2024 FINAL REPORT
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Agricultural Research Fund 2020-2024 project:

Monitoring the Downy Mildew Pathogen for Resistance to Fungicides Using Field and Molecular Tools

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PROBLEM:

Advances have been made in the control of downy mildew (DM) on pickling cucumbers, caused by *Pseudoperonospora cubensis*, including newly developed molecular tools for early detection and annually updated fungicides recommendations based on pathogen resistance. Combining molecular diagnostics with spore traps can now distinguish cucumber DM from hop DM, which has prevented unnecessarily early sprays due to a cross-reaction with hop DM when monitoring airborne sporangia. Also, these molecular assays can distinguish between the two cucurbit DM clades, which provides information regarding which cucurbit crops are likely to become diseased.

While the specificity, reliability, and efficiency of spore trapping information available to the growers has improved dramatically, additional experimentation in commercial fields is still needed to establish spore count thresholds for each clade that would trigger a spray event. While markers for fungicide resistance have been developed for some fungicides, the assays have not yet been transferred to be used on a spore trap and new fungicide resistance markers have been reported that could be tested. New field sampling technologies, such as drones, have become available in recent years that may offer the opportunity to perform spore sampling in a different way. Experiments to monitor pathogen inoculum, host preference, and fungicide resistance in research and commercial fields could support a biosurveillance system for cucurbit DM that along with weather parameters could be adopted by growers to time sprays.

Our overall goal was to reduce the risk and cost that pickling cucumber growers face each year from a rapidly changing, aggressive, and fungicide-resistant DM pathogen.

OBJECTIVES:

1. Develop and implement state-of-the-art molecular tools to detect and characterize the pathogen.
2. Determine the spore thresholds that would trigger a spray event for cucumber DM.
3. Conduct fungicide trials in two geographical regions to develop effective recommendations for this rapidly changing, aggressive, and fungicide-resistant pathogen.

RESULTS:

1. Develop and implement state-of-the-art molecular tools to detect and characterize the pathogen.

NCSU

Host-adapted clade marker (crop risk). The previously identified candidate genomic marker c2555.3e7 of *P. cubensis* exhibited polymorphism by host of origin (Withers et al. 2016) and was used for the development of a diagnostic multiplex qPCR assay. Two host-adapted clades were described for *P. cubensis* by Wallace et al. (2020) and isolates representing each clade were identified for assay design and validation in our study (Figure 1). After confirming clade correspondence, the polymorphic region of c2555.3e7 was used to develop a multiplex qPCR assay to detect and quantify the specific clades of *P. cubensis*. Isolates SC1982 (clade 1) and MSU-1 (clade 2) were used as representative isolates for each clade.

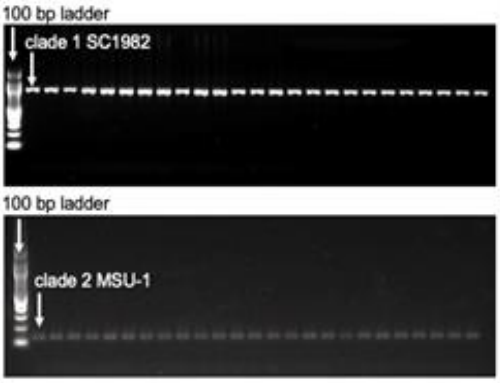

Figure 1: Gel images of c2555.3e7 PCR amplicons of clade 1 (829 bp-top) and clade 2 (128 bp - bottom) *Pseudoperonospora cubensis* sporangia DNA using primers ar1517 and sw553. Clade 1 and clade 2 isolates used in the gel are listed in Table 1 in order of appearance in the gel and noted with an asterisk (*).

Once the primers and probes for the clade-specific qPCR assay were designed, they were used to determine sensitivity and specificity when detecting sporangia in spore trap sample rods and *P. cubensis* DNA. The assay was tested extensively and found to be specific to *P. cubensis* with no cross-reaction to closely related species such as *Pseudoperonospora humuli* (hop downy mildew). As far as sensitivity, none of the qPCR assays were able to detect fewer than 10 sporangia and therefore, the detection threshold is considered to be the DNA concentration associated with $\geq$10 sporangia. This marker was finalized for use in a spore trap detection system.

Quinone outside inhibitor (QoI) fungicide resistance marker. Allele-specific qPCR primers, probes, and assays were developed for previously reported *P. cubensis* mutations (Ishii et al. 2001) in the cytochrome B (CytB) gene known to confer resistance to QoI fungicides. The marker was tested in over 200 *P. cubensis* isolates from diverse hosts to better understand the occurrence of QoI resistance mutations in pathogen populations. Results indicated that QoI resistance is widespread in *P. cubensis* regardless of clade or host. This means that fungicides such as Reason (FRAC 11, fenamidone) with a single FRAC 11 active ingredient, will likely be ineffective for management of cucurbit downy mildew. Similarly, fungicides with two active ingredients and one belonging to FRAC 11, such as Tanos (famoxadone, FRAC 11 and cymoxanil, FRAC 27), may have reduced efficacy due to QoI resistance assuming the second active ingredient has efficacy for *P. cubensis* management. We modified this assay to use in combination with spore traps as an in-season warning system.

Table 1. Occurrence of quinone outside inhibitor (QoI) genotypes expressed as percentages within *Pseudoperonospora cubensis* isolates infecting commercial and wild cucurbits in North Carolina separated by clade.

	Clade 1		Clade 2	
QoI Genotype	**No.**	**(%)**	**No.**	**(%)**
Sensitive - G143	17	14.4	4	3.7
Resistant - G143-143A, 143A	101	85.6	114	96.3
Total	118		108	

Carboxylic acid amide (CAA) fungicide resistance marker. Allele-specific qPCR primers, probes, and assays were developed for previously reported *P. cubensis* mutations (Blum et al. 2011) in the cellulose synthase 3 (CesA3) gene known to confer resistance to CAA fungicides. The marker was tested in over 200 *P. cubensis* isolates from diverse hosts to better understand the occurrence of CAA resistance mutations in pathogen populations. Results indicated that CAA resistance occurs at much higher frequency in clade 2 (~86% of isolates are resistant) than clade 1 (~54% of isolates are resistant). This means that fungicides such as Revus (FRAC 40, mandipropamid) with a single FRAC 40 active ingredient, will likely be ineffective for management of clade 2 isolates but may still be effective for clade 1 isolates. Similarly, fungicides with two active ingredients and one belonging to FRAC 40, such as Zampro (dimethomorph, FRAC 40 and ametoctradin, FRAC 45) or Orondis Ultra (mandipropamid, FRAC 40 and oxathiapiprolin, FRAC 49), may have reduced efficacy to clade 2 isolates due to CAA resistance assuming the second active ingredient has efficacy for *P. cubensis* management. In the case of Orondis Ultra, it is known that oxathiapiprolin has high efficacy for clade 2 and clade 1 isolates. However, this product should then be always treated as a single-active ingredient fungicide for clade 2 isolates since only FRAC 49 would be providing the efficacy and relying on the FRAC 40 premix partner for fungicide resistance management would be insufficient. We have modified this assay to use in combination with spore traps as an in-season warning system.

Table 2. Occurrence of carboxylic acid amide (CAA) genotypes expressed as percentages within *Pseudoperonospora cubensis* isolates infecting commercial and wild cucurbits in North Carolina separated by clade.

	Clade 1		Clade 2	
CAA Genotype	**No.**	**(%)**	**No.**	**(%)**
Sensitive - G1105	54	45.8	15	13.9
Resistant - 1105W, 1105V, G1105-1105V, G1105-1105W, 1105V-1105W, G1105-1105V-1105W	64	54.2	103	86.1
Total	118		108	

Oxathiapiprolin (OXTP) fungicide resistance marker. For this marker, there are no previously reported mutations conferring resistance to OXTP in *P. cubensis*. However, fungicide resistance mutations for OXTP have been reported in other oomycetes (Miao et al. 2016). Thus, our efforts focused on first transferring this marker to *P. cubensis* and then developing assays for detection. Using next generation sequencing data, we successfully reconstructed the oxysterol binding protein (OSBP) gene of *P. cubensis* and *P. humuli*, for comparison purposes and to assist in identifying relevant mutations as we have done in the past with other markers. OSPB is the presumed and reported target of OXTP in other oomycetes, however, the function of this gene in oomycetes is currently unknown. We designed primers for this gene and we screened 25 clade 1 and clade 2 *P. cubensis* isolates from our collection to locate fungicide resistance mutations we can target for assay development. We identified one region that causes a significant change in clade 2 isolates (cucumber) but not in clade 1. We expanded the screening to other 144 isolates and found that the OXTP resistance mutation was not found in isolates from plants

not treated with fungicides prior to 2016. However, in isolates from samples treated with Orondis in 2018 and forward, we found that 82% of clade 2 isolates contained the OXTP mutation. In more recent samples (2021) regardless of isolates coming from Orondis-treated or untreated plants, we found that 100% of clade 2 isolates had the OXTP resistance mutation. We did not find any clade 1 isolates with the OXTP resistant mutation, likely because they do not survive the Orondis treatment. While finding the OXTP resistance mutation as more widespread in clade 2 isolate populations is concerning, field fungicide efficacy data still supports Orondis as an effective partner in a rotational spray program. However, the presence of the OXTP resistance mutation makes it imperative that growers continue to alternate modes of action in a program, do not heavily rely on Orondis for downy mildew control in cucumber, and tank mix Orondis Opti treatments with another protectant, even though Orondis Opti comes pre-mixed with chlorothalonil.

Table 3. Detection of *P. cubensis* clade 2 isolates with oxathiapiprolin (OXTP) fungicide resistance mutation.

Sample origin	No. of isolates with amplification/total isolates tested	Clade	Isolates with mutations found
Quesada Lab CDM Collection (untreated)			
2015-2016	4/12	Clade 1	0
	8/12	Clade 2	0
Fungicide Trials 2018			
Cucumber + Orondis	11/20	Clade 2	9/11 (82%)
Squash + Orondis	0/20	N/A	N/A
Fungicide Trials 2021			
Cucumber + Orondis	25/46	Clade 2	25/25 (100%)
Cucumber non-treated	21/34	Clade 2	21/21 (100%)
Spore trapping plots 2023			
Cucumber non-treated	81/81	Clade 2	76/81 (93%)
Squash non-treated	31/31	Clade 1	0/31 (0%)

We have developed a qPCR assay that can detect the OXTP mutation with higher speed for in-season screening of *P. cubensis* isolates. We tested our assay with samples from fungicide trials in 2018, 2021, and 2023 and found that most clade 2 (cucumber) samples tested had the OXTP resistance mutation. The qPCR assay still has some challenges with reliable amplification so we will continue to improve it for future use in our biosurveillance system.

MSU

Comparing spore traps to increase efficiency and reduce turnaround time and costs. Cucurbit DM relies on fungicide applications which must be initiated when there is an influx of airborne sporangia. In Michigan, timely alerts have assisted growers as to when they should begin crop protection with fungicides. Through this project we adapted quantitative PCR (qPCR)-based assays to distinguish among *P. cubensis* clades I and II and *P. humuli* in spore trap samples from commercial production sites in Michigan and research plots. A multiplex qPCR assay improved the specificity of *P. cubensis* clade II detection accelerating the assessment of field spore trap samples. Each year, monitoring, *P. cubensis* clade II DNA was detected in spore trap samples before CDM symptoms were first observed in cucumber fields (July and August), while *P. cubensis* clade I DNA was not detected in air samples during the growing season.

After evaluating impaction spore traps alongside Burkard traps for detection of airborne sporangia, the Burkard spore trap appeared to be better for recovering sporangia at low concentrations. Detecting low levels of the first sporangia during Michigan's growing season is especially important. This is

summarized in the paper by the MSU graduate student Julian Bello et al. (2022) where a logistic regression was used to model the relationship between the concentration of sporangia (estimated by the Burkard traps) and the probability of detection using qPCR with impaction and Burkard traps. A higher probability of *Pseudoperonospora* spp. detection was predicted for Burkard trap samples with less than 300 sporangia compared to impaction traps. Furthermore, Bello et al. (2022) found that a probability of detection above 90% was predicted for Burkard trap samples with above 50 sporangia, whereas for impaction traps to have a probability of detection above 90%, 300 sporangia or more were needed.

We also tested a new multi-vial cyclone sampler (Figure. 2) at two field sites during 2024. These samplers are of interest as they could provide spore detection results to growers with a reduced turnaround time. The cyclone sampler, collects air samples direct into 1.5 ml Eppendorf vials, allowing sample analysis by qPCR. The multi-vial sampler contains eight Eppendorf, vials mounted on a carousel, allowing up to eight sequential samples to be taken over the course of a week. We tested a free-standing model with a controller and multifunction timer, which collected particles with a sample rate of 16.5 liters per minute. In 2024, the cyclone trap at the Ingham Co. research site was placed in the downy mildew plot and compared to the standard volumetric trap. While the cyclone trap did detect airborne downy mildew sporangia, the frequency of detection was less than that for the volumetric spore that was placed next to it. The second trial located in Berrien Co. compared the cyclone spore trap to the volumetric spore trap with results like those observed in Ingham Co.

Figure 2. Cyclone spore traps tested in Michigan have rotating vials instead of a sticky tape that could lead to quicker processing of air samples to return results to growers quickly.

2. Determine the spore thresholds that would trigger a spray event for cucumber DM.

NCSU

Testing the host-adapted clade marker (crop risk) with spore traps in research stations. Battery-powered impaction-type roto-rod spore samplers (Model 82; Sampler Technologies, Inc.) were placed in

cucurbit sentinel plots for trapping *P. cubensis* aerial sporangia on two sampler rods. The sentinel plots were planted for two years twice per year at the Central Crops Research Station (CCRS; located in Clayton, NC) and the Cunningham Research Station (CURS; located in Kinston, NC), in late April/early May and again in early August of both years. One spore trap was placed adjacent to the cucumber plot and another one adjacent to the butternut squash plot. The traps were operated from May to September each year, and sampler rods from each spore trap at the CCRS were collected 4 days per week (Tuesday – Friday) and 2 days per week at the CURS (Tuesday and Friday). The collected sampler rods were analyzed using the clade-specific multiplex qPCR assay to detect the presence of airborne sporangia.

The clade-specific multiplex qPCR assay detected the presence of airborne *P. cubensis* sporangia from these samples (Figure 3) and the resulting Cq values were used to estimate sporangia counts. In year 1, clade 2 sporangia were first observed on 7 Jul and 5 Jul in spore samples collected from the early-season sentinel plots located at the CCRS and CURS, and the resulting mean Cq values were equal to approximately 67 and 50 sporangia per sample at CCRS and CURS, respectively. Likewise, in the late-season sentinel plots, clade 2 sporangia were first observed in spore samples collected on 7 Sep at the CCRS and on 23 Aug at the CURS, with mean Cq values equivalent to approximately 43 and 50 sporangia per sample in both respective locations. Clade 1 sporangia were not observed in the early-season sentinel plots but were detected in the late-season planting, on 9 Sep at the CCRS and on 26 Aug at the CURS, with mean Cq values equivalent to approximately 51 and 54 sporangia per sample, respectively. In year 2, the early-season sentinel plots saw clade 2 sporangia arrive at the CCRS on 20 Jun and at the CURS on 6 Jun, with mean Cq values equivalent to approximately 31 and 23 sporangia per sample, respectively. Clade 2 sporangia were first detected in the late-season sentinel plots on 1 Sep at the CCRS and on 5 Sep at the CURS, again, with mean Cq values equivalent to 30 and 17 sporangia per sample, respectively. Similar to year 1, clade 1 sporangia were not detected in the early-season sentinel plots at either location but were observed in the late-season sentinel plots at the CCRS on 1 Sep and at the CURS on 5 Sep, with mean Cq values equivalent to 23 and 26 sporangia per sample, respectively.

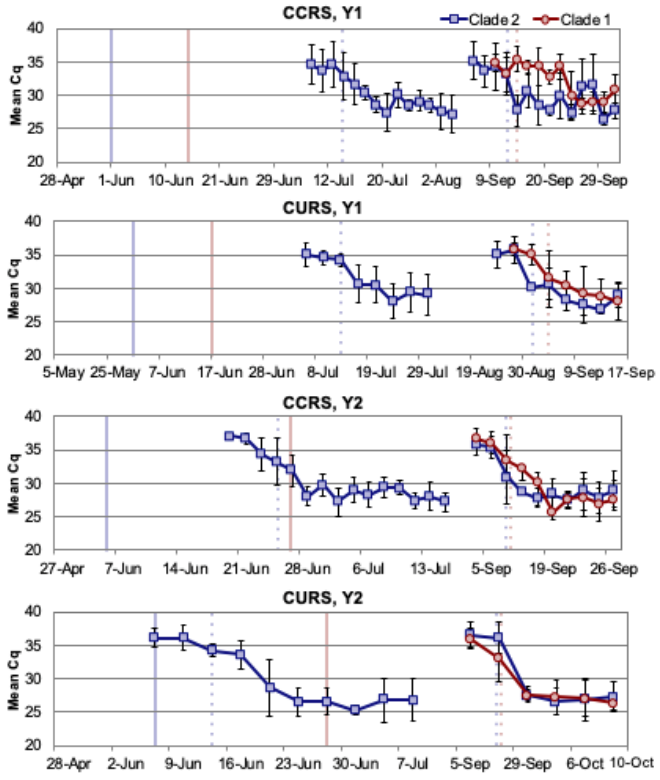

Figure 3: *Pseudoperonospora cubensis* clade 1 and clade 2 sporangia detected with qPCR in spore trap samples collected at the Central Crops Research Station (CCRS) and the Cunningham Research Station (CURS) in year 1 and year 2. The gap between plots represents two plantings of the sentinel plots. Solid vertical lines indicate the first report to the Cucurbit downy mildew IPM pipe of a host susceptible to a clade in North Carolina. Dotted vertical lines indicate visual confirmation (<5% disease) of infection of a host susceptible to a clade in the sentinel plots.

When comparing detection of clade 1 or clade 2 sporangia using the spore samplers and the clade-specific assay with visual confirmation of downy mildew in the sentinel plots and outbreak reports made to the CDM ipmPIPE, we found that disease or sporangia were not detected in the sentinel plots in spite

Figure 4: Spore-trapping drone to sample large-acreage fields for *Pseudoperonospora cubensis* sporangia.

of CDM being reported in the state. Based on the public records of the CDM ipmPIPE, the first detection of disease in North Carolina was on 1 June of year 1 and 5 June of year 2 in cucumber in Duplin County, with cucumber being a clade 2 host. The first detection of CDM on a clade 1 host (watermelon for both reports) was on 17 June of year 1 in Nash County and 27 June of year 2 in Carteret County. In contrast, disease was first confirmed in the cucumber (clade 2) sentinel plot on 13 July and 13 September of year 1 for the first planting and in 23 June and 6 September of year 2 for the second planting at the CCRS, and in 12 July and 30 August of year 1 for the first planting and in 13 June and 8 September of year 2 for the second planting at the CURS. CDM was confirmed in the squash (clade 1) sentinel plot in 14 September of year 1 and 6 September of year 2 for the second planting at the CCRS, and on 2 September of year 1 and 8 September of year 2 for the second planting at the CURS. This means that while the CDM ipmPIPE is a great alert system for large-scale warnings (state level), it is less precise in determining disease onset at the field level. Improving in-field warning systems for CDM could significantly reduce fungicide inputs. In the example field experiment shown here, growers of clade 2 hosts would have saved 6 weeks of sprays (weeks between CDM ipmPIPE report vs. time of spore/symptom detection in-field), while growers of clade 1 hosts would have saved 8 weeks of sprays. Certainly, commercial growers have less spray events than portrayed in this experiment but it does illustrate how in-field monitoring could reduce sprays.

Symptom development (as disease severity <5%) in the sentinel plots was observed between 2 and 4 days after sporangia for the respective clades were detected using the spore samplers and multiplex qPCR. Based on the spore sampler detection trends, CDM symptoms were typically observed within 1 to 2 days after Cq values became lower than approximately 36.7 for both clades and locations, which corresponds to approximately 10 to 20 sporangia. For both clades, the number of detected sporangia increased as CDM progressed throughout the sentinel plots, as indicated by the gradual decrease of Cq values in the assay.

We have tested our biosurveillance package (clade host preference, CAA and QoI sensitivity), into a drone sampling system for sporangia (Figure 4) to facilitate weekly air samplings in a large-scale acreage situation. We performed weekly drone flights in 2021, however, the UAV-trap system was not as effective as the original ground traps used for assay testing. We suspect that it is due to wind currents in combination with trap design, thus, in 2022 we expanded our trap testing to include: a hanging UAV trap, a trap mounted in a robotic rover, and a ground trap to be used as a control since we know we can detect spore with that system. We also added a wind sensor to understand how we need to modify the trap design to make the trap robust to changes in the wind (Figure 5). In those tests we were able to detect the pathogen prior to disease but we did not consistently detect it in samplings later in the season, indicating that perhaps the ground traps mounted on the drone or rover were not sampling as efficiently as those on the ground. Thus, we re-designed traps that are capable of vacuuming and mounted those in the drone and rover for 2023 tests. We saw similar findings than in 2022 but noticed that if we increased the DNA used

Figure 5. Spore trap in rover, hanging from UAV, with a wind sensor.

for the assay, we could increase our detection comparable to the ground traps we initially tested. We are processed all the 2023 samples after these modifications and found that the vacuum and UAV combination had the earliest and most consistent detection of CDM out of all the trap-vehicle combinations tested. We repeated those tests in 2024, both in the research plot and in a grower farm and are currently processing those samples.

MSU

Using environmental conditions to determine fungicide application intervals once airborne *P. cubensis* sporangia have been detected is preferable to continual monitoring of aerial spore loads due to control and cost. Data from weather monitoring systems may be accessed remotely and some include certain disease forecasting programs that could enable crop consultants and other field personnel to guide cucumber growers' management decisions without a significant financial investment or access to a laboratory. We evaluated the disease forecasting models, TOM-CAST, BLITE-CAST, and DM-CAST, to schedule fungicide applications for CDM control. Field trials were conducted from 2021-2024 at the MSU Plant Pathology Research Farm. A WatchDog® Wireless Weather Station and a leaf wetness sensor (Spectrum Technologies Inc.) were mounted on a steel pole and placed in the corner of the research plots each year to collect temperature, relative humidity, rainfall, and leaf wetness data. Weather data were automatically collected and sent to a corresponding SpecConnect® account (Spectrum Technologies Inc.) at 15-minute intervals.

In our 2021 and 2022 studies, the BLITE-CAST 15DSV treatment was like the 7-day treatment according to rAUDPC data in both years with fewer (2021) or the same number of applications (2022). The rAUDPC data indicated that the BLITE-CAST 18DSV treatment was similar to the 7-day treatment in 2021 and required two fewer applications. The 2022 rAUDPC data indicated that BLITE-CAST 18DSV had significantly more CDM disease than the 7-day treatment but required one less application. The amount of disease observed in 2022 for the BLITE-CAST 18DSV treatment was commercially acceptable with less than 20% CDM at the last assessment. The disease pressure in the 2022 trial was high with >75% disease severity in the control at the final assessment which would likely translate into crop failure. In 2021, the DM-CAST (3/5 day) model required one more application but controlled disease better than the 7-day treatment based on the rAUDPC data whereas in 2022 it had the same number of applications and significantly more disease. In 2022, two treatments were added to expand upon the DM-CAST model in an effort to reduce the number of fungicide applications required by the program, The DM-CAST 7/10 day treatment reduced the number of applications compared to the 7-day treatment (4 to 6) but had significantly more disease. The DM-CAST 5/7-day treatment had one less spray than the 7-day treatment but also resulted in significantly more disease than the 7-day treatment according to the rAUDPC data. However, this treatment had less disease than the DM-CAST 7/10 day treatment and a similar level of disease to the DM-CAST 3/5 day treatment. Similar to the BLITE-CAST 18DSV treatment, the amount of disease at the last assessment (<10%) was commercially acceptable. The TOM-CAST model, developed for use on fungal pathogens, was not as effective in limiting CDM as the other two models included in our study.

In our 2023 and 2024 studies, BLITE-CAST 18DSV, BLITE-CAST 15DSV, and the 7-day calendar programs were tested. The fungicides, Renaz SC + Bravo Weather Stik SC, alternated with Orondis Opti SC, alternated with Zampro SC + Bravo Weather Stik SC, were applied to 'Peacemaker' and 'Vlaspik'. The 7-day program received 7 applications. BLITE-CAST 15DSV had 7 applications in 2023 and 6 in 2024; BLITE-CAST 18DSV had 6 applications in 2023 and 5 in 2024. Across all programs, 'Vlaspik' had higher disease than 'Peacemaker' (Table 4). In 2023, the BLITE-CAST 15 DSV and the 7-day program were not significantly different from each other nor were the BLITE-CAST 18DSV and BLITE-CAST 15DSV programs. All programs effectively limited disease for both cultivars. The 7-day calendar program was significantly better than the BLITE-CAST 18DSV program in limiting CDM. In 2024, the 7-day and BLITE-CAST

programs had a similar level of CDM and were effective. Across all programs, 'Vlaspik' had a higher CDM compared to 'Peacemaker'. CDM was controlled for both cultivars using a 7-day or BLITECAST 15DSV program. BLITE-CAST 15 DSV could provide effective control and reduce spray applications.

Table 4. Downy mildew disease severity when fungicides were applied according to various programs based on weather conditions or applied every 7 days.

		Disease severity (%)			
Cultivar	Program	9 Sep	16 Sep	24 Sep	27 Sep
Peacemaker	Untreated	3.0	10.8	23.8	12.0
	7-day calendar	0.0	0.0	0.3	0.5
	15DSV BLITE-CAST	0.0	0.3	1.8	1.3
	18SDV BLITE-CAST	0.3	1.3	8.8	6.0
Vlaspik	Untreated	4.0	33.8	56.3	55.0
	7-day calendar	0.0	0.0	2.0	4.5
	15DSV BLITE-CAST	0.3	1.8	8.8	7.3
	18SDV BLITE-CAST	1.3	13.8	28.0	18.0

Using spore trapping to identify an influx of sporangia indicating that a spray program should begin. Spore traps were placed in Michigan counties at the beginning of the cucumber growing season and were used to detect sporangia of CDM. Burkhard volumetric spore traps, were maintained in seven Michigan counties. Molecular diagnostics using qPCR was used to identify the presence of *P. cubensis* sporangia collected in the spore trap.

Spore traps in Ingham and Berrien counties were located at research plots. Spore traps in Bay, Saginaw, Muskegon, and Allegan counties were located adjacent to commercial cucumber fields and the spore trap in Monroe County was placed next to a commercial squash field. Spore traps were placed at their locations once cucumbers (or squash) had been planted and the seedlings had emerged.

Air is pulled into the spore trap and any airborne sporangia and other particles (dirt, pollen, dust) are impacted onto a reel coated in sticky tape inside the spore trap (Figure 6). The tape slowly rotates throughout the week so that the day/time that the sporangia are impacted onto the tape can be recorded. The reel was collected weekly. A portion of the tape on the reel was mounted on a

Figure 6. Burkard spore trap deployment at a commercial cucumber field in Saginaw County, MI (**A**). Air flow is measured using a meter (**B**) before and after a reel with sticky tape (**C**) is mounted inside the drum of the trap.

microscope slide where sporangia are visible using a light microscope. The remaining tape was processed by DNA extraction and qPCR reaction that detects the presence of *P. cubensis* clade 1, *P. cubensis* clade 2 and *P. humuli* (pathogen that causes hop downy mildew). Sporangia of *P. cubensis* and *P. humuli* cannot be distinguished by their morphology on a microscope slide but can be distinguished in the qPCR reaction. Weather data was also collected throughout the season using a weather station at each location.

Sporangia of *P. cubensis* clade 2 were first detected on 5 June in Bay County (Table 5). *P. cubensis* DNA was next detected in Allegan County on 6 June. Occasional detection of both *P. cubensis* and *P. humuli* was observed between 21 July and 14 August after the first symptoms of cucurbit downy mildew were observed in Saginaw County on cucumber. Increased detection of *P. cubensis* was observed as disease symptoms were found across the state; field symptoms were first observed on the west side of the state. ***See Appendix 1 for spore trapping results.***

Spore traps collected increasing amounts of *P. cubensis* clade 2 sporangia as pathogen pressure increased throughout the state. Spore traps were used to monitor for *P. cubensis* clade 1, which infects acorn squash, butternut squash, pumpkin, and watermelon, throughout June and through September. *P. cubensis* clade 1 was not detected in the 2023 season.

Table 5. Statewide spore trapping in Michigan provided an early warning system for the development of cucumber downy mildew.

County:	Allegan	Berrien	Muskegon	Bay	Monroe	Saginaw	Ingham
First Confirmed Positive (qPCR):	July 9th	July 23rd	June 16th	June 20th	June 14th	June 6th	July 8th
First Confirmed Positive (Scouting):	July 30th	August 7th	July 18th	July 15th	N/A*	July 12th	July 15th

3. Conduct fungicide trials in two geographical regions to develop effective recommendations for this rapidly changing, aggressive, and fungicide-resistant pathogen.

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The experiment was performed at the Horticultural Crops Research Station in Clinton, NC in 2024. Experimental plots were single raised beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. Cucumber (Lizst) was directly seeded on 1 Aug (2-ft in-row spacing, 2 seed/hill) and thinned to one plant per hill after emergence (7 plants/plot). Regular cultural practices like irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Seven treatments and the control were tested in a randomized complete block design with four repetitions. Fungicide treatments were applied using a CO2-pressurized backpack sprayer equipped with a single-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 35 psi. Disease severity per plot was assessed every week and it is still ongoing. Preliminary data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's protected least significant differences (LSD) test to separate means.

Downy mildew was first detected on 29 Aug at approximately 2% disease severity in the field. Disease pressure was lower than usual for this time of the year in North Carolina due to hot and dry conditions during the field season. The disease severity data obtained on 13 Sept showed that all treatments were statistically better than the non-treated control. The disease summary using Area Under the Disease Progress Curve values (AUDPC) showed no statistical differences.

Table 6. NCSU 2024 fungicide trial.

Treatments	Rate (fl oz /acre)	Disease Severity[z] (%) 13 Sept. – Week 3	AUDPC[y]
Non-treated control	–	12.0 a[w]	236.63 a
Ranman	2.75	3.8 cd	70.13 a
Ordonis Opti	32.0	4.8 cd	478.38 a
Previcur Flex	19.2	5.3 bcd	84.38 a
Omega 500	24.0	6.3 bc	169.00 a
Zampro	14.0	6.5 bc	203.25 a
Presidio	4.0	2.8 d	102.38 a
Revus	8.0	8.3 b	184.63 a

[z]Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.
[y]Area under disease progress curve for total of all the foliar diseases present.
[w]Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Fisher's Protected LSD).

MSU

Fungicides are currently the primary way to control CDM and yearly efficacy is likely influenced by the sensitivity of the pathogen population(s) that enter Michigan as *P. cubensis* does not overwinter in the state and must be reintroduced each growing season. The fungicide sensitivity of *P. cubensis* populations causing disease in Michigan later in the season is influenced initially by the fungicides used in the southeastern U.S. or in northern production greenhouses.

Field trials were conducted at the MSU's Plant Pathology Research Farm each year of this project. Fungicides were applied weekly, and disease severity was evaluated by visually assessing the percentage of foliar area with symptoms. All fungicides tested limited CDM compared to the control but were not as effective as Orondis Opti. Ranman (Renaz), Zampro, Previcur Flex, and Elumin effectively controlled CDM. In 2021, Elumin had significantly less disease than zoxamide premixed with Bravo WeatherStik and a similar level of disease to zoxamide premixed with mancozeb. In 2022, Elumin had significantly less disease than the two premixes including zoxamide. This is the first report of Elumin showing improved efficacy compared to zoxamide under field conditions. Orondis Opti was the most effective fungicide in our trial. Overall, the two multisite fungicides (Bravo WeatherStik and mancozeb) effectively controlled CDM but were not as effective under high pathogen pressure compared to Orondis Opti, Previcur Flex, Ranman, Omega, Zampro, and Elumin.

The 2024 studies were conducted at MSU's Southwest Research and Extension Center near Benton Harbor, MI, using 'Vlaspik' and were comprised of five fungicide plots. While data collection and analysis is ongoing at the time of this report, it is clear our recommended fungicides have remained effective.

Tables 7-11. Fungicide efficacy studies were conducted across several field trials to test the ability of various products to limit cucurbit downy mildew on 'Vlaspik' cucumber.

Table 7. Trial 1

Treatment and rate/A, applied at 7-day intervals	Foliar Disease (%)[z]						
	23 Aug	29 Aug	6 Sep	12 Sep	19 Sep	26 Sep	AUDPC[y]
Untreated	3.3	7.0	18.8	23.8	26.3	38.8	663.8
Orondis Opti 2.5 pt	0.0	0.0	0.0	0.0	0.8	0.8	7.9
Omega 24.0 fl oz	0.5	0.3	0.3	1.8	0.5	1.0	23.4

Previcur Flex 1.2 pt	0.3	0.5	1.0	1.0	1.5	1.0	31.8
Zampro 14.0 fl oz	0.8	0.8	1.3	0.3	1.0	2.8	34.5
RenaZ 2.75 fl oz	0.3	0.5	1.8	2.8	0.5	2.0	44.9
Orbus 4F 24.0 fl oz	0.3	0.3	2.5	2.8	1.5	0.3	49.3
Elumin 8.0 fl oz	0.3	1.8	4.0	4.0	1.5	3.0	88.0

[z]Based on visual assessment foliage diseased.
[y]Area Under the Disease Progress Curve.

Table 8. Trial 2.

Treatment[z] and rate/A, applied at 7-14 day intervals	Foliar Disease (%)[z]						AUDPC[y]
	23 Aug	29 Aug	6 Sep	12 Sep	19 Sep	26 Sep	
Untreated	1.0	6.3	16.3	18.8	31.5	38.8	638.5
Orondis Opti 40.0 fl oz *-alt-* Ranman 2.75 fl oz + BWS	0.0	0.0	0.0	2.0	0.3	0.3	15.6
Zampro 14.0 fl oz + Cevya 5.0 fl oz	0.0	0.0	0.5	2.0	0.0	1.0	20.0
Zampro 14.0 fl oz + Activator 90	0.5	0.0	0.0	0.5	2.0	0.8	21.4
Cevya 5.0 fl oz + Activator 90	0.5	1.3	6.0	6.5	10.3	19.5	234.5

[z]Based on visual assessment foliage diseased.
[y]Area Under the Disease Progress Curve.
[x]BWS = Bravo WeatherStik SC32 fl oz.

Table 9. Trial 3.

Treatment and rate/A, applied at 7-day intervals	Foliar Disease (%)[z]						AUDPC[y]
	23 Aug	29 Aug	6 Sep	12 Sep	19 Sep	26 Sep	
Untreated	2.8	9.8	18.5	25.0	41.3	46.3	819.1
Orondis Opti 2.5 pt *-alt-* Ranman 2.75 fl oz + BWS[x] 2.0 pt *-alt-* Previcur Flex 1.2 pt + BWS 2.0 pt *-alt-* Zampro 14.0 fl oz + BWS 2.0 pt	0.0	0.5	1.5	5.5	1.0	1.5	62.0
Orondis Opti 2.5 pt + Curezin XT 6.0 pt *-alt-* Ranman 2.75 fl oz + Curezin XT 6.0 pt + Dyne-Amic 0.375% V/V *-alt-* Previcur Flex 1.2 pt + Curezin XT 6.0 pt + Dyne-Amic 0.375% V/V *-alt-* Zampro 14.0 fl oz + Curezin XT 6.0 pt + Dyne-Amic 0.375% V/V	0.0	1.3	2.8	4.5	3.8	2.5	92.3
Curezin XT 6.0 pt + Salia 12.0 fl oz	0.3	6.0	5.5	18.3	10.5	13.5	320.6
Howler EVO 2.5 lb + Dyne-Amic 0.375% V/V *-alt-* Curezin XT 6.0 pt + Dyne-Amic 0.375% V/V	0.8	10.3	10.3	25.0	11.3	14.3	436.9

[z]Based on visual assessment foliage diseased.
[y]Area Under the Disease Progress Curve.
[x]BWS = Bravo Weatherstik SC.

Table 10. Trial 4.

Treatment and rate/A, applied at 7-day intervals	Foliar Disease (%)[z]						AUDPC[y]
	23 Aug	29 Aug	6 Sep	12 Sep	19 Sep	26 Sep	
Untreated	3.0	13.8	18.0	31.3	47.5	56.3	963.8
Orondis Opti 40.0 fl oz	0.0	0.3	0.8	2.8	15.3	3.0	142.1
Ranman 2.75 fl oz + BWS[x] 32.0 fl oz + Reason 500 5.5 fl oz + Orondis Opti 40.0 fl oz + Zing 36.0 fl oz + Zampro 14.0 fl oz + Gavel 908.0 g	0.3	0.0	0.3	5.5	12.5	6.0	146.8
Orondis Opti 40.0 fl oz + Gavel 908.0 g + Zampro 14.0 fl oz + Reason 500 5.5 fl oz + Bagde 32.0 fl oz + Ranman 2.75 fl oz + BWS 32.0 fl oz + Zing 36.0 fl oz	0.0	1.8	1.3	4.8	14.3	11.0	190.1
Orondis Opti 40.0 fl oz *-alt-* Ranman 2.75 fl oz + BWS 32.0 fl oz	1.3	3.8	0.0	3.0	22.8	12.3	251.6
Latitude 29.0 fl oz	0.0	0.8	0.3	8.5	20.0	18.8	267.9

[z]Based on visual assessment foliage diseased.
[y]Area Under the Disease Progress Curve.
[x]BWS = Bravo WeatherStik SC

Table 11. Trial 5.

Treatment and rate/A, applied at 7–14-day intervals	Foliar Disease (%)[z]						AUDPC[y]
	23 Aug	29 Aug	6 Sep	12 Sep	19 Sep	26 Sep	
Untreated	3.0	16.0	21.8	13.5	43.8	51.3	846.6
Orondis Opti 40.0 fl oz -alt- Ranman 2.75 fl oz + BWS[x] 32.0 fl oz	0.0	0.0	0.0	11.0	0.3	0.0	73.3
SA-0650120 55.0 fl oz	2.3	11.3	21.5	2.5	33.8	46.3	650.4
SA-0650120 28.0 fl oz	4.8	13.0	19.3	9.5	32.5	41.3	673.6
SA-0650120 28.0 fl oz + Arius 250 12.0 fl oz	3.0	14.8	20.8	8.3	31.3	48.8	700.5
SA-0650120 41.0 fl oz	4.3	12.0	19.8	11.0	35.8	52.5	740.5

[z]Based on visual assessment foliage diseased.
[y]Area Under the Disease Progress Curve.
[x]BWS = Bravo Weatherstik SC

TIMELINE:

Activity	Y1	Y2	Y3	Y4	Y5
Developing and implementing state-of-the-art molecular tools to detect and characterize the pathogen	MI,NC	MI,NC			
Determine the spore thresholds that would trigger a spray event for cucumber DM	MI	MI,NC	MI,NC	MI,NC	MI,NC
Conduct fungicide trials in two geographical regions to develop effective recommendations for this rapidly-changing, aggressive, and fungicide-resistant pathogen.	MI,NC	MI,NC	MI,NC	MI,NC	MI,NC

CUMMULATIVE OUTPUTS OVER THE FULL GRANT PERIOD:

Publications:

1. Bello Rodriguez, J.C. 2020. Genetic monitoring of cucurbit downy mildew in Michigan. Doctoral dissertation, Michigan State University, East Lansing, MI.
2. Salcedo, A., Hausbeck, M., Pigg, S., and Quesada-Ocampo, L.M. 2020. Diagnostic guide for cucurbit downy mildew. Plant Health Progress 21:166-172. *Editor's pick
3. Wallace E., D'Arcangelo K. N., and Quesada-Ocampo L. M. 2020. Population analyses reveal two host-adapted clades of *Pseudoperonospora cubensis,* the causal agent of cucurbit downy mildew, on commercial and wild cucurbits. Phytopathology 110: 15-78-1587. *Editor's pick
4. Bello Rodriguez, J.C., Hausbeck, M., and Sakalidis, M.L. 2021. Application of target enrichment sequencing for population genetic analyses of the obligate plant pathogens *Pseudoperonospora cubensis* and *P. humuli* in Michigan. Molecular Plant-Microbiome Interactions 34(10): 1103-1118.
5. Bello Rodriguez, J.C., Sakalidis, M.L., Perla, D., and Hausbeck, M.K. 2021. Detection of airborne sporangia of *Pseudoperonospora cubensis* and *P. humuli* in Michigan using Burkard spore traps couple to qPCR. Plant Disease 105(5):1373-1381.
6. Crandall S. G., Ramon M. L., Burkhardt A. K., Bello-Rodriguez J. C., Adair N., Gent D. H., Hausbeck M. K., Quesada-Ocampo L. M., and Martin F. N. 2021. A multiplex TaqMan qPCR assay for detection and quantification of clade 1 and clade 2 isolates of *Pseudoperonospora cubensis* and *P. humuli*. Plant Disease 105: 3154-3161.
7. D'Arcangelo K. N., Adams M. L., Kerns J. P., and Quesada-Ocampo L. M. 2021. Assessment of fungicide product applications and program approaches for control of downy mildew on pickling cucumber in North Carolina. Crop Protection 140: 105412.
8. Govindasamy, R., Hausbeck, M.K., Simon, J., and Wyenandt, A. 2021. Downy Mildew Impacts and Control Measure on Cucurbits in the United States. Journal of the American Society of Farm Managers and Rural Appraisers 2021:78-88.
9. Kousik C., Quesada-Ocampo L. M., Keinath A., Hausbeck M., Granke L., Naegele R., and Ji P. 2021. Managing stubborn oomycete plant pathogens. Plant Health Progress 22: 215-218.
10. Rahman A., Standish J.R., D'Arcangelo K. N., and Quesada-Ocampo L. M. 2021. Clade-specific biosurveillance of *Pseudoperonospora cubensis* using spore traps for precision disease management of cucurbit downy mildew. Phytopathology 111: 312-320.

11. Salcedo A., Purayannur S., Standish J. R., Miles T., Thiessen L., and Quesada-Ocampo L. M. 2021. Fantastic downy mildew pathogens and how to find them: Advances in detection and diagnostics. Plants 10: 435. *Invited paper for *Detection and Diagnostics of Fungal and Oomycete Plant Pathogens* Special Issue
12. Kenny, G. 2021. Controlling downy mildew incited by *Pseudoperonospora* cubensis on pickling cucumbers with fungicides. Master's thesis, Michigan State University, East Lansing, MI.
13. Bello-Rodriguez J. C., Higgins D. S., Sakalidis M., Quesada-Ocampo L. M., Martin F. M., and Hausbeck M. K. 2022. Clade-Specific Monitoring of Airborne *Pseudoperonospora* spp. Sporangia Using Mitochondrial DNA Markers for Disease Management of Cucurbit Downy Mildew. Phytopathology 112(10):2110-2125. DOI: 10.1094/PHYTO-12-21-0500-R
14. D'Arcangelo K.N., Wallace E.C., Miles T.D., and Quesada-Ocampo L. M. 2022. Carboxylic acid amides but not Quinone outside Inhibitor fungicide resistance mutations show clade-specific occurrence in *Pseudoperonospora cubensis* causing downy mildew in commercial and wild cucurbits. Phytopathology 113: 80-89..
15. Egel S. E., Adkins S. T., Wintermantel W. M., Keinath A. P., D'Arcangelo K. N., Parada-Rojas C. H., Rennberger, G., Toporek S. M., Hausbeck M. K., and Quesada-Ocampo L. M. 2022. Diseases of Cucumbers, Melons, Pumpkins, Squash, and Watermelons. Handbook of Vegetable and Herb Diseases: 1-105.
16. Govindasamy R., Arumugam S., Hausbeck M., Wyenandt A., and Simon J. E. 2022. The impact of downy mildew on high-value cucurbit crops in the US: an econometric analysis. Agricultural Economics Research Review 35: 37-44. DOI: 10.5958/0974-0279.2022.00003.9
17. Shirley A. M., Vallad G. E., Dufault N., Raid R., and Quesada-Ocampo L. M. 2022. Duration of disease control for fungicides against cucurbit downy mildew under Florida field conditions. Plant Disease 106: 1167-1174.
18. Shirley A. M., Vallad G. E., Quesada-Ocampo L. M., Dufault N., and Raid R. 2023. Effect of cucurbit host, production region, and season on the population structure of *Pseudoperonospora cubensis* in Florida. Plant Disease 108: 442-450.
19. Uebbing, M.R. 2023. Managing cucurbit downy mildew on cucumber using disease forecasters and fungicides. Master's thesis, Michigan State University, East Lansing, MI.
20. Uebbing, M.R., Hayden, Z.D., and Hausbeck, M.K. 2023. Conventional and Biopesticide Fungicides for Cucurbit Downy Mildew Control on Cucumber in Michigan. Plant Health Progress (First Look). DOI: 10.1094/PHP-03-23-0024-RS.
21. Higgins, D.S., Goldenhar, K.E., Kenny, G.E., Perla, D.E., and Hausbeck, M.K. 2023. An evaluation of year-to-year fungicide efficacy and cultivar resistance combined with fungicide programs to manage cucumber downy mildew. Crop Protection 168:106176. DOI: 10.1016/j.cropro.2022.106176.
22. Uebbing, M.R., Hayden, Z.D., and Hausbeck, M.K. 2024. Conventional and Biopesticide Fungicides for Cucurbit Downy Mildew Control on Cucumber in Michigan. Plant Health Progress 25(1): 9-18. DOI: 10.1094/PHP-03-23-0024-RS.

Abstracts:

1. Adams, M. L., D'Arcangelo, K. N., Quesada-Ocampo, L. M. (2020) Evaluation of fungicides and cultivars for control of cucumber downy mildew. Phytopathology 110: S1.21.
2. Bhuiyan, M. Z. R., D'Arcangelo, K. N. and Quesada-Ocampo L. M. (2023) Populations of *Pseudoperonospora cubensis* causing downy mildew in squash and cucumbers are structured by host genotype. Phytopathology 113: S3.108.

3. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. (2020) Leveraging population genetics to develop disease control practices: a study in the crop-specific management of cucurbit downy mildew. Phytopathology 110: S2.203.
4. D'Arcangelo, K. N., Rahman, A., Miles, T. D., and Quesada-Ocampo, L. M. (2020) Utilizing a population genetics approach to provide crop-specific management strategies for cucurbit downy mildew. Phytopathology 110: S1.7.
5. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. (2021) Utilizing a population genetics approach to facilitate crop-specific management of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*. Phytopathology 111: S1.14.
6. D'Arcangelo, K. N., Rahman, A., Miles, T. D., and Quesada-Ocampo, L. M. (2021) Distribution of alleles related to carboxylic acid amide and quinone outside inhibitor resistance in host-adapted clades of *Pseudoperonospora cubensis*. Phytopathology 111: S2.114.
7. Prieto-Torres, M. and Quesada-Ocampo L. M. (2023) Monitoring for mutations related to oxathiapiprolin fungicide resistance in *Pseudoperonospora cubensis* populations. Phytopathology 113: S2.31.
8. Prieto-Torres, M., and Quesada-Ocampo L. M. (2023) Monitoring oxathiapiprolin fungicide resistance mutations in *Pseudoperonospora cubensis* populations in North Carolina. Phytopathology 113: S3.18.
9. Purayannur, S., Cano, L.M., Bowman, M., Childs, K.L., and Quesada, L.M. (2020) Host-specific effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Phytopathology 110: S1.3.
10. Purayannur, S., Cano, L. M., Bowman, M. J., Childs, K. L., and Quesada-Ocampo, L. M. (2020) Clade-specific RXLR effectorome of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Phytopathology 110: S2.6.
11. Purayannur S., Cano L.M., Bowman M.J, Childs K.L., and Quesada-Ocampo, L.M. (2021) Effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Phytopathology 111: S2.7.
12. Uebbing, M.R. and Hausbeck, M.K. 2022. Using weather conditions to time fungicide application intervals for control of downy mildew on cucumber. Phytopathology 113(9s): S2.9.
13. Prieto Torres, M. and Quesada-Ocampo, L.M. (2024) Optimizing a mobile spore trapping system for detection of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew. Phytopathology: in press.
14. Prieto Torres M and Quesada-Ocampo, L.M. (2024) Optimizing a mobile spore trapping system for detection of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew. (Plant Health) Phytopathology: in press.

Oral Research Presentations:

1. D'Arcangelo K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo L. M. Utilizing a population genetics approach to provide crop-specific management strategies for cucurbit downy mildew. Annual Southern Division American Phytopathological Society Meeting, Charleston, SC, February 2020.
2. Purayannur, S., Cano, L. M., Bowman, M. J., Childs, K. L., and Quesada-Ocampo, L. M. Host-specific effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Annual Southern Division American Phytopathological Society Meeting, Charleston, SC. February, 2020.

3. Quesada-Ocampo L. M. Leveraging population genetics, epidemiology, and genomics to improve management of cucurbit downy mildew. Department of Plant Pathology, University of Florida, Gainesville, FL, February 2020.
4. Quesada-Ocampo L. M. Management of re-emerging pathogens of vegetable crops through translational approaches. Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA, March 2020*. (*Cancelled due to COVID-19)
5. D'Arcangelo, K. N., Rahman, A., Miles, T. D., and Quesada-Ocampo, L. M. Utilizing a population genetics approach to enable crop-specific management of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*. NC State Plant Pathology Graduate Student Symposium. Raleigh, NC. July 2020.
6. D'Arcangelo, K. N. and Quesada-Ocampo, L. M. Population genetics and genomics strategies for biosurveillance and improved management of cucurbit downy mildew. North Carolina State University 3rd Annual Genetics & Genomics Initiative Retreat. Raleigh, NC, August 2020.
7. D'Arcangelo, K. N., Rahman, A., Miles, T., and Quesada-Ocampo, L. M. Leveraging population genetics to develop disease control practices: A study in crop-specific management of cucurbit downy mildew. Annual American Phytopathological Society Meeting, Denver, CO. August, 2020.
8. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve disease control in vegetable crops. Department of Plant Pathology, Washington State University, Pullman, WA, September 2020.
9. Hausbeck, M.K. and Kenny, G. 2020. From the Field to the Lab and Back: Monitoring Fungicide Resistance in Cucurbit Downy Mildew. Pickle Packers International Annual Meeting. Virtual, 19 Oct.
10. Quesada-Ocampo L. M. From the field to the lab and back: monitoring fungicide resistance in cucurbit downy mildew. Pickle Packers International Annual Meeting. Virtual Meeting, October 2020.
11. Quesada-Ocampo L. M. Population genetics and epidemiology approaches for management of re-emerging pathogens of vegetable crops. Department of Plant Pathology, University of Minnesota, St. Paul, MN, October 2020.
12. Quesada-Ocampo L. M. Population genetics and epidemiology approaches for management of re-emerging pathogens of vegetable crops. Department of Plant Pathology, University of Minnesota, St. Paul, MN, October 2020.
13. Quesada-Ocampo L. M. Leveraging population genetics, epidemiology, and genomics to improve management of re-emerging pathogens of vegetable crops. Department of Plant Pathology, Kansas State University, Manhattan, KS, November 2020.
14. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. Utilizing a population genetics approach to facilitate crop-specific management of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*. Annual Southern Division American Phytopathological Society Meeting, Virtual Meeting. February, 2021.
15. D'Arcangelo, K. N., Rahman, A., Miles, T. D. and Quesada-Ocampo, L. M. Utilizing a population genetics approach to facilitate crop-specific management of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*. Annual Southern Division American Phytopathological Society Meeting, Virtual Meeting. February, 2021.
16. Hausbeck, M.K. 2021. A Partnership to protect Michigan's Cucumber Industry. Farm Lane Society meeting, Virtual, 5 Mar.

17. Hausbeck, M.K., Harlan, B.R., Bello, J.C., and Kenny, G. 2021. Downy Mildew Management in Pickling Cucumbers. Agriculture Agri-Food Canada, Ontario, Canada. Virtual, 8 Apr. 105 attendees.
18. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve management of cucurbit downy mildew. Agriculture Agri-Food Canada, Ontario, Canada, April 2021.
19. Hausbeck, M.K., Perla, D., Spafford, J., and Uebbing, M. 2021. CucCAP2: Cucumber and Squash. CucCAP Grant Meeting, Virtual, spring 2021.
20. Purayannur S., Cano L.M., Bowman M.J, Childs K.L., Quesada-Ocampo L.M. Effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Annual American Phytopathological Society Meeting, Virtual Meeting. August, 2021.
21. Purayannur S., Cano L.M., Bowman M.J, Childs K.L., Quesada-Ocampo L.M. Effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Annual American Phytopathological Society Meeting, Virtual Meeting. August, 2021
22. Quesada-Ocampo L. M. From the field to the lab and back: translational strategies to improve disease management in vegetable crops. Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, September 2021.
23. D'Arcangelo, K. N. and Quesada-Ocampo L.M. Characterization of the population dynamics of alleles related to Carboxilic Acid Amide and Quinone Outside Inhibitor resistance in the host-adapted clades of *Pseudoperononspora cubensis* to facilitate crop-specific management of cucurbit downy mildew. Department of Entomology and Plant Pathology Seminar. Raleigh, NC, October, 2021.
24. Salcedo, A., Parada-Rojas C. H., Purayannur S.,Quesada-Ocampo L. M. Accelerating Resistance Breeding in Cucurbits. CucCAP2 meeting, Virtual Meeting, October 2021.
25. Quesada-Ocampo L. M. Translational strategies to improve management of re-emerging pathogens of vegetable crops. Australasian Plant Pathology Society, Australia, November 2021.
26. Quesada-Ocampo L. M. Translational strategies to improve management of re-emerging pathogens of vegetable crops. Australasian Plant Pathology Society, Australia, November 2021.
27. Uebbing M.R., Hausbeck M.K. Managing Cucurbit Downy Mildew in Pickling cucumber using disease forecasters and fungicides. Department of Plant Soil and Microbial Sciences, Michigan State University, March 2022.
28. Peterson, A.M., Bello, J.C., Kenny, G., Perla, D., Uebbing, M., Hausbeck, M.,K. 2022. Burkard spore traps for detection of *Pseudoperonospora cubensis* sporangia in cucurbit production. 10th International IPM Symposium, Denver, CO, 28 Feb-3 Mar. Poster presentation.
29. Perla D. and Hausbeck M.K. Vegetable Disease Management using host resistance and fungicides. American Phytopathological Society North Central Division Meeting, Lincoln, NE, June 2022.
30. Uebbing M.R., Hausbeck M.K. Using weather conditions to time fungicide application intervals for control of downy mildew on cucumber. American Phytopathological Society North Central Division Meeting, Lincoln, NE, June 2022.
31. Quesada-Ocampo L. M. Disease management in vegetable crops. AgBiome seminar, July 2022.
32. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Biology Seminar, University of Massachusetts, September 2022.

33. Quesada-Ocampo L. M. Translational research for detection and management of diseases of vegetable crops. Universidad Nacional Mayor de San Marcos. Lima, Peru, November 2022.
34. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Genetics and Genomics Academy Seminar, North Carolina State University, Raleigh, NC, November 2022.
35. Quesada-Ocampo L. M. Next generation technologies for plant pathogen detection. Commercializing Academic Research Showcase & Innovation Expo. North Carolina Plant Sciences Initiative. North Carolina State University, Raleigh, NC, November 2022.
36. Prieto-Torres, M. and Quesada-Ocampo L. M. Monitoring for mutations related to oxathiapiprolin fungicide resistance in *Pseudoperonospora cubensis* populations. American Phytopathological Society-Southern Division, Durham, NC, February 2023.
37. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Pathology Seminar, University of Georgia, Athens, GA, March 2023.
38. Prieto Torres M, Quesada-Ocampo L. M. PhD Proposal Seminar: Biosurveillance and disease management for cucurbit downy mildew *(Pseudoperonospora cubensis*). Department of Entomology and Plant Pathology. Raleigh, NC, April 2023.
39. Hausbeck, M.K. 2023. Grower-Driven Research Leads to Integrated Disease Management. Departmental Seminar, Michigan State University. East Lansing, MI, 27 Apr.
40. Quesada-Ocampo L. M. Translational research for detection and management of diseases of vegetable crops. NC Plant Sciences Initiative Partners Event, Raleigh, NC, May 2023.
41. Quesada-Ocampo L. M., Xiang, L., Brown H., and Vijapurapu R. Evaluation of commercially available spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits. NC State Chancellor Innovation Fund Meeting, Raleigh, NC, May 2023.
42. Quesada-Ocampo L. M. Translational research for detection and management of diseases of vegetable crops. NC Plant Sciences Initiative Senator Visit, Raleigh, NC, June 2023.
43. Prieto Torres M, Quesada-Ocampo L. M. Monitoring oxathiapiprolin fungicide resistance mutations in *Pseudoperonospora cubensis* populations in North Carolina. American Phytopathological Society, Plant Health 2023, Denver, CO, August 2023.
44. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Biochemistry and Molecular Biology Seminar, Reno, NV, September 2023.
45. Quesada-Ocampo L. M. Next generation technologies for plant pathogen detection. NC Plant Sciences Initiative State of the Union. North Carolina Plant Sciences Initiative. North Carolina State University, Raleigh, NC, February 2023. Quesada-Ocampo L. M., Xiang, L., Brown H., and Vijapurapu R. Evaluation of commercially available spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits. CALS Tailgate Chancellor Suite, Raleigh, NC, October 2023.
46. Prieto Torres, M. and Quesada-Ocampo L. M. Monitoreo y biovigilancia en poblaciones de mildeo velloso en cucurbitáceas (*Pseudoperonospora cubensis*), en Carolina del Norte. I Simposio Internacional de Fitopatología y Microbiología Agrícola, Universidad Nacional Mayor de San Marcos, Peru, November 2023.
47. Prieto Torres M. and Quesada-Ocampo L. M. Optimizing a mobile spore trapping system for detection of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew. American Phytopathological Society, Plant Health 2024, Memphis, TN, July 2024.
48. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Plant Pathology, Gainesville, FL, November 2023.
49. Quesada-Ocampo L. M. Applied genomics for disease management in vegetable crops. Department of Botany and Plant Pathology, West Lafayette, IN, November 2023.

50. Quesada-Ocampo L. M. Population structure of *Pseudoperonospora cubensis*. FRAC-APS Pathogen Resistance Committee Workshop. Oomycete Fungicides. American Phytopathological Society Annual Meeting. Memphis, TN, July 2024.
51. Hausbeck, M.K. 2024. Grower-Driven Research Leads to Integrated Disease Management. Nebraska Plant Science Symposium. Lincoln, NE, 29 April.

Oral Extension Presentations:

1. Quesada-Ocampo, L. M. Cucumber diseases in the greenhouse. Greenhouse vegetable production agent training. Raleigh, NC, January 2020.
2. Hausbeck, M.K. and Kenny, G. 2020. From the Field to the Lab and Back: Monitoring Fungicide Resistance in Cucurbit Downy Mildew. Pickle Packers International Annual Meeting. Virtual, 19 Oct. 78 attendees.
3. Hausbeck, M.K. 2020. Vegetable and Root Crop Field Day: Disease control of Vegetables. Virtual, Sept. https://www.canr.msu.edu/events/oceana-research-tour-virtual-field-day
4. Hausbeck, M.K. 2020. 2021 Spray Program. Southeast Vegetable Meeting. Virtual, 4 Nov.
5. Quesada-Ocampo L. M. Never a dill moment when managing cucumber downy mildew. 2020 Eastern NC Certified Crop Adviser Training. Virtual Meeting, December 2020.
6. Hausbeck, M.K. Downy Mildew Management in Pickling Cucumbers. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 8 Dec 2020.
7. Hausbeck, M.K. and Higgins, D.S. The Grounder, the Line Drive, and the Pop Fly: Fielding Three Very Different Vine Crop Diseases. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 9 Dec 2020.
8. Quesada-Ocampo L. M. Pickles in a pickle: trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Pickle Packers International Spring Meeting. Virtual, April 2021*.
9. Hausbeck, M.K. and Uebbing M.R. 2021. Pickles in a pickle: Trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Annual Meeting of the Pickle Packers International. Virtual, 19 Oct. 78 attendees.
10. Quesada-Ocampo, L. M. Pickles in a pickle: trying to outsmart *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. North Carolina Vegetable Growers Association Ag Expo. Wilmington, NC, November 2021.
11. Hausbeck, M.K. and Uebbing, M. Downy Mildew: New Insights on Control. Great Lakes Farm, Fruit and Vegetable Expo. Grand Rapids, MI, 8 Dec 2021.
12. Quesada-Ocampo L. M. IPM Q & A, Vegetable diseases hot topics update facilitated by Dr. Adrienne Gorny. DEPP IPM calls. April 2022.
13. Hausbeck, M.K. 2022. Developments in downy mildew and Phytophthora capsici control in pickling cucumber. Annual Meeting of the Pickle Packers International. Las Vegas, NV, 19 Oct. 75 attendees.
14. Uebbing, M. and Hausbeck, M.K. 2022. Using Disease Forecasters to Time Fungicide Applications to Control Downy Mildew in Pickling Cucumber. MSU Pickle & Pepper Research Committee meeting. Grand Rapids, MI. 6 Dec. 55 attendees.
15. Uebbing, M. and Hausbeck, M.K. 2022. Downy Mildew Update in Pickling Cucumber. Great Lakes Farm, Fruit and Vegetable Expo. Grand Rapids, MI. 6 Dec. 55 attendees.
16. Quesada-Ocampo L. M., Rosado-Rivera Y.I., and Prieto M. Management of downy mildew in cucurbit crops. 35th Annual Southeast Vegetable and Fruit Expo. Durham, NC, December 2022.

17. Quesada-Ocampo L. M. Field monitoring of the cucurbit downy mildew pathogen: the next frontier. Pickle Packers International Spring Meeting. Raleigh NC, April 2023.
18. Hausbeck, M.K. 2023. Research Advances in Controlling Downy Mildew. Annual Meeting of Pickle Packers International. Austin, TX, 31 October.
19. Quesada-Ocampo L. M. Management of cucurbit downy mildew. 36th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, November 2023.
20. Hausbeck, M.K. 2024. Downy mildew control. Cucumber Day presented by Ontario Processing Vegetable Growers. London, ON, Canada, 3 April 2024.

Plant Disease Management Reports:

1. Adams M. L., Collins H., and Quesada-Ocampo L. M. (2020) Evaluation of fungicides for control of downy mildew on cucumber, Clayton, 2019. Plant Disease Management Reports 14: V116.
2. Adams M. L., Collins H., and Quesada-Ocampo L. M. (2020) Evaluation of cultivars in combination with fungicides for control of downy mildew and yield effects on cucumber, Clinton, 2019. Plant Disease Management Reports 14: V117.
3. Adams M. L., Collins H., and Quesada-Ocampo L. M. (2020) Evaluation of fungicides and cultivars for control of downy mildew on cucumber, Kinston 2019. Plant Disease Management Reports 14: V107.
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7. Bello, J. C., Engfehr, C.L. and Hausbeck, M.K. 2020. Evaluation of alternating programs of fungicides for control of downy mildew on pickling cucumber, 2019. PDMR 14: V163.
8. Kenny, G. E., Engfehr, C.L., and Hausbeck, M.K. 2020. Evaluation of 9 alternating programs of fungicides for control of downy mildew on pickling cucumbers, 2019. PDMR 14: V216.
9. Kenny, G.E., Engfehr, C.L. and Hausbeck, M.K. 2020. Evaluation of single product treatments for control of downy mildew on pickling cucumbers, 2019. PDMR 14: V183.
10. Shrestha, S., Perla, D.E., Harlan, B.R., and Hausbeck, M.K. 2021. Field evaluation of basil cultivars for susceptibility to downy mildew, 2020. PDMR 15: V066.
11. Adams M. L., Collins H., Quesada-Ocampo L. M. (2021) Evaluation of fungicides for control of downy mildew of winter squash, Kinston 2020. Plant Disease Management Reports 15: V167.
12. Adams M. L., Collins H., Quesada-Ocampo L. M. (2021) Evaluation of fungicides for control of downy mildew on cucumber, Clayton, NC 2020. Plant Disease Management Reports: 15: V125.
13. Adams M. L., Collins H., Quesada-Ocampo L. M. (2021) Evaluation of fungicides for control of downy mildew on cucumber, Clinton, NC 2020. Plant Disease Management Reports: 15: V124.
14. Adams M. L., Collins H., Quesada-Ocampo L. M. (2021) Evaluation of cultivars in combination with fungicides for control of downy mildew and yield effects on cucumber, Clinton, NC II 2020. Plant Disease Management Reports: 15: V140.

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16. Adams M. L., Batts T. M., Collins H., Quesada-Ocampo L. M. (2021) Evaluation of fungicides for control of downy mildew on cucumber, Saratoga, NC 2020. Plant Disease Management Reports: 15: V106.
17. Uebbing, M.R and Hausbeck, M.K. 2022. Evaluation of disease forecasters for control of downy mildew on pickling cucumber, 2021. PDMR 16: V136.
18. Uebbing, M.R and Hausbeck, M.K. 2022. Evaluation of single product treatments for control of downy mildew on pickling cucumbers, 2021. PDMR 16: V137.
19. Rosado-Rivera Y. I., Adams M. L., Collins H., Quesada-Ocampo L. M. (2022) Evaluation of fungicides for control for management of cucumber downy mildew, Clinton, NC 2021. Plant Disease Management Reports: 16: V180.
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21. Rosado-Rivera Y.I., Adams L.M., Collins H., Quesada-Ocampo L.M. (2023) Evaluation of varieties for control of downy mildew on cucumber, Clinton, NC 2021. Plant Disease Management Reports 17: V168.
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25. Rosado-Rivera Y.I., Quesada-Ocampo L.M. (2023) Evaluation of fungicides for management of cucumber downy mildew, Clinton 2022. Plant Disease Management Reports 17: V100.
26. Rosado-Rivera Y.I., Quesada-Ocampo L.M. (2023) Evaluation of fungicide rotation for downy mildew management on cucumbers, Clinton 2022. Plant Disease Management Reports 17: V097.
27. Uebbing, M.R. and Hausbeck, M.K. 2023. Evaluating pickling cucumber cultivars for resistance to downy mildew under natural infection conditions, 2022. Plant Disease Management Reports 17: V157.
28. Uebbing, M.R. and Hausbeck, M.K. 2023. Efficacy of organic products for control of powdery mildew on moderately resistant acorn squash, 2022. Plant Disease Management Reports 17: V158.
29. Uebbing, M.R. and Hausbeck, M.K. 2023. Evaluation of biological and conventional products to control downy mildew on pickling cucumbers, 2022. Plant Disease Management Reports 17: V156.
30. Rosado-Rivera Y. I., Collins, H., Quesada-Ocampo L. M. (2024) Cucumber downy mildew management, Clinton 2023. Plant Disease Management Reports 18: V054
31. Rosado-Rivera Y. I.[T], Collins H[T]., Quesada-Ocampo L. M. (2024) Management of cucumber downy mildew using cultivars and fungicide programs, Clinton, NC 2023. Plant Disease Management Reports 18: V054
32. Rosado-Rivera Y. I.[T], Collins H[T]., Quesada-Ocampo L. M. (2024) Chemical management for downy mildew on cucumbers, Clinton, NC 2023. Plant Disease Management Reports 18: V055

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2. Quesada-Ocampo L. M., Meadows I., and Louws F. (2020) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Quesada-Ocampo L. M. contributed 11 tables total).
3. Southeastern Vegetable Extension Workers. Kemble J., Meadows I., Jennings K. M., and Walgenbach J. F., Eds. (2020) Southeastern US 2020 Vegetable Crop Handbook. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Quesada-Ocampo L. M. contributed 11 tables total).
4. Quesada-Ocampo L. M., Meadows I., and Gorny A. (2021) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Quesada-Ocampo L. M. contributed 11 tables total).
5. Southeastern Vegetable Extension Workers. Kemble J., Meadows I., Jennings K. M., and Walgenbach J. F., Eds. (2021) Southeastern US 2021 Vegetable Crop Handbook. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Quesada-Ocampo L. M. contributed 11 tables total).
6. Quesada-Ocampo L. M., Meadows I., and Gorny A. (2022) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual. Basil, cucurbits, hop, lettuce, endive, sweetpotato, and fungicide resistance tables (Quesada-Ocampo L. M. contributed 11 tables total).
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8. Adams R., Britton W., Jennings K., Quesada-Ocampo L. M., Schultheis J., and Walgenbach J. (2022) 2022 Crop Profile for Cucumber in North Carolina. Southern Integrated Pest Management Center. URL: https://ipmdata.ipmcenters.org/source_report.cfm?sourceid=1452&view=yes
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5. Quesada-Ocampo L. M. (Updated yearly) Cucurbit downy mildew. Vegetable Pathology Factsheets. NC State Extension Publications. URL: http://content.ces.ncsu.edu/cucurbit-downy-mildew
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2. MSU: Confirmed Downy Mildew Reports in Michigan: https://veggies.msu.edu/downy-mildew-news/.
3. MSU: Downy Mildew Spore Trap Daily Totals (Counts/day by Michigan Counties): https://veggies.msu.edu/downy-mildew-news/.

4. MSU: Hausbeck Lab Facebook page: https://www.facebook.com/MSUveggieornamentalpathology.
5. NCSU: Extension Plant Pathology Portal: http://plantpathology.ces.ncsu.edu/.
6. NCSU: Vegetable Pathology lab website: http://go.ncsu.edu/veggiepathology/.
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Proceedings/Online/Magazine Publications:

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4. Hausbeck, M. 2020. First cucumber downy mildew outbreak of 2020 confirmed in Berrien County. MSU Extension News: Vegetables:22 Jun.
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7. Hausbeck, M.K. 2020. Monitoring and managing cucumber downy mildew. Interview by Alex Tekip. Michigan State University AgBioResearch, Futures: Summer 2020.
8. Higgins, D., Miles, T., Byrne, J. and Hausbeck, M.K. 2020. Finding and fighting a new hop disease in Michigan. Compiled by Matt Davenport. MSUTODAY: Oct 1, 2020.
9. Hausbeck, M.K. 2020. Downy Mildew Management in Pickling Cucumbers. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 8 Dec. Pickling Cucumber 1 Session Summaries.
10. Hausbeck, M.K. and Higgins, D.S. 2020. The Grounder, the Line Drive, and the Pop Fly: Fielding Three Very Different Vine Crop Diseases. Great Lakes Farm, Fruit and Vegetable Expo. Virtual, 9 Dec. Vine Crops 1 Session Summaries.
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LEVERAGED FUNDING

Hausbeck = $410,000 in current state and federal funding, $200,000 in PPI funding

Quesada = $869,729 in current state and federal funding, $200,000 in PPI funding

Current Funding

A cucurbit downy mildew early warning network for Michigan's growers. PI Hausbeck, M.K. MSU AgBioResearch GREEEN Grant, 7/1/23-6/30/25, **$60,000 to Hausbeck** ($60,000 total award).

QuSeC-TAQS: Sensing-Intelligence on The Move: Quantum-Enhanced Optical Diagnosis of Crop Diseases. PI Liu J. and Co-PI Quesada-Ocampo L. M., Zhang Z., Zhuang Q., Xiang L. NSF, 08/01/23-07/31/27, **$75,000 to Quesada-Ocampo** ($1,075,000 total award).

Evaluation of commercially viable spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits. PI Xiang L. and Co-PI Quesada-Ocampo L. M. North Carolina Biotechnology Center, 07/01/23-6/30/25, **$25,000 to Quesada-Ocampo** ($50,000 total award).

Innovations in Workforce Development: FFAR Fellowship for Prieto M. PI Quesada-Ocampo L. M. Foundation for Food and Agriculture Research, 9/1/22-6/30/25, **$195,000 to Quesada-Ocampo.**

CucCAP2: Harnessing genomic resources for disease resistance and management in cucurbit crops – bringing the tools to the field. PI Grumet R. and Co-PIs Fei Z., Hausbeck M., Havey M., Kousik S., Levi A., Linares A., Ling K., Loy B., Mazourek M., McCreight J., McGregor C., Quesada-Ocampo L. M., Reddy U., Schultheis J., Smart C., Wechter P., Weng Y., Wessel-Beaver L., and Wintermantel B. USDA SCRI, 9/1/20-8/31/25, **$574,729 to Quesada-Ocampo, $350,000 to Hausbeck** ($7,050,603 total award).

Past Funding

Biosurveillance of cucurbit downy mildew. PI Quesada-Ocampo L.M. NCDA Specialty Crop Block Grant, 1/1/19-12/31/20, **$70,745**.

Enhanced mitigation and detection of basil, cucurbit, and impatiens downy mildew. PI Palmer C. and Co-PIs Crouch J. A., Daughtrey M., Hausbeck M. K., Quesada-Ocampo L. M., Shishkoff N. APHIS PPQ Farm Bill Grant, 1/1/21-12/31/21, **$108,063 to Quesada-Ocampo** ($309,626 total award).

Biosurveillance of cucurbit downy mildew. PI Quesada-Ocampo L.M. NCDA Specialty Crop Block Grant, 1/1/19-12/31/20, **$70,745 to Quesada-Ocampo**.

Strategies are needed to protect Michigan's cucurbits from fungicide resistant downy mildew. PI Hausbeck, M.K. MDARD Specialty Crop Block Grant, 10/1/20-9/30/22, **$91,577 to Hausbeck** ($91,577 total award).

Advancing monitoring and early detection of downy mildew in cucumbers using spore traps and molecular tools. PI Hausbeck, M.K. MSU AgBioReasearch GREEEN Grant, 10/1/2020-6/30/2023, **$80,000 to Hausbeck** ($80,000 total award).

UAV biosurveillance of cucurbit downy mildew. PI Quesada-Ocampo L.M. and Co-PI Young S. NCDA Specialty Crop Block Grant, 1/1/22-12/31/23, **$29,817 to Quesada-Ocampo** ($77,850 total award).

Evaluation of commercially viable spore traps for detection of clade 1 and clade 2 downy mildew on cucurbits, NC 2024. PI Quesada-Ocampo L. M. and Co-PI Xiang L. Chancellor's Innovation Fund Grant Program, 06/01/23-5/31/24, **$25,000 to Quesada-Ocampo** ($50,000 total award).

Testing new ways to monitor and manage cucurbit downy mildew. PI Hausbeck, M.K. MDARD Specialty Crop Block Grant, 10/1/22-9/30/24, **$96,486 to Hausbeck** ($96,486 total award).

Appendix 1. Detection of *Pseudoperonospora* sporangia in Michigan counties monitored using spore traps, 2024.

Site updated: 8/16/24

KEY:	
P.Cub1	*Pseudoperonospora cubensis* clade 1 (downy mildew of curcurbits; impacts **acorn squash, butternut squash, pumpkin, watermelon**)
P.Cub2	*Pseudoperonospora cubensis* clade 2 (downy mildew of curcurbits; impacts **cucumber, cantaloupe**)
★	Spore Trapping **Started**
☒	Spore Trapping **Ended**
[DM]	Visual positive identification of downy mildew in field (scouting)
-	**Negative**; No confirmed airborne presence of pathogen
POS	**Positive**; Confirmed airborne presence of pathogen

Quick Tips:

Confirmed airborne presence indicates possible rapid onset of downy mildew in target crops. It is recommended to take immediate action in preventative treatment of plants if positive airborne presence is confirmed within your county.

For a list of products that have been tested and are effective against DM in field trials, or for more information on downy mildew management, click here.

*Spore trapping was temporarily halted for repairs to spore trap.

County:	Allegan		Berrien		Muskegon		Bay		Monroe		Saginaw		Ingham	
DM Class:	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2
20-May														
21-May							★ -	★ -						
22-May							-	-						
23-May							-	-						
24-May							-	-						
25-May							-	-						
26-May							-	-						
27-May							-	-						
28-May							-	-						
29-May					★ -	★ -	-	-						
30-May					-	-	-	-						
31-May					-	-	-	-			★ -	★ -		
1-Jun					-	-	-	-			-	-		
2-Jun					-	-	-	-			-	-		
3-Jun					-	-	-	-			-	-		
4-Jun					-	-	-	-	★ -	★ -	-	-		
5-Jun	★ -	★ -			-	-	-	-	-	-	-	-		
6-Jun	-	-			-	-	-	-	-	-	-	POS		
7-Jun	-	-			-	-	-	-	-	-	-	-		
8-Jun	-	-			-	-	-	-	-	-	-	-		
9-Jun	-	-			-	-	-	-	-	-	-	-		
10-Jun	-	-			-	-	-	-	-	-	-	-	★ -	★ -
11-Jun	-	-			-	-	-	-	-	-	-	-	-	-
12-Jun	-	-	★ -	★ -	-	-	-	-	-	-	-	-	-	-
13-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14-Jun	-	-	-	-	-	-	-	-	-	POS	-	-	-	-
15-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16-Jun	-	-	-	-	-	POS	-	-	-	-	-	-	-	-
17-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19-Jun	-	-	-	-	-	-	-	-	-	-	-	POS	-	-
20-Jun	-	-	-	-	-	-	-	POS	-	-	-	-	-	-
21-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-

County:	Allegan		Berrien		Muskegon		Bay		Monroe		Saginaw		Ingham	
DM Class:	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2	P.Cub1	P.Cub2
4-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-Jul	-	-	-	-	-	-	-	-	-	-	-	POS	-	-
6-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8-Jul	-	-	-	-	-	-	-	-	-	-	-	-	-	POS
9-Jul	-	POS	-	-	-	-	-	POS	-	-	-	-	-	-
10-Jul	-	-	-	-	-	-	-	-	-	-	-	POS	-	-
11-Jul	-	-	-	-	-	-	-	POS	-	-	-	-	-	-
12-Jul	-	-	-	-	-	-	-	POS	-	-	-	[DM] -	-	POS
13-Jul	-	-	-	-	-	-	-	POS	-	POS	-	-	-	POS
14-Jul	-	-	-	-	-	-	-	-	-	POS	-	-	-	-
15-Jul	-	POS	-	-	-	-	-	[DM] -	-	POS	-	-	-	[DM] POS
16-Jul	-	-	-	-	-	-	-	-	-	POS	-	POS	-	POS
17-Jul	-	POS	-	-	*	*	-	-	-	-	-	-	-	POS
18-Jul	-	-	-	-	*	[DM] *	-	-	-	-	-	-	-	POS
19-Jul	-	-	-	-	*	*	-	-	-	-	-	-	-	POS
20-Jul	-	POS	-	-	*	*	-	-	-	-	-	-	-	-
21-Jul	-	POS	-	-	*	*	-	-	-	-	-	-	-	POS
22-Jul	-	POS	-	-	-	-	-	POS	-	POS	-	POS	-	POS
23-Jul	-	POS	-	POS	-	POS	-	POS	-	-	-	-	-	POS
24-Jul	-	POS	-	-	-	POS	-	-	-	-	-	POS	-	POS
25-Jul	-	POS	-	-	-	POS	-	POS	-	-	-	-	-	POS
26-Jul	-	POS	-	POS	-	POS	-	POS	-	-	-	-	-	POS
27-Jul	-	POS	-	-	-	POS	-	-	-	POS	-	-	-	POS
28-Jul	-	POS	-	-	-	POS	-	POS	-	-	-	-	-	POS
29-Jul	-	POS	-	POS	-	POS	-	POS	-	-	-	-	-	POS
30-Jul	-	[DM] POS	-	POS	-	[DM] POS	-	-	-	-	-	-	-	POS
31-Jul	-	POS	-	POS	-	POS	-	POS	-	-	-	POS	-	POS
1-Aug	-	POS	-	-	-	POS	-	POS	-	-	-	-	-	POS
2-Aug	-	POS	-	-	-	POS	-	POS	-	-	-	POS	-	POS
3-Aug	-	POS	-	-	-	POS	-	-	-	-	-	POS	-	POS
4-Aug	-	POS	-	-	-	POS	-	-	-	POS	-	POS	-	POS
5-Aug	-	POS	-	-	-	POS	-	-	-	-	-		-	POS
6-Aug	-	POS	-	-	-	POS	-	POS	-	-	-	POS	-	POS
7-Aug	-	POS	-	[DM]	-	POS	-	POS	-	-	-	POS	-	POS
8-Aug	-	POS	-	-	-	POS	-	POS	-	-	-	POS	-	POS
9-Aug	-	POS	-	-	-	POS	-	POS	-	POS	-	POS	-	POS
10-Aug	-	POS	-	-	-	POS	-	POS	-	POS	-	-	-	POS
11-Aug	-	POS	-	-	-	POS	-	-	-	-	-	-	-	POS
12-Aug	-	POS	-	POS	-	POS	-	-	-	-	-	-	-	POS
13-Aug														
14-Aug														
15-Aug														
16-Aug														
17-Aug														
18-Aug														

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PPI Agricultural Research Committee Proposals and Project Reports

Ag Research Fund (ARF): ☐ ARF Pre-Proposal ☐ **ARF Annual Report**

Field Research (FR): ☐ FR Proposal ☐ FR Progress Report

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1. Research Project

   Date First Approved: **2022** Number of Renewals: **0**

   Project Term (# of years): **THREE** Date of Completion: **12/31/2024**

2. Report period: **January 1, 2024 – October 21, 2024**
3. Research Agency: **USDA-ARS**
4. Department or Unit: **Vegetable Crops Res Unit**
5. Investigator(s): **Yiqun Weng**
6. Project Leader(s): **Yiqun Weng**
7. Project Title: **Development of pickling cucumber inbreds with multiple disease resistances conferred by a novel mutant**
8. Amount of research funding requested for 2024: **$20,000**
9. Amount of research funding received for 2022: **$20,000**
10. Total research funding given to this project to date: **$20,000**
11. Percent of project's total yearly funds represented by PPI research grant (est.): 40**%**
12. Signature of person preparing report:

Date: October 23, 2024

PPI ARF Research Project

2024 Progress Report

This project has three specific objectives:

1) Evaluation of *speckled and virescent leaf* (*svl*) mutant for its resistances against major foliage diseases. Clarify the genetic basis.
2) Develop molecular markers for this valuable attribute, which will be used to introgress this trait into Gy14 pickling cucumber genetic background through marker-assisted selection.
3) Develop and deliver to the industry an improved pickling cucumber inbred line with multiple disease resistance (MDR) and associated know-how for commercial cucumber breeding.

1. Cloning and functional characterization of *svl* gene

In 2023-2024, we continued work on map-based cloning of the *svl* mutant gene. Fine genetic mapping placed the gene into a small region containing five predicted genes. Multiple lines of evidence suggest that second gene is the best candidate for *svl* (Figure 1A) which is a homolog of Arabidopsis gene *CsStic2* (*suppressor of tic40*). We cloned this gene from both WI7642M (mutant, *svl*) and WI7642W (normal leaf color, *SVL*). It turned out that there is a 4884 bp (base pair) retrotransposon insertion inside the 5th exon of this gene in the mutant. This insertion resulted in loss of the entire 72-bp of the 5th exon in its cDNA (Figure 1B).

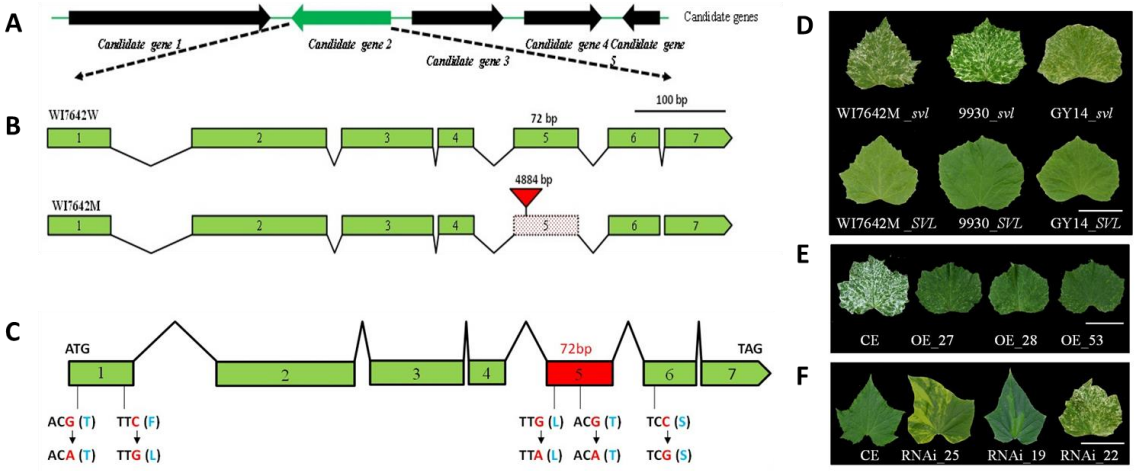

Figure 1. Cloning and functional characterization of *svl* gene. The gene was mapped in a 40kb region containing five gene (A). The second gene is the candidate for the *svl* mutation, and the leaf color mutation is due to the insertion of a retrotransposon sequence in the 5th exon of *SVL*(B). Although there are multiple sequence variations inside this gene in some cucumber lines, only WI7642M shows the svl mutant appearances. (C). When the mutant gene is brought into different genetic backgrounds (Asian Long type 9930 and US pickle cucumber Gy14), the resulting introgression lines exhibit the svl mutant phenotype (D). Cucumber plants over-expressing the SVL gene can rescue the mutant phenotype (E). Conversely, inhibition of the gene expression will result in speckled or chlorotic leaves (F).

We examined sequence variation of this gene in many other cucumber lines and found that some lines also carry SNPs (single nucleotide polymorphisms) in its promoter or coding regions (Figure 1C). However, all lines carrying these variants have normal green leaves. This suggests that the large retrotransposon insertion and loss of 72bp coding sequence is critical for the *svl* appearances in WI7642M.

We developed homozygous introgression lines by marker-assisted selection (MAS) to bring the *svl* mutant allele into Gy14 (US pickle), 9930 (Asian Long) and Poinsett 76 (US slicer). All the introgression lines show speckled and virescent leaves (Figure 1D) indicating strong effect of this mutation. We developed transgenic cucumber plants by increasing expression of the *SVL* gene (over-expression, OE) in the mutant, which can reverse the mutant phenotype back to normal greenhouse appearances (Figure 1E). When the expression of the *SVL* gene was inhibited with the RNAi (RNA interface) technology in normal plants, the resulting plant shows speckled leave or chlorotic leave depending on the genetic backgrounds (Figure 1F). Thus, results from these experiments further validate the function of the *svl* gene.

2. Evaluation of WI7642M mutant for disease resistances and abiotic stress tolerance

Our studies in previous years found that the *svl* mutant exhibits resistance to multiple pathogens including downy mildew (DM), anthracnose (AR), powdery mildew (PM), as well as angular leaf spot (ALS). These pathogens have different life styles: DM, PM and AR are biotrophic, and ALS is hemibiotrophic. We further tested the resistance of WI7642M (svl) and WI7642W (SVL) to target leaf spot (TLS) which is cause by a necrotrophic pathogen. We found WI7642M has much better resistance than WI7642W (Figure 2).

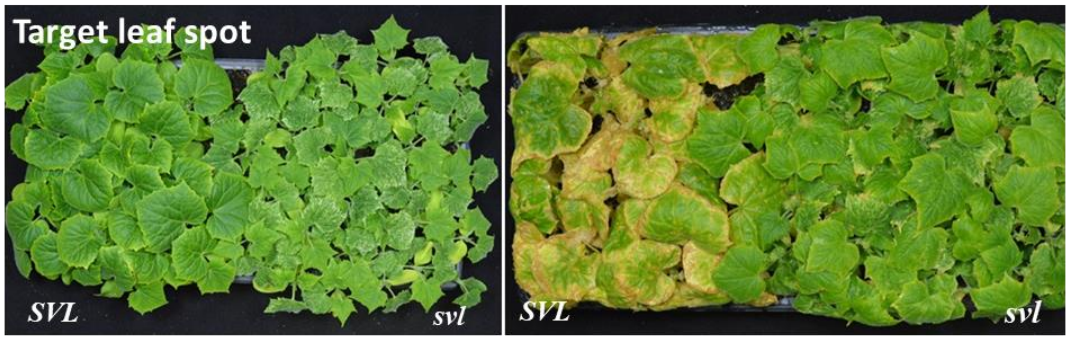

Figure 2. A: The mutant WI7642M (svl) exhibits high resistance to TLS than WI7642W (SVL). The left and right panels are images taken before and one week after the inoculation of the TLS pathogen, respectively.

The resistance to cucumber pathogens of the two lines were also tested by an industry collaborator, and it was found that WI7642M has highly resistance to scab, which is caused by a hemibiotrophic fungal pathogen, and intermediate resistance to PM and AR. These experiments further confirmed the multiple disease resistance of the *svl* mutant. However. The degree of resistance varies depending on the lifestyles of the pathogen. It seems WI7642M has much better resistance to necrotrophic and hemibiotrophic fungal pathogens.

Our work in 2022 and 2023 indicated that WI7642M has better tolerance to water stresses. Our industry collaborator conducted a series experiments systematically investigated performance

of WI7642M and WI7642W under control soil water conditions. Figure 3 shows the whole-plant drought response profiles (θ_{crit}) of WI7642M and WI7642, which suggests that WI7642M has significantly better tolerance than WI7642W.

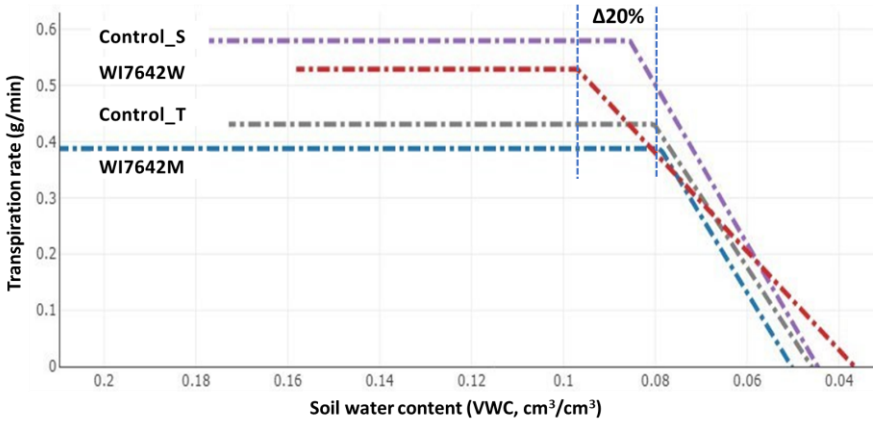

Figure 3. Whole-plant drought response profile (θ_{crit}) of WI7642M and WI7642W. Control_S and Control_T are drought susceptible and drought tolerant control lines, respectively. Graph curtesy of industry collaborator.

3. Reactive oxygen species (ROS) accumulation in WI7642M and WI7642W

We speculate the resistance to multiple diseases and tolerance to abiotic stresses of WI7642M may be associated with differential accumulation of ROS (for example, hydroperoxide or H_2O_2, superoxide, hydroxyl radical, and singlet oxygen). We measured the concentrations of H_2O_2 and superoxide radical (O_2^-) in the cotyledons and young leaves of WI7642M and WI7642W, and found that the mutant (WI7642M) accumulates more of the two ROS species (Figure 4).

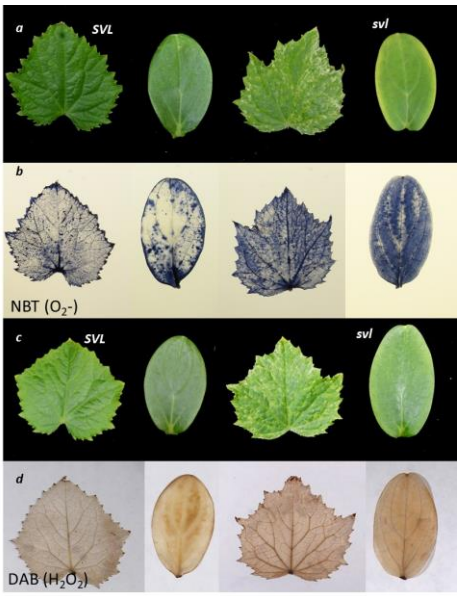

Figure 4. The cotyledons and true leaves of WI7642M (svl) seems to accumulate more ROS (H_2O_2 and O_2^-) than WI7642W (SVL).

4. Development of introgression lines and germplasm release

We conducted marker-assisted backcrossing (MABC) aiming to bring the mutant *svl* allele into different genetic backgrounds. A molecular marker was developed based on the 4.9kb insertion. We used three varieties: Gy14 (pickling cucumber), Poinsett 76 (a slicer) and 9930 (a Chinese Long cucumber). In 2024, homozygous introgression lines in the genetic backgrounds of the three varieties were obtained. Test of these lines for resistance against multiple diseases is underway. Preliminary results are consistent the early notion that the mutant seems to be more resistance to necrotrophic pathogens.

A USDA germplasm release of the two lines is in preparation.

2024 PROGRESS REPORT

Report period: 4.1.24 – 9.30.24

Agricultural Research Fund 2024-2028 project:

Monitoring *Phytophthora capsici* for Resistance to Fungicides Using Field and Molecular Tools

Mary Hausbeck, Professor & Extension Specialist, Michigan State University
Lina Quesada, Professor & Extension Specialist, North Carolina State University

PROBLEM:

Phytophthora capsici causes root and crown rot in pepper, and fruit rot in pepper and cucumber. In the last few years, it has been especially devastating due to the heavy and unexpected rainfall throughout the growing season that favor this "water mold" pathogen and disease. A key interest of the processing industry is identifying whether some fungicides can be effective as foliar applications. Some production systems do not use drip irrigation and must rely on foliar fungicide applications. Developing an overall program that alternates among registered fungicides and maximizes their effectiveness is needed to protect the crop through an entire season. In some U.S. growing regions *P. capsici* is resistant to mefenoxam (Ridomil), cyazofamid (Ranman), and fluopicolide (Presidio). Additional complicating factors include the development of fungicide resistance within the pathogen population to commonly used products. In Michigan, there are fields infested by *P. capsici* that has high resistance to the key fungicide Ridomil where in other regions of the state, the fungicide can still be used judiciously. Before registration of the fungicides Revus (mandipropamid) and Presidio (fluopicolide), the arsenal of effective active ingredients for the control of *P. capsici* was limited to just a few products. The risk of resistance to newer fungicides can be lessened with research informing management decisions.

We have made significant progress in understanding fungicide resistance in the cucurbit downy mildew pathogen. By transferring the tools developed for downy mildew to *P. capsici*, we can understand the fungicide sensitivity of Michigan and North Carolina pathogen populations and generate the best science-based recommendations for producers. Better disease management will also positively impact other growing regions from which processors may source products.

Our goal is to reduce the risk and cost that pickling cucumber and pepper growers face each year because of a fungicide-resistant Phytophthora pathogen.

OBJECTIVES

1. Develop and implement state-of-the-art molecular tools to diagnose fungicide-resistance for fungicides with available markers
2. Conduct fungicide trials in two states to develop effective recommendations
3. Disseminate findings to producers in state and national meetings.

RESULTS

1. Develop and implement state-of-the-art molecular tools to diagnose fungicide-resistance for fungicides with available markers

<u>Michigan</u>

From our collection of *P. capsici* cultures (>300) that were obtained from diseased cucurbit fruit growing in commercial fields, select isolates were selected for fungicide sensitivity. For Ridomil (mefenoxam) and Presidio (fluopicolide) sensitivity determination, we used tested a high-throughput fungicide phenotypic assay recently developed for testing pathogen related to *P. capsici*. The distribution of fungicide sensitivity was measured as the effective concentration for 50% inhibition of

mycelial growth (EC_{50}) of each isolate growing in fungicide-amended liquid media (0, 0.1, 1, 2, 5, 10, and 20 µl/ml). In each assay run, a *P. capsici* standard strain SP98 sensitive to mefenoxam and fluopicolide was included as the sensitive control. The calculated EC_{50} value of SP98 was recorded and used as the sensitivity baseline for each assay run. Each assay run included Three replicates of each fungicide concentration were included. Every isolate was classified as: sensitive if the EC_{50} value was < 0.2 µl/ml, intermediately sensitive if the EC_{50} value was > 0.2 µl/ml and < 10 µl/ml, or resistant if the EC_{50} value was > 10 µl/ml. The distribution of the calculated EC_{50} values of mefenoxam and fluopicolide sensitivity follow unimodal curves. Within the 2022 isolates tested (29) for sensitivity to mefenoxam, 3% of were sensitive, 69% were intermediately sensitive, and 28% were resistant. A similar frequency (%) was observed for the sensitivity to fluopicolide; 12% (6/51) were sensitive, 63% (32/51) were intermediately sensitive, and 25% (13/51) were fully resistant to fluopicolide. Among isolates tested (50) for sensitivity to mefenoxam; 4% were sensitive, 78% were intermediately resistant, and 18% were fully resistant. A similar frequency was observed for sensitivity of the *P. capsici* population to fluopicolide with 2% (1/55) sensitive, 80% (44/55) intermediately resistant, and 18% (10/55) fully resistant.

North Carolina

P. capsici can develop resistance to fungicides, thus, we are developing molecular assays to quickly diagnose fungicide resistance. The first step, is to identify fungicide resistance isolates and then, identify a marker in their genome that is specific to that fungicide resistance. To identify fungicide resistant isolates, we have evaluated 72 *P. capsici* isolates from different states in the US including NC, SC, TN, NY, and MI, against the fungicides Subdue Maxx (mefenoxam), Presidio (fluopicolide), and Forum (dimetomorph). Evaluations for Orondis (oxathiapiprolin) and Quadris (azoxystrobin) are ongoing. Fungicides to screen were chosen because they are either actively used for *P. capsici* management and/or because markers related to fungicide resistance have been reported in other oomycetes that we could potentially transfer to *P. capsici*.

Markers related to fungicide resistance have been reported in other oomycetes for dimetomorph, fluopicolide, oxathiapiprolin, and azoxystrobin. If we find them to also be associated with fungicide resistance in *P. capsici*, we will develop rapid diagnostic assays. Our initial evaluation of 72 isolates using agar plates amended with fungicides, revealed no resistance to dimetomorph, 2 resistant isolates to fluopicolide, and 15 resistant isolates to mefenoxam, experiments for oxathiapiprolin and azoxystrobin are still ongoing. Sequencing of reported oomycete markers for the isolates resistant to fluopicolide did not show the presence of the reported mutation, indicating that a new marker may be needed to diagnose fluopicolide resistance to *P. capsici*. We are in the process of reconstructing the gene related to fluopicolide resistance in *P. capsici* to try and identify such a marker. We are taking a similar approach for oxathiapiprolin in case the reported markers are also not informative for *P. capsici*. An update on this progress will be given at the PCIC session during the PPI meeting in Chicago.

2. Conduct fungicide trials in two states to develop effective recommendations

Michigan

Pepper Trial 1: This study was conducted at the Michigan State University Southwest Research and Extension Center near Benton Harbor, MI, on sandy soil (soil pH=6.1, CEC meq/100g=2.5, Oakville fine sand) previously planted to peppers. Preplant fertilizer (nitrogen 100 lb/A, potassium 180 lb/A, sulfur 25 lb/A, and boron 2.0 lb/A) was applied and incorporated on June 4. Raised plant beds (6 in.) covered with black polyethylene plastic and spaced 8 ft apart were formed on June 6 with a single drip tape (0.65 gpm/100 ft) installed under the plastic mulch for plot irrigation. On June 11, 6-week-old 'Red Knight' pepper plants were transplanted 12 in. apart into the raised beds. Fertilizer (nitrogen 28%) was applied weekly at one gal/A/day through drip irrigation. Treatments were arranged in a randomized complete block design with four replicates. A replicate consisted of a single 20-ft row with a 5-ft buffer within the

row to separate treatments. *P. capsici* isolates 23-11-2, 23-12-33, and 23-13-10 (Isolated from cucumbers) and 23-1-8 (isolated from yellow squash) were used to infect cucumbers fruits in ratio of 1:1:1:1. Fruits (15) were incubated under direct light at room temperature for a week until sporulation. Fruits with the sporulating pathogen were washed in tap water to dislodge sporangia to induce the production of zoospores for inoculum. Plants were inoculated with a liquid solution with zoospores on 26 July by drenching 20 ml of the zoospores solution in the root zone using a manual backpack sprayer (Stihl SG 20, Waiblingen, Germany) with a hand-wand without nozzle at 20 psi and calibrated to deliver approximately 20 ml of inoculum per plant. The treatments were drenched using a CO2 backpack sprayer and broadcast with a single XR8006 flat fan nozzle calibrated at 35 psi, and delivered 100 gal/A. Fungicide treatments were applied on 17, and 25 July; 1, 8, 16 and 22 Aug. Number of dead plants were counted on 8,15, 22 and 29 Aug. The number of dead plants were used to calculate the area under the disease progress curve (AUDPC). Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).

Disease pressure was severe with nearly all (93%) of the untreated plants dying. Except for a couple of biocontrol products including AVIV and AC203, all other treatments were significantly effective in liming Phytophthora compared to the untreated control. Among the most effective treatments were those that had Orondis Gold as part of the treatment program. Revus was also among the most effective fungicide treatments. While Theia and YSY products were not as effective as the Orondis-based programs and Revus, they were significantly more effective than the untreated control. When looking at the AUDPC, which illustrates plant death over the duration of the study, the Orondis-based programs and Revus were the most effective. The YSY product was better than the untreated control and also better than the AC203 treatment.

Treatment and rate/A, applied at 7-day intervals[z]	Dead Plants (%)[y]				
	8 Aug	15 Aug	22 Aug	29 Aug	AUDPC[x]
Untreated	40.3 bc	68.1 a	84.7 a	93.1 a	1536.1 a
Presidio 4.0 fl oz *-alt-* Elumin 8.0 fl oz *-alt-* Orondis Gold 55.0 fl oz	0.0 d	0.0 b	1.4 c	1.4 d	14.6 c
Howler 2.5lbs + A+Dyne-Amic 0.375% V/V *-alt-* Orondis Gold 2.5 pt	0.0 d	1.4 b	2.8 c	1.4 d	34.0 c
Revus 8.0 fl oz	2.8 d	2.8 b	1.4 c	2.8 d	48.6 c
YSY 5.68kg/100 gal	31.9 c	48.6 a	54.2 b	56.9 c	1030.6 b
Theia 3lb + A+Dyne-Amic 0.375% V/V	47.2 ac	54.2 a	63.9 ab	68.1 bc	1229.9 ab
AVIV 30.0 fl oz	56.9 ab	63.9 a	69.4 ab	88.9 a	1443.8 ab
AC203 24 fl oz/A in 100 GPA	65.3 a	70.8 a	76.4 ab	83.3 ab	1550.7 a

[z]Treatments applied at same volume per acre, 100 gal/A.
[y]Based on visual count of dead plants in field.
[x]Area under the disease progress curve

Pepper Trial 2: This study was conducted at the Michigan State University Southwest Research and Extension Center near Benton Harbor, MI, on sandy soil (soil pH=6.1, CEC meq/100g=2.5, Oakville fine sand) previously planted to peppers. Preplant fertilizer (nitrogen 100 lb/A, potassium 180 lb/A, sulfur 25 lb/A, and boron 2.0 lb/A) was applied and incorporated on June 4. Raised plant beds (6 in.) covered with black polyethylene plastic and spaced 8 ft apart were formed on June 6 with a single drip tape (0.65 gpm/100 ft) installed under the plastic mulch for plot irrigation. On June 11, 6-week-old 'Red Knight' pepper plants were transplanted 12 in. apart into the raised beds. Fertilizer (nitrogen 28%) was applied

weekly at one gal/A/day through drip irrigation. Treatments were arranged in a randomized complete block design with four replicates. A replicate consisted of a single 20-ft row with a 5-ft buffer within the row to separate treatments. *P. capsici* isolates 23-11-2, 23-12-33, and 23-13-10 (Isolated from cucumbers) and 23-1-8 (isolated from yellow squash) were used to infect cucumbers fruits in ratio of 1:1:1:1. Fruits (15) were incubated under direct light at room temperature for a week. Fruits with the sporulating pathogen were washed in tap water to dislodge sporangia to induce the production of zoospores for inoculation. Plants were inoculated with a liquid solution with zoospores on 15 Aug by spraying the plant foliage with 20 ml of the zoospore's solution using a manual backpack sprayer (Stihl SG 20, Waiblingen, Germany) equipped with a hollow cone nozzle (Stihl 408BA015KN, Waiblingen, Germany), and calibrated to spray 15 ml of inoculum per plant. After inoculation, overhead irrigation was used early each morning and late each evening to create conditions favorable for disease development. Fungicides were applied using a CO_2 backpack sprayer and a broadcast boom equipped with four XR8003 flat-fan nozzles spaced 18 in. apart, calibrated at 35 psi, and delivering 50 gal/A. Fungicide treatments were applied on 8, 16, 22 and 29 Aug. Fruits and leaves with lesions and dead plants were rated on 22 and 29 Aug; and 6 September. Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD). No phytotoxicity or tank-mix incompatibility was observed.

Foliar blighting from Phytophthora was relatively severe in this trial with 57% of the plants having foliar symptoms by the last rating date. The only treatment that was effective in protecting the plants was Presidio. None of the experimental treatments were effective.

Treatment and rate/A, applied at 7-14 day intervals	Fruits (#)			Plants with Symptoms (%)			
	22 Aug	29 Aug	6 Sep	22 Aug	29 Aug	6 Sep	AUDPC[z]
Untreated	1.5 ab[y]	13.3 ab	16.3 ab	2.8 a	20.8 ab	56.9 a	63.9 a
Presidio 4.0 fl oz	0.0 b	4.0 b	4.8 b	0.0 a	4.2 b	11.1 b	12.3 b
Experimental 2 (medium) + Activator 90 8.0 fl oz	2.5 a	12.0 a	18.8 ab	0.0 a	16.7 ab	52.8 a	54.3 a
Experimental 2 (low) + Activator 90 8.0 fl oz	0.8 ab	13.8 ab	21.0 ab	0.0 a	22.2 ab	59.7 a	65.6 a
Experimental 2 (high) + Activator 90 8.0 fl oz	2.5 a	16.3 a	29.5 a	0.0 a	29.2 a	70.8 a	81.4 a

[z]Area under the disease progress curve.
[y]Data was analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).

Pepper Trial 3: This study was conducted at the Michigan State University Southwest Research and Extension Center near Benton Harbor, MI, on sandy soil (soil ph=6.1, CEC meq/100g=2.5, Oakville fine sand) previously planted to peppers. Preplant fertilizer (nitrogen 100 lb/A, potassium 180 lb/A, sulfur 25 lb/A, and boron 2.0 lb/A) was applied and incorporated on June 4. Raised plant beds (6 in.) covered with black polyethylene plastic and spaced 8 ft apart were formed on June 6 with a single drip tape (0.65 gpm/100 ft) installed under the plastic mulch for plot irrigation. On June 11, 6-week-old 'Red Knight' pepper plants were transplanted 12 in. apart into the raised beds. Fertilizer (nitrogen 28%) was applied weekly at one gal/A/day through drip irrigation. Treatments were arranged in a randomized complete block design with four replicates. A replicate consisted of a single 20-ft row with a 5-ft buffer within the row to separate treatments. *P. capsici* isolates 23-11-2, 23-12-33, and 23-13-10 (Isolated from cucumbers) and 23-1-8 (isolated from yellow squash) were used to infect cucumbers fruits in ratio of 1:1:1:1. Fruits (15) were incubated under direct light at room temperature for a week until sporulation.

Fruit were pathogen sporulation were washed in tap water to dislodge sporangia to induce the production of zoospores for inoculation. Plants were inoculated with a liquid solution with zoospores on Aug 15 by spraying the plant foliage with 20 ml of the zoospore's solution using a manual backpack sprayer (Stihl SG 20, Waiblingen, Germany) equipped with a hollow cone nozzle (Stihl 408BA015KN, Waiblingen, Germany), and calibrated to spray 15 ml of inoculum per plant. After inoculation, overhead irrigation was used early each morning and late each evening to create conditions favorable for disease development. Fungicides were applied using a CO_2 backpack sprayer and a broadcast boom equipped with four XR8003 flat-fan nozzles spaced 18 in. apart, calibrated at 35 psi, and delivering 50 gal/A. Fungicide treatments were applied on 8 and 16 Aug. Fruits and leaves with lesions were rated 22 and 29 Aug; and 6 September. Data were analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD). No phytotoxicity or tank-mix incompatibility was observed.

In the untreated control, 68% of the plants developed Phytophthora symptoms including blighting of the foliage. All treatments were significantly effective in limiting foliar lesions. The treatments of Orondis, the fungicide program with Previcur Flex, and Bravo WeatherStik were all significantly effective compared to the untreated control. While Bravo WeatherStik was similar to the other treatments according to the statistics, that treatment resulted in 18% of the plants with foliar blighting.

Treatment and rate/A, applied at 7-day intervals	Fruits (#)			Plants with Symptoms (%)			
	22 Aug	29 Aug	6 Sep	22 Aug	29 Aug	6 Sep	AUDPC[z]
Untreated Control	1.8a[y]	19.3a	16.3a	0.0a	36.1a	68.1a	491.0a
Orondis Ultra 8.0 fl oz + Activator 90 0.125% V/V	0.0a	1.0b	0.5b	2.8a	1.4b	1.4b	24.3b
Experimental 1 13.7 fl oz + Previcur Flex 11.2 fl oz + Activator 90 0.125% V/V	0.3a	1.8b	2.3b	0.0a	2.8b	2.8b	29.2b
BWS[x] 1.5 pt	0.3a	4.3b	7.5b	0.0a	5.6b	18.1b	102.1b

[z]Area under the disease progress curve.
[y]Data was analyzed using an analysis of variance (ANOVA) with means separation performed using Fisher's protected least significant difference (LSD).
[x]BWS = Bravo WeatherStik SC

North Carolina

Unfortunately, the notice of funding for this project was received too late in the field season to establish a pepper trial in NC. This objective will be completed next year.

3. Disseminate findings to producers in state and national meetings

Both PIs maintain lab websites with up to date *Phytophthora* information and publications.
MSU: veggies.msu.edu
NCSU: go.ncsu.edu/veggiepathology

Cumulative outputs over the full grant period

Publications

Extension/Outreach

1. Egel, D.S., Foster, R., Maynard, E., Weller, S., Babadoost, M., Nair, A., Rivard, C., Kennelly, M., Hausbeck, M., Szendrei, Z., Hutchison, B., Orshinsky, A., Eaton, T., Welty, C., Miller, S., eds. 2016-24. Midwest Vegetable Production Guide for Commercial Growers. Michigan State University Extension Bulletin 0312. Updated yearly.

2. Hausbeck, M.K. 2024. Managing *Phytophthora* on cucumber. Updated Jun 2024. Online at https://veggies.msu.edu/extension-publications/#FactSheets.

Presentations

Professional Meetings

1. Cochran-Murray, S., Miles. T, and Quesada-Ocampo L. M. Evaluating a Recombinase Polymerase Amplification (RPA) assay for detection of *Phytophthora capsici* in on-farm water sources. American Phytopathological Society Annual Meeting, Plant Health 2024, Memphis, TN, July 2024. Poster presentation.

Extension/Outreach

1. Hausbeck, M.K. 2024. Grower-Driven Research Leads to Integrated Disease Management. Nebraska Plant Science Symposium, Lincoln, NE, 29 Apr.
2. Hausbeck, M.K. 2024. Phytophthora fruit rot of cucumber: the menace that has not gone away. Cucumber Day presented by Ontario Processing Vegetable Growers. London, ON, Canada, 2 Apr.
3. Hausbeck, M.K. 2024. Grower-Driven Research Leads to Integrated Disease Management. Nebraska Plant Science Symposium, Lincoln, NE, 29 Apr.
4. Quesada-Ocampo L. M. Vegetable Disease Management. NC State Extension Horticulture Program Team Statewide Agent Training. Raleigh, NC, August 2024.

TIMELINE

Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Develop and implement state-of-the-art molecular tools to diagnose fungicide-resistance for fungicides with available markers.	NC	NC	NC	NC	NC
Collect *P. capsici* isolates and test for fungicide resistance. Conduct fungicide trials in two states to develop effective recommendations (MI-Pepper, Cucumber; NC-Pepper)	MI,NC	MI,NC	MI,NC	MI,NC	MI,NC
Disseminate findings to producers in state and national meetings	MI,NC	MI,NC	MI,NC	MI,NC	MI,NC

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PPI Agricultural Research Committee Proposals and Project Reports

Ag Research Fund (ARF): ❑ ARF Pre-Proposal ❑ ARF Annual Report
Field Research (FR): ❑ FR Proposal ☑ FR Progress Report

1. Research Project
   Date First Approved: 2024 Number of Renewals: 3
   Project Term (# of years): 3 (2024-226) Date of Completion: _____
2. Report Period: 2024 (Year 1)
3. Research Agency: University of Guelph
4. Department or Unit: Ontario Crops Research Centre - Simcoe
5. Investigator(s): Rachel Riddle, Rene Van Acker
6. Project Leader(s): Rachel Riddle
7. Project Title: Cucumber Variety Evaluation for the North American Pickling Industry
8. Amount of research funding requested for the coming year: $7,000
9. Amount of research funding received for the past year: $7,000
10. Total research funding given to this project to date: $7,000
11. Percent of project's total yearly funds represented by PPI research grant (est.): 6%
12. Signature of person preparing report: _____ Date: Sept 30/24

Please complete items 13 and 14 on a separate page

13. Progress Toward Objectives
14. Significance of Results

13. Progress Toward Objectives

The objective of this research project was to evaluate new cucumber varieties for enhanced yield, superior performance, fruit quality (fresh and brined), disease tolerance, adaptability and acceptability to local processors. In addition, a further objective was to identify varieties with plant characteristics that include high fruit-to-vine ratio and high yield potential with processing qualities suited to the North American pickle processing industry. The main focus of the trials was on parthenocarpic varieties for evaluation. These cucumber types have become more important to the North American Pickle Industry because of superior fruit quality, higher yield potential and the fact that no bees are required for pollination. The Ontario Industry is also interested in these varieties.

Research trials were conducted to evaluate new processing cucumber varieties. Trials were set up as a randomized complete block design. Plot size for hand pick trials were 20 ft. x 5 ft. and replicated three times and for machine harvest trials were 20 ft. x 2.5 ft. and replicated four times. New varieties were requested from seed companies, worldwide. There were 7 conventional and 65 parthenocarpic varieties, including varieties suitable for hand and machine harvest. The crop was seeded from May 29 to June 26 and grown according to accepted commercial practices. Each variety was thinned to a known plant stand – 18,000 plants/acre for the parth hand pick varieties; and 55,000 and 28,000 plants per acre for the standard and parth machine harvest varieties, respectively. Crops were harvested between July 15 and August 13 and graded according to commercial industry standards. Crop yield ($ and ton/acre) was computed for each variety. Length-diameter (LD) ratios were also measured. Varieties were put in brine at the Simcoe Research Station and will be evaluated for brining characteristics by a panel of research, seed company and industry personnel in early October. Data will be statistically analyzed and summarized in a final report that will be distributed to the research committee and interested members of PPI in mid-October.

14. Significance of Results

Processing cucumbers are an important high-dollar value crop. New superior yielding varieties are essential to ensure the industry's competitiveness. Many new varieties are being released by the seed companies; therefore, variety evaluation is essential in order to recommend the best of these varieties to the industry. In particular, varieties that have local adaptability and market acceptance together with higher yields, improved fresh quality, improved brining quality and better disease resistance/tolerance are needed. Based on these field trials, superior yielding processing cucumber varieties with improved fresh quality and yield were identified for conventional and parthenocarpic varieties for both the handpick and machine-harvest market. A complete report including yield data, LD ratios, fresh internal quality and brine quality data on varieties evaluated in these trials will be available mid-October. This research will enhance competitiveness of the North American Pickle Industry by providing superior varieties that are higher yielding; have improved quality and better disease tolerance/resistance.

Funding was used to support a summer student to assist with variety evaluation including trial maintenance, harvesting and brining.

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Ag Research Fund (ARF): ❑ ARF Pre-Proposal ❑ ARF Annual Report
Field Research (FR): ☑ FR Proposal ❑ FR Progress Report

1. Research Project
   Date First Approved: 2024 - Asking for a deferral to 2025 Number of Renewals: _____
   Project Term (# of years): 3 (2025-2027) Date of Completion: _____
2. Report Period: NA
3. Research Agency: University of Guelph
4. Department or Unit: Ontario Crops Research Centre - Simcoe
5. Investigator(s): Rachel Riddle, Lisa Weber, Rene Van Acker
6. Project Leader(s): Rachel Riddle
7. Project Title: Processing Pepper Cultivar Evaluation
8. Amount of research funding requested for the coming year: $7,000
9. Amount of research funding received for the past year: $0
10. Total research funding given to this project to date: $0
11. Percent of project's total yearly funds represented by PPI research grant (est.): 30%
12. Signature of person preparing report: _____ Date: _____

Please complete items 13 and 14 on a separate page

13. Progress Toward Objectives
14. Significance of Results

Objectives

The objective of this research proposal is to evaluate existing and new pepper hybrids for yield, fruit quality, disease susceptibility, adaptability and acceptability for North American processors. The main focus will be on banana and jalapeno type cultivars.

This research will provide the North American processing industry with cultivars that have:

- Increased yields
- Improved fruit characteristics important for the processing industry including:
    - Fruit wall thickness
    - Fruit length/width
    - Ease of stem and calyx removal during picking
- Improved disease tolerance/resistance
    - Anthracnose (*Colletotrichum scovillei*) – new species reported in the U.S. and Ontario

Significance of Results

Jalapeno and banana peppers are an important crop for vegetable growers who are growing for processing purposes. There is a continued need for processing pepper cultivar research in North America. Improved fruit quality such as thicker walls and longer fruit for jalapenos used for slicing are traits that need to be evaluated. Also important is evaluating cultivars for improved disease resistance to *Colletotrichum scovill*e, a new species of anthracnose that has become a very concerning problem for growers across the U.S. and in Ontario. The continued evaluation of processing peppers that have local adaptability and market acceptance together with higher yields, improved fresh quality, and better disease resistance/tolerance are needed.

Research trials are proposed to evaluate banana and jalapeno pepper cultivars. Plants will be started by seed in the greenhouse late April and transplanted in the field early June. Trials will be set up as a randomized complete block design with four replications. Transplants will be planted on double rows, 23" inches between double rows, with 5' centers and 18" between plants. New varieties will be requested from seed companies globally and compared to standard commercial cultivars. The crop will be grown according to accepted commercial practices. Data will be collected on disease severity from natural infection, with the focus on anthracnose. Fruit will be harvested at crop maturity and graded according to commercial industry standards. Crop yield, will be computed for each variety. Fruit wall thickness and fruit length/width will be measured. Counts of fruit with and without stems will be recorded. Data will be statistically analyzed and reported.

Funding will be used to support a research assistant to assist with variety evaluation including trial set-up, planting, maintenance, harvesting, grading, and fruit assessments. The funding will supplement funding provided by the Vegetable Seed Industry. The request is for more than the $5,000 outline in proposal instructions due to significant increases in minimum wage. The request for funding for this project was approved in 2024, but the research was not completed. We are asking for a deferral of the funds and that the project be funded for the 2025 season instead.

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PPI Agricultural Research Committee Proposals and Project Reports

Ag Research Fund (ARF): ❑ ARF Pre-Proposal ❑ ARF Annual Report
Field Research (FR): ☒ FR Proposal ❑ FR Progress Report

1. Research Project
   Date First Approved: _____ Number of Renewals: 0
   Project Term (# of years): 3 Date of Completion: New project
2. Report Period: New project
3. Research Agency: North Carolina State University
4. Department or Unit: Entomology and Plant Pathology and NC Plant Sciences Initiative
5. Investigator(s): Lina Quesada-Ocampo
6. Project Leader(s): Lina Quesada-Ocampo
7. Project Title: Monitoring downy mildew risk in cucurbit crops affected by Pseudoperonospora cubensis
8. Amount of research funding requested for the coming year: $5,000
9. Amount of research funding received for the past year: $45,000 from other PPI grants
10. Total research funding given to this project to date: $0 for this new project
11. Percent of project's total yearly funds represented by PPI research grant (est.): 4.5%
12. Signature of person preparing report: Lina Quesada-Ocampo Date: 09/30/2024

Please complete items 13 and 14 on a separate page

13. Progress Toward Objectives
14. Significance of Results

Agricultural Research Fund – Field Research Pre-proposal

Monitoring downy mildew risk in cucurbit crops affected by *Pseudoperonospora cubensis*

Lina Quesada-Ocampo, WNR Distinguished Professor & Extension Specialist
North Carolina State University

PROBLEM:

Downy mildew (DM, *Pseudoperonospora cubensis*) has become a devastating disease in cucurbit crops every year since its re-emergence in 2004, when it overcame host resistance in cucumber. In response to the re-emergence of *P. cubensis*, the Cucurbit Downy Mildew IPMpipe was created to provide an alert system to growers and warn them when outbreaks were identified near their farms. Unfortunately, funding for the CDM IPMpipe first through USDA support and later through competitive grants, ended fully two years ago. The website for the alter system was moved to the Southern Region IPM center and will continue to be supported. However, the sentinel plots and the forecasting system are no longer supported. This has resulted in several states becoming disengaged in reporting CDM outbreaks, which puts NC cucurbit crops at risk.

There is a clear and urgent need for local pathogen monitoring to ensure timely disease management, while minimizing the number of fungicide applications needed to obtain a high-quality crop. Fungicide resistance monitoring has also become an urgent need since the pathogen can quickly become insensitive to fungicides. Through previous PPI funding, we have developed molecular assays that can be used to analyze select samples in sentinel plots to: 1) provide a pathogen alert system, and 2) determine the fungicide sensitivity of the isolates causing disease for fungicides with developed assays. In this project, we propose to continue our effort of deploying sentinel plots in NC for pathogen monitoring, but adding additional information provided by clade and fungicide resistance markers developed in previous PPI projects. Due to costs, we will not be able to analyze every sample found in the sentinel plots, but we will be able to provide detailed information of initial outbreaks per host per NC region to give growers as much information as we can so they can plan their fungicide programs.

SPECIFIC METHODS AND PROCEDURES:

Year 1-3: NC will establish three small field trials in three regions representing the climates and soils found in NC (Eastern, Western, and Piedmont). These fields will contain commercial cucurbit hosts. All hosts will be scouted weekly for the presence of any downy mildew pathogens. Samples will be taken from hosts infected for the first time in a season with downy mildew to genetically identify the pathogen and obtain information about clade and fungicide sensitivity to fungicides including oxathiapiprolin (Orondis), carboxylic acid amides, and quinone outside inhibitors.

OUTCOMES:

- We will monitor for downy mildew every year for three years to warn growers when the pathogen is in NC.
- We will characterize the downy mildew genetically to identify clade and sensitivity to select fungicides, including Orondis.
- This research will support grower downy mildew management efforts and minimize the risk of infection and development of fungicide resistance.

FUNDING REQUEST: $5,000 per year for 3 years

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PPI Agricultural Research Committee Proposals and Project Reports
Ag Research Fund (ARF): ❑ ARF Pre-Proposal ☒ ARF Annual Report
Field Research (FR): ☒ FR Proposal ❑ FR Progress Report

Research Project
Date First Approved: ____March 2011 ____ Number of Renewals: __11_____
Project Term (# of years): ___11_____ Date of Completion: __ Sep 2023_____

Report Period: _____Sept 2022- Sept 2024_____

Research Agency: _____Texas A&M AgriLife Research_____

Department or Unit: _____Horticultural Sciences, VFIC_____

Investigator(s): _____Kevin Crosby _____

Project Leader(s): _____Kevin Crosby _____

Project Title: _____ Improving quality and disease resistance in pickling peppers_____

Amount of research funding received for the past year: _____$4000_____

Total research funding given to this project to date: ____$44,000_____

Percent of project's total yearly funds represented by PPI research grant (est.): ___3%_____

Signature of person preparing report: ____  Date: __Sep 26, 2024

Progress Towards objectives

During the 2022-2024 project period, we completed five field cycles of selection, at Weslaco, Uvalde and College Station. We continued several GH experiments to improve both disease resistance and quality of pickling pepper breeding lines. A total of 128 jalapeno and hot wax breeding lines and hybrids were planted in the spring 2023 and 2024 field plots at Weslaco, Uvalde and College Station to select for fruit quality, earliness, bacterial leaf spot and virus resistance. These included F1 hybrids and some inbreds- F5-F8 generations. There was moderate TSWV pressure but heat stress was the most serious problem. Twelve jalapeño F1 hybrids and two hot wax F1 hybrids were heat tolerant based on high fruit set, early maturity, and yield compared to checks, 'Rio de Oro,' and 'Mixteco.' Three jalapeño breeding lines with suitable fruit size (2-3") for whole pickles, and good disease resistance (BLS and PVY) were identified and can serve as parents to create F1 hybrids. Fruit quality attributes measured were firmness, size, wall thickness, seed and placenta content, color and pungency. Capsaicin content was measured at the Vegetable and Fruit Improvement Center at Texas A&M. The jalapenos ranged from 0 to 80,000 Scoville units based on capsaicin content on a dry weight basis.
During the 2022-24 winter and early spring we conducted controlled crosses between elite jalapeño, banana, and hot wax breeding lines in the greenhouse. This allowed us to create F1 hybrid, backcross and F2 generation seed for each of these pickling pepper types. In addition, controlled inoculation experiments were conducted to select for resistance genes against TSWV, powdery mildew (PM), and *Phytophthora capsici*. A total of 75 breeding lines with jalapeño, banana, and hot wax type fruits were screened with these diseases. Several backcross families were carried forward to introgress PM and TSWV resistance into elite banana and jalapeño (both highly susceptible) inbred lines. We utilized the resistance genes from our *C. annuum* x *C. baccatum* hybrids. Thrips and TSWV pressure in the greenhouse was also severe, allowing for selection of lines carrying the *Tsw* resistance gene. Each resistant plant was selfed or backcrossed to an elite inbred line with high quality fruit traits. Seeds of 80 new, experimental hybrids were generated for field testing in the Spring and Fall of 2024.

During the Spring/Summer of 2023-24 we also planted field trials at Pearce, AZ to screen jalapeños for processing quality attributes. Three selections were made, 2 had high yield and high capsaicin levels of 11,000 ppm and 13,000 ppm, and one had no capsaicin but very high yields. All had very healthy plants with no virus symptoms. Two were for whole pickle and one was long and slender for making nacho rings.

Table 1. Jalapeño fruit quality attributes from Uvalde, TX field trial, June 2023.

Line	Class	Fruit size[z] (g)	Fruit length (cm)	Fruit Color	Capsaicin (ppm)[x]	Disease Resistance[y]
9	F1	24	10.2	Dark green	1,200	BLS1-3, PVY
25	F1	25	10.0	Dark green	1,280	BLS1-3, TSWV
35	F1	28	9.2	Dark green	1,370	BLS1-3
36	F1	22	8.8	Dark green	1,440	BLS1-3
51	F1	20	9.8	Med green	1,200	TMV
57	F1	20	5.5	Dark green	1,300	BLS1-3, PVY, TSWV

[z]Weight and length based on average of 10 fruits
[y]Resistance legend: BLS= bacterial leaf spot, PVY= potato virus Y,
TEV= tobacco etch virus, TMV= tobacco mosaic virus, TSWV= tomato spotted wilt virus
[x]Dry weight basis

Fig 1. Fruit of some TAMU hybrid jalapenos- 25 TSWVR, and 57 BLSR for nacho rings.

F1 Hybrid 25

F1 Hybrid 57

Significance of Results

Resistance to viruses and powdery mildew in TAMU pepper lines should be very useful in banana pepper, as commercial cultivars are highly susceptible. The low pungency jalapeños in the breeding program should be useful for the market segment that does not favor hot peppers. Extremely firm fruit in some TAMU jalapeño lines is desirable for post-harvest handling and integrity of the flesh after pickling. The field performance of some of our elite inbred lines and test hybrids at multiple locations demonstrates the commercial potential of TAMU germplasm (Table 1). Elite selections of both open-pollinated and F1 hybrid jalapeños, sweet banana and hot wax peppers are available for commercial testing with seed companies and growers through a materials transfer agreement with Texas A&M University Office of Innovation. We will continue to increase seeds of elite parental lines in our cages or isolation plots. The effort to integrate resistance to both leafminer and aphids in some of our jalapeño and wax breeding lines is somewhat unique. We will continue to select for these resistance traits in jalapeño and banana backgrounds after several backcross generations. During the report period, we achieved 4 more generations of selection to introgress multiple resistance traits into elite jalapeño and banana lines. Advanced selections are currently being planted in our Fall/Winter GH for disease and quality screening as well as to increase seeds.

Proposed Objectives for 2025-26

1. Advance powdery mildew and TSWV resistance in elite quality jalapeño and banana lines with virus and BLS resistance genes. Field and controlled inoculation screening.
2. Conduct 2 cycles of selection/breeding in field plots and controlled pollinations in the GH. This will include controlled inoculations, fruit quality analysis and yield/maturity assessment. In addition, test hybrids will be generated to assess heterosis for important quality traits and yield.
3. Continue selection for fruit quality, yield and resistance to insects (aphids and leafminer) in south Texas. Test our interspecific *C. annuum x C. chinense* families for pepper weevil resistance or tolerance.
4. Plant trials of advanced hybrids in multiple locations.

We will conduct field and greenhouse selection for resistance to powdery mildew on several interspecific pepper families, and backcross resistant plants to elite jalapeño and sweet banana inbred lines. Selection for bacterial leaf spot resistance in lines carrying both dominant and recessive resistance genes will be conducted in the field at College Station where disease pressure is high. Selection for powdery mildew resistance will be conducted at Uvalde, where this disease occurs in the field each year. Resistant plant selections will be self-pollinated to maintain uniformity. Field selections will be conducted in both the spring and fall seasons. Test hybrids will be generated through controlled pollinations in a greenhouse to produce seed for commercial trials. All seed will be cleaned and treated with TSP to prevent contamination by bacteria or tobamoviruses.

Fruit quality traits to be measured will include color, size, shape, weight, mesocarp thickness, pungency, and firmness. Additional traits to be measured will include yield per plant, maturity date, plant height and heat tolerance based on fruit set.

Resistance to aphids, leaf miners and mites will be evaluated based on number of affected leaves per plant in the field plots if these pests are present. This screening will take place at Weslaco and College Station where pest pressure is high. Interspecific resistance we have introgressed into our jalapeño lines will be transferred into our banana lines.

Budget- $4000

Labor (technician and student worker salaries+ benefits)- $2500
Supplies (fertilizer, potting mix)- $500
Travel- to evaluate field trial at Weslaco and Uvalde- $1000