Arusha, Tanzania. Feb.