Research Project Report – Final Summary

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Title: Genetic Detection of Downy Mildew and Epidemiology and Mitigation Strategies for Impatiens Downy Mildew
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Abstract

Over a 5-year timeframe starting in 2013, the team of researchers listed above investigated several downy mildews – Impatiens and Cucurbit, primarily, for biology, epidemiology, genomics, diagnostics, and mitigation. USDA-APHIS provided funding for this research via a series of cooperative agreements with Michigan State University and Rutgers University (the IR-4 Project). Throughout this project, this team made several key discoveries. First, Impatiens Downy Mildew (IDM) overwinters as oospores – thick walled resting spores - in stems of common garden impatiens (*I. walleriana*) and in seeds of balsam impatiens (*I. balsamina*). Second, alternative hosts are available and can potentially serve as reservoirs for inoculum for both IDM and cucurbit downy mildew (CDM). Third, collecting diverse populations of downy mildews (IDM, CDM and downy mildews of hops, basil) have yielded richer genomic resources that in turn has fostered improved understanding of population dynamics and has provided the basis for new genomic-based diagnostic tools (spore trapping + PCR for CDM, and FISH assay for IDM). Genomics has also identified several new downy mildew species affecting cucurbit hosts. Fourth, screening for mitigation options highlighted the critical need to develop and maintain robust rotational programs to minimize resistance development and spread. Pockets of transient resistance were noted for mefenoxam and fluopicolide in *Plasmopara obuscens*, the IDM pathogen.

In addition to being highly prolific researchers, this team has been highly prolific in outreach. This group has prepared and published or presented 25 scientific abstracts/posters, 51 oral scientific presentations, 27 scientific manuscripts, 24 Plant Disease management Reports, 163 oral extension presentations, 14 trade journal articles, 26 online & print extensions bulletins, plus field days, news releases, and popular press interviews. Plus, this team hosted a final summary to APHIS personnel to present outcomes and future research pathways.

Table 1. Project Objectives and Milestones

Area	Category	Objective	Objective#	Revised Header	Revised Objective Code
1. Biology and Epidemiology of IDM and CDM	a. Method Development	Develop method for maintaining different strains of *P. obducens* on impatiens seedlings	FY13-Obj3: Epi-ARSFD-2	Method development for maintaining *P. obducens* strains	Bio-ARSFD-1
		n/a	n/a	Method development for maintaining *P. obducens* strains	Bio-CU-1
		Develop methodology to assess oospore viability and ability to infect *I. walleriana*	FY14-Obj2: EPI-ARSFD-2, EPI-HC-2	Methodology to assess oospore viability and infection ability	Bio-ARSFD-2, Bio-HC-2
		n/a	n/a	Method Development for *P. obduscens* baiting	Bio-ARSFD-3, Bio-HC-3
	b. Collection Maintenance	Establish and maintain collection of *P. obducens* strains from diverse geographies	FY13-Obj3: Epi-ARSFD-3	Establishment of *P. obducens* collection	Bio-ARSFD-4
	c. Homothally	Determine whether *P. obducens* is self-fertile or requires mating types for oospore formation	FY13-Obj3: Epi-ARSFD-4	Mating type investigations	Bio-ARSFD-5
		Examining whether mating is important for development of viable oospores	FY14-Obj4: EPI-ARSFD-4, EPI-HC-4	Importance of mating for oospore development	Bio-ARSFD-6, Bio-HC-6
	d. Fungal Development	Determine the effect of temperature on infection by sporangia	FY13-Obj2: Epi-MSU-1	Sporangia: Effect of temperature on infection	Bio-MSU-1
		Determine the effect of temperature on infection by zoospores	FY13-Obj2: Epi-ARSFD-1	n/c	n/c
		Determine optimal temperature and humidity for sporangiospore development	FY14-Obj6: EPI-MSU-1	Sporangia: Optimal temperature and humidity for formation	Bio-MSU-2
		Track infections (from leaf infection to stem)	FY15-Obj3: Epi-CU-3	Oospores: Time course of formation in Impatiens sp.	Bio-CU-2
		Determine time course of oospore formation in *I. balsamina* and *I. capensis*	FY15-Obj: Epi-CU-2		
		P. obducens oospore formation in several Impatiens sp.	FY16-Obj3: Bio-CU-2		
		Determine whether oospore formation is triggered by day length	FY13-Obj3: Epi-ARSFD-5	n/c	n/c

Area	Category	Objective	Objective#	Revised Header	Revised Objective Code
1. Biology and Epidemiology of IDM and CDM, *continued*	d. Fungal Development, *continued*	Length of *P. obducens* oospore survival	FY16-Obj1 Bio-CU-1, Bio-ARSFD-1, Bio-HC-1	Oospores: Length of survival	Bio-ARSFD-7, Bio-HC-7
		Temperature requirements for *P. obducens* oospore germination	FY16-Obj2: Bio-ARSFD-2, Bio-HC-2	Oospores: Temperature requirements for germination	Bio-ARSFD-8, Bio-HC-8
		n/a	n/a	Sporangia and oospore inoculation of roots and systemic infection	Bio-ARSFD-9, Bio-HC-9
	e. Overwintering	n/a	n/a	IDM Survival in Monitor Plots with Natural Infections or Inoculation	Bio-ARSFD-10, Bio-HC-10, Bio-ARSFD-11, Bio-CU-3
		Determine whether oospores overwinter	FY13-Obj4: Epi-CU-1 FY14-Obj3: EPI-CU-2; EPI-ARSFD-3, EPI-HC-3 FY15-Obj1: Epi-CU-1, Epi-ARSFD-1, Epi-HC-1	Determine whether oospores survive over winter	Bio-CU-4
		Determine whether IDM overwinters in seed of *I. balsamina* and *I. capensis*	FY15-Obj4: Epi-CU-4, Epi-ARSFD-2, Epi-HC-2	Seed infection in naturally infected plants of I. balsamina	Bio-CU-5, Bio-ARSFD-12, Bio-HC-12
		Simulate winter conditions and examine whether oospores are viable	FY14-Obj5; EPI-ARSFD-5, EPI-HC-5	See *Oospores: temperature requirements for germination*	See *Bio-ARSFD-8, Bio-HC-8*
	f. Alternate Hosts	Determine role of alternate hosts and overwintering inoculum in IDM	FY14-Obj1 EPI-CU-1; EPI-ARSFD-1, EPI-HC-1	Role of alternate hosts for IDM	Bio-CU-6
		n/a	n/a	*Impatiens walleriana* cultivar screening	Bio-CU-7, Bio-MSU-3
		Determine susceptibility of alternate hosts to CDM	FY14-Obj7: EPI-NCSU-1	Role of alternate hosts in CDM outbreaks by using monitor plots	Bio-NCSU-1, Bio-MSU-4
		Determine role of alternate hosts in CDM outbreaks by using monitor plots	FY15-Obj5: Epi-NCSU-1, Epi-MSU-1		
		Determine role of alternate host in CDM	FY16-Obj4: Bio-NCSU-1, Bio-MSU-1		

Area	Category	Objective	Objective#	Revised Header	Revised Objective Code
2. Genomic Resources & Diagnostics	a. Sampling	Sampling of CDM and IDM	FY13-Obj5: Gen-CU-1, Gen-MSU-1, Gen-NSCU-1, Gen-UF-1	Collect and process CDM and IDM	Gen-CU-1, Gen-MSU-1, Gen-NCSU-1, Gen-UF-1
	b. Genetic Resources	Genomic resources for CDM	FY13-Obj6: Gen-NCSU-2	Genomic resources for CDM	Gen-NCSU-2
		Genomic resources for IDM.	FY13-Obj7: Gen-ARSB-1	Genomic resources for IDM	Gen-ARSB-1
		Permanent *P. obducens* genetic resources and pathogen population assessments using simple sequence repeat (SSR) markers	FY14-Obj14: Gen-ARSB-2 Gen-Rut-2		
		Development of genomic resources for diagnostics of basil and impatiens downy mildews	FY14-Obj12: Diag-NCSU-2	Genomic resources for *P. belbahri* and *P. obducens*	Gen-NCSU-3
		Pending *P. obducens* mating type analysis, identify mating type determinants	FY14-Obj13: Gen-ARSB-1 Gen-Rut-1	Identify mating type determinants	Gen-ARSB-2, Gen-Rut-2
	c. Primer/Probe Development	Test candidate genes for diagnostics of CDM	FY14-Obj11: Diag-NCSU-1	Test candidate genes for CDM diagnostics	Gen-NCSU-4, Gen-MSU-2
	d. Detection	Develop field-friendly molecular diagnostics of *P. cubensis* for early warning systems in conjunction with spore trapping	FY15-Obj9: Gen-NSCU-1, Gen-MSU-1	Field-friendly molecular diagnostics of P. cubensis for early warning systems	Gen-NSCU-5, Gen-MSU-3
		Early detection tools using spore trapping and realtime PCR	FY16-Obj9: Gen-NSCU-1, Gen-MSU-1		
		Development of Illumina metagenomic sequencing and fluorescence in situ hybridization-catalyzed reporter deposition (CARD-FISH) assays to visualize and genotype *P. obducens* in situ from environmental	FY14-Obj10: Diag-ARSB-1, Diag-Rut-1	Illumina sequencing and FISH assays for IDM	Gen-ARSB-3, Gen-Rut-3
		Monitor *P. obducens* populations; persistence of oospores in field soil using genomic diagnostic tools	FY16-Obj10: Gen-ARSB-1, Gen-Rut-1	Monitor P. obducens populations	Gen-ARSB-4, Gen-Rut-4
	e. Population Characterization	Herbarium and modern sample assessment to determine whether IDM and CDM outbreaks are caused by endemic or introduced pathogen genotypes	FY13-Obj6: Gen-NCSU-2 FY13-Obj8: Gen-ARSB-2	Characterize IDM populations	Gen-ARSB-5, Gen-Rut-5
				Characterize CDM populations	Gen-MSU-4

Area	Category	Objective	Objective#	Revised Header	Revised Objective Code
2. Genomic Resources & Diagnostics, *continued*	e. Population Characterization, *continued*	Determine IDM pathogen abundance and developmental changes throughout the growing season and dormancy using metagenomic and fluorescence in situ hybridization assays	FY15-Obj10: Gen-ARSB-1, Gen-Rut-1	See: *Monitor P. obducens populations*	See: *Gen-ARSB-4, Gen-Rut-4*
3. Mitigation Strategies	a. Management	Efficacy of biological and chemical mitigation strategies for IDM	FY13-Obj1: Mit-MSU-1, Mit-Rut-1, Mit-UF-1	Efficacy of biological and chemical mitigation strategies for IDM	Mit-FL-1, Mit-FL-2, Mit-MSU-1, Mit-Rut-1, Mit-Rut-2, Mit-CU-1
		Screen new fungicides and test fungicide rotation programs for IDM	FY14-Obj8: Mit-Rut-1		
		To cope with resistant *P. obducens* populations, screen new fungicides and test fungicide rotation programs	FY15-Obj7: Mit-MSU-2, Mit-UF-2, Mit-CU-1, Mit-Rut-1 FY16-Obj7: : Mit-MSU-3, Mit-UF-2, Mit-CU-1, Mit-Rut-1		
	b. Management Longevity	Continue IDM landscape studies to monitor performance of mitigation tools and environmental impacts for disease development	FY15-Obj8: Mit-UF-1, Mit-MSU-1 FY16-Obj8: Mit-UF-3, Mit-MSU-4	Longevity of tools applied during production after transplanting to the landscape	Mit-UF-3, Mit-MSU-2
	c. Fungal Impact	Fungicide impact on sporangia formation	FY13-Obj1: Mit-MSU-2	n/c	n/c
		Assess impact of fungicides on *P. obducens* oospore formation	FY13-Obj1: Mit-CU-1, Mit-ARSFD-1 FY14-Obj9: Mit-CU-1	Fungicide impact on oospore formation in leaves	Mit-CU-2
	d. Plant Impact	Determine impact of mitigation strategies on plant growth and development	FY15-Obj6: Mit-MSU-1, Mit-UF-1 FY16-Obj6: Mit-MSU-2, Mit-UF-1	Plant impact	Mit-MSU-3
	e. Monitoring Resistance	n/a	n/a	Monitoring P. obducens resistance	Mit-FL-4, Mit-MSU-4
		Monitor fungicide resistance of CDM	FY16-Obj5: Mit-MSU-1, Mit-NCSU-1	Monitoring P. cubensis resistance	Mit-NCSU-1, Mit-MSU-5
Outreach		Disseminate findings to stakeholders	All Years	Outreach	All
		Present findings at trade shows, grower and landscape manager education seminars and scientific meetings			

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Objective 1a. Biology and Epidemiology of IDM and CDM: Method Development

Method development for maintaining P. obducens strains (Bio-ARSFD-1)

In, 2013, Shishkoff acquired two Maryland isolates of the impatiens downy mildew and established methodologies to maintain single-spored isolates in culture via inoculation of stems with sporangiophores and maintaining them prior to transferring to new stems.

Method development for maintaining P. obducens strains (Bio-CU-1)

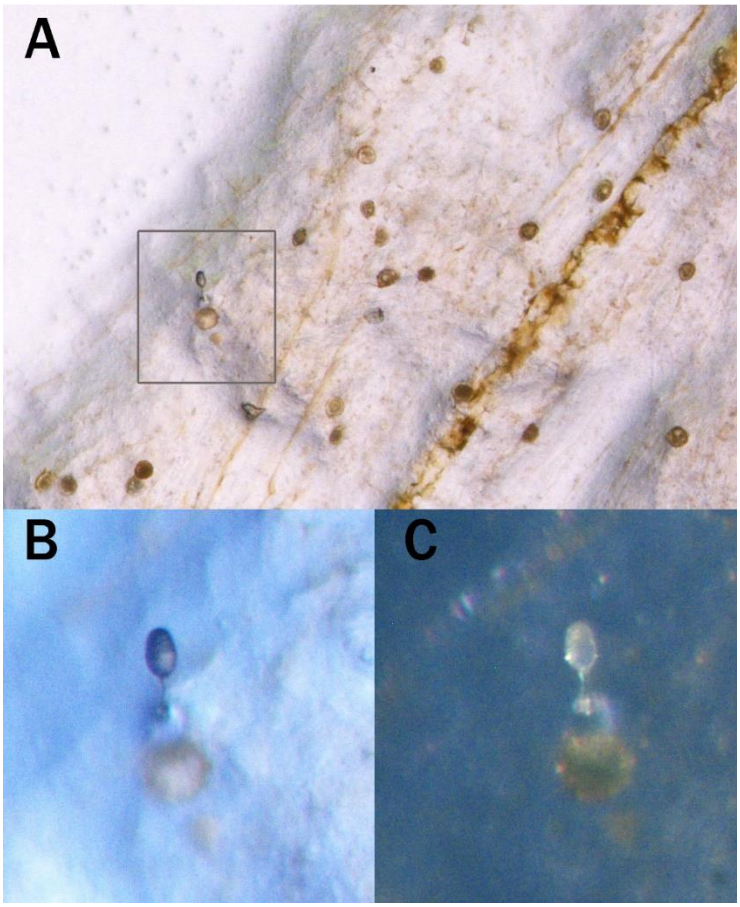
Daughtrey developed axenic cultures of *Impatiens walleriana* on quarter-strength Murashige-Skoog medium and in non-sterile peatlite growing medium in Magenta jars: plant material has successfully been maintained in the laboratory under fluorescent lights or grow-lights for 3 months. Excessive leaf curling was noted in the cultured plants, which was more severe under the fluorescent lights.

Methodology to assess oospore viability and infection ability (Bio-ARSFD-2, Bio-HC-2)

Methods were developed to produce and collect oospores of IDM (using sand to macerate tissue and nested sieves to collect oospores). Oospores were treated using various stimulants (cold temperature, electrolyzed acidified water (AEW), bleach, plant exudates, wetting and drying) to try to elicit germination with little success. Additional research examined whether utilizing snail digestive tracts would stimulate germination.

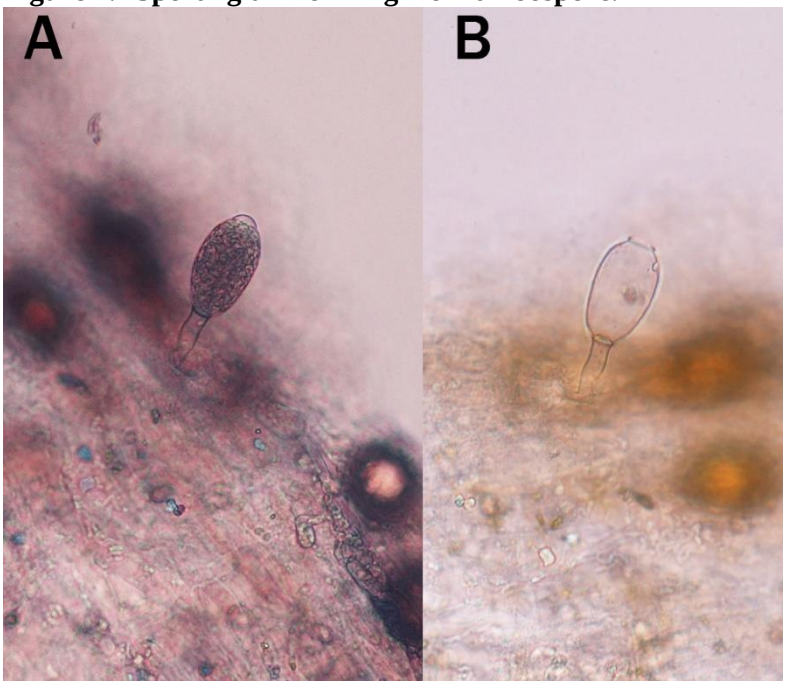
However, a breakthrough occurred summer of 2015. Stem segments of *I. balsamina* were inoculated with various isolates of IDM, incubated at 20 C in a growth chamber with a 14-hr photoperiod for one month (for oospore maturation) and then treated variously: incubated at 20C for an additional month or incubated at 0C for a month, treated with acidic electrolyzed water (AEW, known to stimulate germination of thick-walled smut teliospores), enzymatically treated with cellulase (to change the permeability of the oospore wall), fed to ramshorn snails (to partially digest the cell wall), or simply floated in water, plated on water agar or plated on Synthetic Nutrient Deficient (SNA) medium. It became clear that oospores would germinate after a month's incubation at 0 C no matter what other treatments they were given (Figure 1, Figure 2, Figure 3).

Figure 1. Oospores in a tissue fragment after one month at 0C.



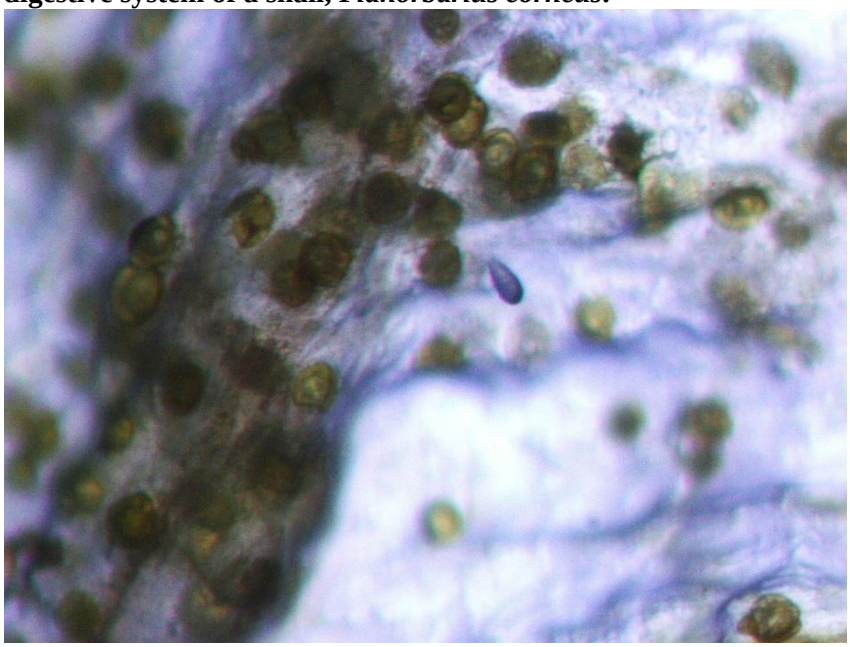
A) Oospore germinating to produce a sporangium. B) Close-up with bright field illumination. C) Close-up with dark-field illumination. The golden oospore is visible immersed in plant tissue, with the sporangium growing up out of the tissue. The sporangia forming from oospores are larger than those forming vegetatively and the sporangiophore typically gives rise to a single sporangium, unlike the vegetative sporangiophore, which is highly branched and bears many small sporangia. (photographs by Dr. Farivar Eskandari)

Figure 2. Sporangium forming from an oospore.



A) A sporangium filled with cytoplasmic contents. B) The same sporangium the following day, empty, zoospores presumably having been released. (photographs by Dr. Farivar Eskandari.)

Figure 3. Oospore germinating after having incubated for a month at 0 C and then passing through the digestive system of a snail, *Planorbarius corneus*.



Photograph by Dr. Farivar Eskandari

Oospores of a number of *Plasmopara obducens* isolates have been made to germinate, and measurements have been made of the resulting megasporangia. Stem segments of *Impatiens balsamina* were surface-sterilized for 60 sec in 10% bleach (0.01% sodium hypochlorite) and placed on moist filter paper in a 9 cm diameter Petri dish, 4-5 segments per plate. For each of the 16 isolates a suspension of sporangia in water was poured over the stems until they were 1/4-1/2 immersed. The plates were left overnight at 20 C. The following day the inoculum suspension was decanted and plates stacked in a one gallon ziplock bag. The stems were allowed to incubate in a growth chamber with an 18-hr photoperiod for approximately one month to allow oospores to develop and mature. The bag of Petri plates was then placed in an incubator set at 0 C for at least one month. After plates of stem tissue were removed from the incubator, the plates were placed under a dissecting microscope and stems dissected to reveal areas dense with oospores. Strips of oospore-laden tissue were moved to a Petri dish half-filled with water. After 3-6 days at room temperature, the submerged stem tissue was again examined and tissue with germinating oospores was placed on a microscope slide and examined at 40x. The dimensions of the oogonium, oospore, and antheridium were recorded, and for germinated oospores, the length and width of the megasporangium.

There was some variation from isolate to isolate, but overall, for 229 oogonia, oogonial length was 43.3 $\pm$ 5.43 um (from base to top). Antheridia tended (when origin could be determined) to arise just below the oogonium. Oospores were subglobose with a diameter of 28.2 $\pm$ 2.88 (n = 229). The wall of mature but not germinating oospores was thick, two-layered and refractive, 2.65 $\pm$ 0.66 (n=70). In samples with many germinating oospores, the contents of many oospores appeared opaque and the oospore wall thinner (Figure 4a). Germination commenced with a cytoplasm-dense hypha emerging from the oogonium (Figure 4b); an apical swelling (Figure 4c) became obovate and was walled off with a septum (Figure 4d). Inside this sessile sporangium, zoospores formed and were released from an apical pore. These megasporangia were 38.01 $\pm$ 7.48 in length and 20.65 $\pm$ 2.99 in width (n=303). Vegetative sporangia were formed on branched sporangiophores. The vegetative sporangia were smaller than megasporangia: 14.7 $\pm$ 1.64 in length and 11.7 $\pm$ 1.08 in width and easily detached from the sporangiophore.

Figure 4. Germination progression of *P. obducens* oospores

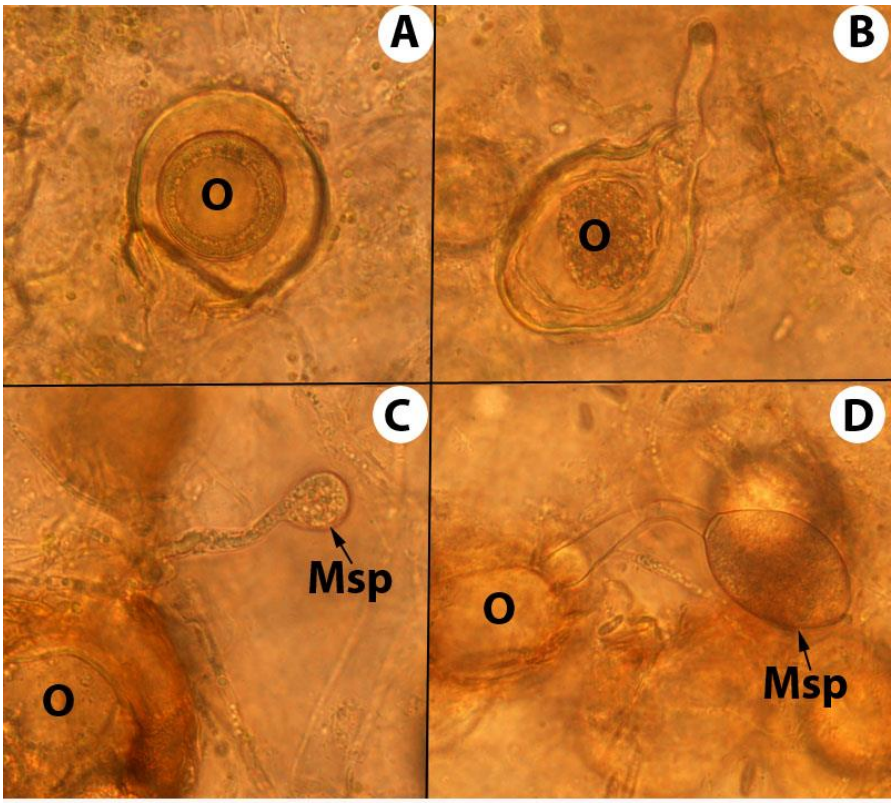


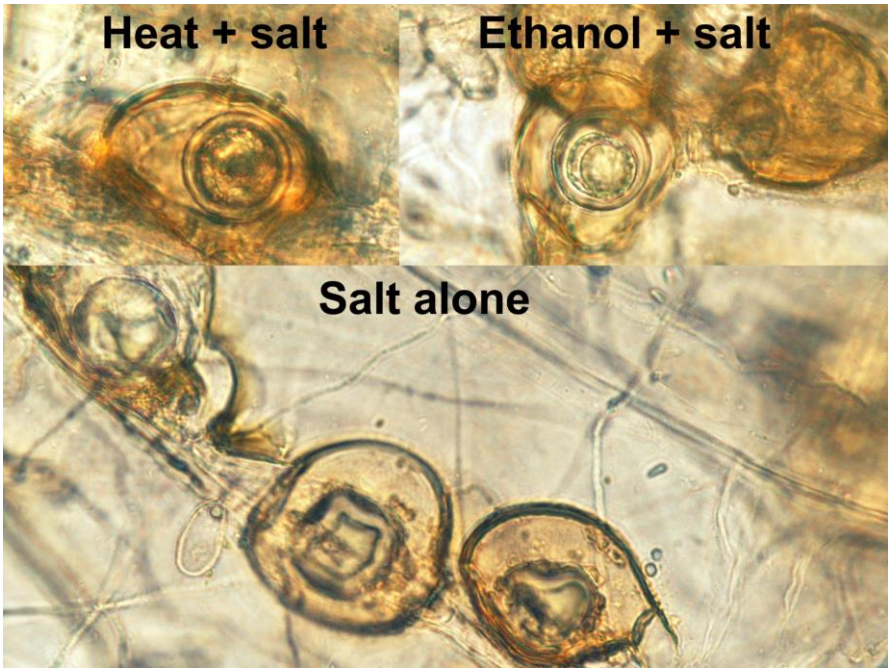
Fig 1. Germination of oospore of Plasmopara obducens. A) Oospore (O). B) Germinating oospore. C)Apical swelling that will become a megasporangium (Msp). D) Mature megasporangium.

Koch's postulates were confirmed three times with germinating oospores of *P. obducens* transferred to leaf tissue of *I. balsamina*. Our work showed that oospores can germinate to form megasporangia, which then produced zoospores that infect impatiens.

Assessing viability of oospores has proven challenging above and beyond developing a technique to foster oospore germination. Vital stains have been used to find a method to determine viability, but oospores so far have proved impermeable.

To aid in the determination of overwintering of oospores, it was necessary to develop a technique to identify living oospores. One of the challenges of working with oospores is that, unless they germinate, it is difficult to tell if they are alive or dead. There are a number of vital staining techniques that have been used with oospores, but they are often subjective. they have been investigating the use of 4 M sodium chloride to plasmolyze living oospores while having no effect on dead ones. Samples of oospores were autoclaved to heat-kill them, treated with 70% ethanol for 2 hr to kill them, or left untreated. The oospores were then immersed in 4 M NaCl and examined at 40 x with a microscope. Figure 5 shows the results. Killed oospores remained spherical with salt treatment, while untreated oospores buckled and became highly refractive. Additional activities will be reported under 2015-8130-274-CA.

Figure 5. Oospores killed by autoclaving or ethanol, or left untreated, and then immersed in a 4 M NaCl solution.



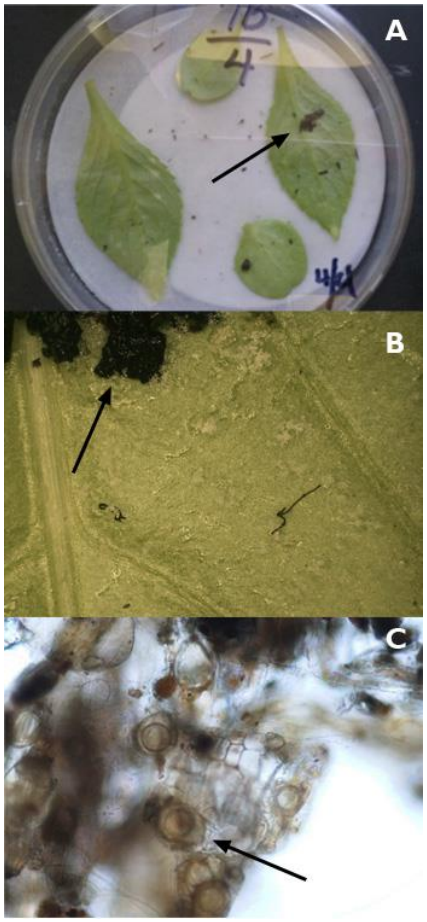
Untreated oospores buckled as the cytoplasm shrunk under osmotic pressure.

Method Development for P. obduscens baiting (Bio-ARSFD-3, Bio-HC-3)

At USDA-ARS-Fort Detrick, they developed a soil-baiting technique to assess whether *P. obduscens* oospores were present. they used soils from where known infections had occurred including soil sent from M. Daughtrey in 2013 as test materials. These soil samples were been stored in the refrigerator at 4 C. Soil was added to water in Petri dishes and allowed to incubate at room temperature for four or six days, then drops of water were pipette onto detached leaves of *I. balsamina* in moist chambers. The first trial turned up nothing, but in the second trial, one plate turned up positive for a NY soil (Table 2). they observed that the center of the infection site contained a piece of plant tissue loaded with oospores (Figure 6).

After this initial observation, they refined the soil baiting technique to use nested sieves to collect organic debris from soil for subsequent tests. In the first trial using organic material (second experiment), samples of soil from a local monitor plot yielded positive samples (Table 3). In two instances the pathogen was recovered, not only suggesting that a baiting assay was a viable methodology but that oospores can remain alive in soil for long periods of time and then infect host tissue.

Figure 6. Impatiens downy mildew *(Plasmopara obducens*) isolated from infested soil collected in 2013.



A) Moist chamber containing leaves treated with water from moistened soil sample. Arrow indicates infected leaf. B) Close-up of infected leaf showing sporangiophores of *P. obducens*. Arrow indicated piece of organic debris included in the water sample. C) Root piece in organic debris containing oospores with dimension consistent with those of *P. obducens*. Arrow points to one of the oospores.

Table 2. Results of first experiment inoculating detached leaves with a soil from sites that contained mildew-infected Impatiens plants stored at 4C.

Sample ID	Year of soil collection	Soil Location	Day 4 Inoculation		Day 6 Inoculation	
			Rating on Day 7[x]	Rating on Day 10[x]	Rating on Day 7[x]	Rating on Day 10[x]
Control	2016	USDA, MD	----	----	----	----
1B	No Date	Gansevoort, NY	----	----	----	----
2B	2013	Riverhead, NY	----	----	----	----
7B	2013	Mt. Sinai, NY	---+	---+	----	----
14B	2013	Rochester, NY	----	----	----	----
15B	2013	Rochester, NY	----	----	----	----
18B	2014	Riverhead, NY	----	----	----	----
19B	2013	Long Island, NY	----	----	----	----
20B	2014	Oyster Bay, NY	----	----	----	----
N	2015	Mt. Airy, MD	----	----	----	----
K	2015	Walkersville, MD	----	----	----	----

Soils were added to water, incubated for 4-6 days at room temperature, and then pipette as a soil-water slurry onto detached Impatiens leaves which were incubated for 10 days and rated for presence of IDM.

Table 3. Results of second experiment inoculating detached leaves with a soil from sites that contained mildew-infected Impatiens plants stored at 4C.

Sample ID	Year of soil collection	Soil Location	Day 4 Inoculation		Day 6 Inoculation	
			Rating on Day 7[x]	Rating on Day 10[x]	Rating on Day 7[x]	Rating on Day 10[x]
Control	2016	USDA, MD	----	----	----	----
9B-Control	2012	Oyster Bay, NY	----	----	----	----
1B	No Date	Gansevoort, NY	----	----	----	----
3B	2013	Riverhead, NY	----	----	----	----
4B	2013	Riverhead, NY	----	----	----	----
5B	2013	Riverhead, NY	----	----	----	----
6B	2013	Long Island, NY	----	----	----	----
8B	2013	Port Jefferson, NY	----	----	----	----
10B	2013	Ithaca, NY	----	----	----	----
11B	2013	Ithaca, NY	----	----	----	----
12B	2013	Ithaca, NY	----	----	----	----
13B	2013	Geneva, NY	----	----	----	----
16B	2013	Rochester, NY	----	----	----	----
17B	2013	Rochester, NY	----	----	----	----
18B	2014	Riverhead, NY	----	----	----	----
19B	2013	Long Island, NY	----	----	----	----
N	2015	Mt. Airy, MD	---+	---+	----	---+
K	2015	Walkersville, MD	----	----	----	----
FD	2015	Fort Detrick, MD	----	----	----	----
M(a)	2015	USDA, MD	----	----	----	----
M(b)	2015	USDA, MD	----	----	----	----

Soils were passed through nested sieves to catch debris on the 53 um sieve and the debris was added to water, incubated for 4-6 days at room temperature, and then pipette as a soil-water slurry onto detached Impatiens leaves which were incubated for 10 days and rated for presence of IDM.

Objective 1b: Biology and Epidemiology of IDM and CDM: Collection Maintenance

Establishment of P. obducens collection (Bio-ARSFD-4)

Cultures of downy mildew were established and have been maintained from two Maryland locations and one Long Island location at Fort Detrick, Frederick, MD.

Objective 1c: Biology and Epidemiology of IDM and CDM: Homothally

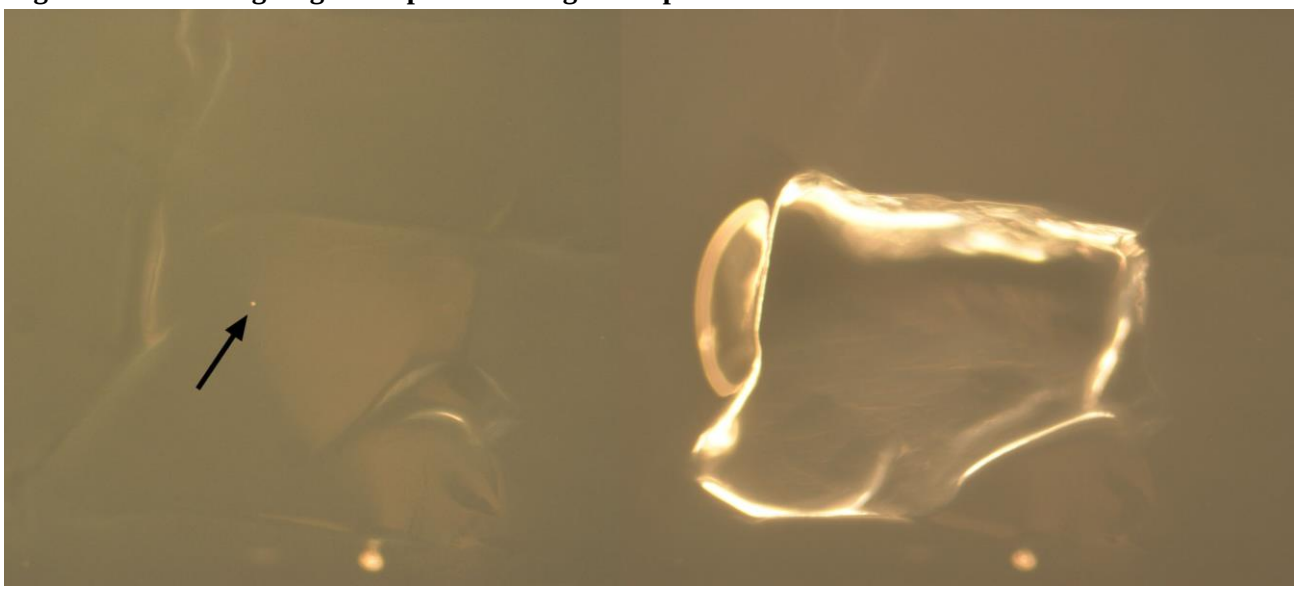
Mating type investigations (Bio-ARSFD-5)

Shishkoff developed a methodologies to determine whether *P. obduscens* is homothallic or heterothallic: that is, whether isolates of different mating type are required for oospore formation. Methodologies were developed for single-sporangium isolates and single zoospore isolates.

Subsequently, two hundred mating tests have been performed on 14 single-sporangium isolates of IDM from three populations collected from New York and Maryland. Results indicate homothalism.

To obtain single-zoospore isolates, zoospores were poured over water agar in a thin film of water and a small scalpel was used to cut a block of agar around the zoospore to transfer the block to a detached impatiens leaf (Figure 7). Four downy mildew isolates were derived from single zoospores and mating tests occurred to confirm that IDM is a homothallic organism.

Figure 7. Collecting single zoospores for single-zoospore isolates.



A) A block cut around a zoospore (arrow). B) block removed.

Importance of mating for oospore development (Bio-ARSFD-6, Bio-HC-6)

In an attempt to better understand the oospore stage, 14 single-sporangium isolates and three single-zoospore isolates were used in single and dual inoculations of stem tissue to see if the pathogen was homothallic or heterothallic; all isolates tested were able to produce oospores when inoculated singly, suggesting homothally.

Objective 1d: Biology and Epidemiology of IDM and CDM: Fungal Development

Sporangia: effect of temperature on infection (Bio-MSU-1)

Hausbeck studied temperature and relative humidity during infection of IDM. Impatiens 'Accent Premium White' plants in 4-inch pots were arranged in a split plot design in growth chambers with a 16/8 hour photoperiod with temperature (15, 20, 25, 30°C) as the whole plot treatment. Plants were inoculated with *P.* at 6 x 10^4 sporangia/ml. Nine days later, the total number of leaves and the number of leaves with *P. obducens* sporulation were counted per plant. The experiment was conducted twice. Temperature and relative humidity in each chamber was monitored for the duration of each experiment using a WatchDog model 450 data logger.

Statistical analysis of the temperature and leaf wetness data was completed, and information was put in table and graph form. Sporulation was not observed on any plants incubated at 30ºC (Table 4, Table 5). Following the initial rating, the plants that had been incubated at 30ºC were moved to the 20ºC incubator to determine if plants had a latent infection, but the pathogen was unable to sporulate at 30ºC. A leaf wetness period of ≥6 hours yielded a greater proportion of leaves containing sporangia than a leaf wetness period of 3 hours.

Hausbeck examined sporangia production within a greenhouse containing infected impatiens. A Burkard volumetric spore sampler was set up in each of two greenhouses with impatiens. Spore reels were changed weekly, and tapes from the reel were cut into 7 pieces representing 24 hours, and each hour was marked. Tapes were mounted on microscope slides, stained, examined under a microscope, and *P. obducens* spores were counted. Environmental data was monitored using a WatchDog model 450 data logger. The experiment in Greenhouse 11A ran from 30 Apr through 28 Jun, and Greenhouse 11B from 1 May through 7 Jul (Figure 8).

Table 4. Differences in mean proportion leaves infected (number of leaves with sporulation/total number of leaves) for impatiens plants exposed to different temperatures following inoculation with *P. obducens* sporangia.

Temperature (°C)	Proportion infected*
15	0.02 b
20	0.59 a
25	0.05 b
30	0.00

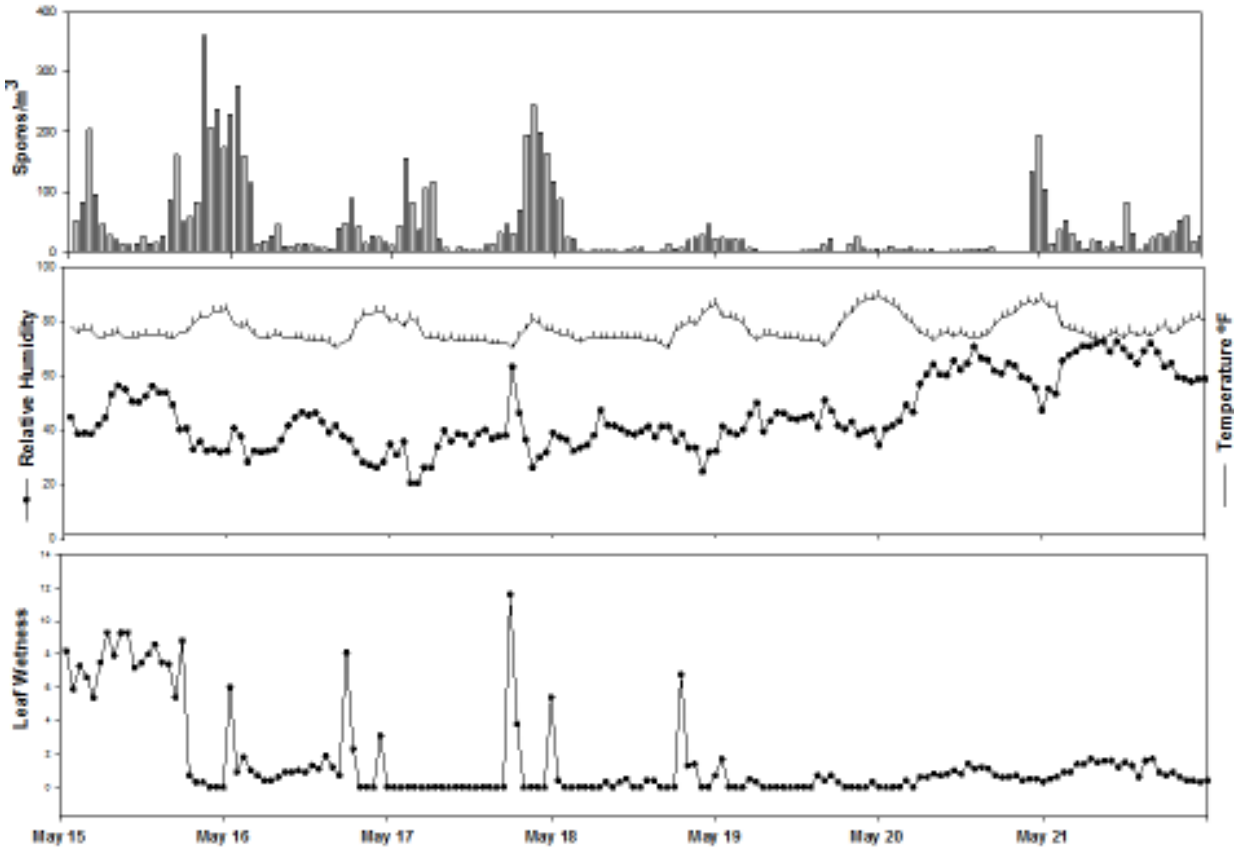
*means that share a letter are not statistically different (P<0.05). No leaves showed signs of infection following incubation at 30°C and these data were excluded from statistical analysis.

Table 5. Differences in mean proportion leaves infected (number of leaves with sporulation/total number of leaves) for impatiens plants exposed to different leaf wetness periods following inoculation with *P. obducens* sporangia.

Leaf Wetness (h)	Proportion infected*
control	0.04 c
3	0.21 bc
6	0.25 ab
12	0.30 a
24	0.28 ab

*means that share a letter are not statistically different (P<0.05). No leaves showed signs of infection following incubation at 30°C and these data were excluded from statistical analysis.

Figure 8. Comparison of sporangia release with relative humidity and leaf wetness, Hausbeck, 2013



Sporangia: optimal temperature and humidity for formation (Bio-MSU-2)

Concentrations of airborne sporangia were monitored in two greenhouses from late April into July 2013 using 7-day volumetric spore samplers (Burkard Mfg. Co. Ltd., Rickmansworth, Herfordshire, England) placed between greenhouse benches holding downy mildew infected impatiens. To determine the correlation of environmental parameters and sporangia in the atmosphere, temperature and relative humidity (RH) were recorded hourly. Data was analyzed by determining if weekly spore counts were influenced by the number of hours of low (<25%), moderate (25-49% and 50-75%) or high (>75%) RH (Table 6). In greenhouse 11A, the smaller amount of sporangia observed in week 3 when compared to week 2 may correlate to lower RH observed for the week. A large decrease in spores was observed between weeks 5 and 6 as the number of hours of RH >50% decreased from 130 hours in week 5 to just 44 hours in week 6. In greenhouse 11B, spore counts increased by >400% from week 4 to week 5, which correlates to increased RH observed during week 5 (38 hrs with >75% RH) versus week 4 (8 hrs with >75% RH). A similar increase in sporangia was observed between weeks 6 and 7, which also correspond with increased RH observed during the week with the larger spore counts. Although RH remained high in greenhouse during weeks 7-9, the high number of hours above 25^{o}C likely account for the reduced number of sporangia collected by the spore sampler. Temperatures above 25^{o}C were negatively correlated to observed sporulation in growth chamber studies conducted by the Hausbeck Lab. The negative effect of higher temperatures on sporangia formation and release events is highly evident as counts remained low in greenhouse 11A during week 9 despite the highest number of hours with RH >75%.

Table 6. Total Sporangial counts by week for greenhouses 11A and 11B. Sum of recorded hours of selected extreme weather variables of high and low humidity, and high temperatures in the respective greenhouses are listed.

Week	Dates	Total spore count	Hours of RH (%)				Hours of temperature (°C)			
			<25	25-49	50-75	>75	15-20	20-25	25-30	>30
Greenhouse 11A										
1	30 Apr-4 May	10,985	1	64	39	0	0	67	31	6
2	5 May-11 May	11,410	3	117	45	3	20	112	35	0
3	12 May-18 May	6,952	18	142	12	0	36	92	39	1
4	19 May-25 May	1,505	5	55	102	7	19	105	35	9
5	26 May-1 Jun	1,225	8	30	101	29	3	106	54	5
6	2 Jun-8 Jun	184	9	116	44	0	0	118	49	1
7	9 Jun-15 Jun	271	0	35	110	23	0	84	84	0
8	16 Jun-22 Jun	56	0	66	95	7	0	80	85	3
9	23 Jun-28 Jun	12	0	0	89	44	0	44	83	6
Greenhouse 11B										
1	1 May-4 May	4,277	4	68	10	0	0	55	26	1
2	5 May-11 May	834	10	93	62	3	16	137	15	0
3	12 May-18 May	3,137	21	119	29	0	19	144	5	0
4	19 May-25 May	603	4	48	109	8	7	122	39	0
5	26 May-1 Jun	2,472	6	36	89	38	1	110	54	3
6	2 Jun-8 Jun	141	5	108	56	0	1	156	11	0
7	9 Jun-15 Jun	471	0	12	118	38	0	144	24	0
8	16 Jun-19 Jun	52	1	36	45	4	0	62	22	2
9	28 Jun-29 Jun	38	0	1	32	0	0	29	4	0
10	30 Jun-6 Jul	18	0	34	128	6	0	101	62	4

As with most downy mildews, *P. obducens* sporangial releases followed a diurnal pattern. The number of atmospheric sporangia increased greatly starting at 09:00 and peak counts were observed around 12:00. Sporangia counts slowly decreased after 12:00 with few spores observed after 21:00. The diurnal pattern is likely associated with decreasing RH observed in the late morning, causing liberation of the sporangia.

The information collected and analyzed in the greenhouse spore sampling experiment may help impatiens growers optimize their scouting and fungicide application schedules. Changes to current production methods include limiting the time foliage remains wet by timing irrigations events accordingly and increased venting to remove humid air. If a grower suspects that his impatiens may have become infected with downy mildew, he may consider growing the crop at higher temperatures (>25^{o}C) to reduce the number of atmospheric sporangia, therefore reducing the chance of infection to spread to surrounding healthy impatiens. The higher number of relative spore counts observed in April-May when compared to June-July can help growers understand when a more intensive fungicide program may be necessary to produce a disease free crop.

Oospores: time course of formation in Impatiens sp. (Bio-CU-2)

During summer 2015, three trials with jewelweed (*I. capensis*) failed to produce any downy mildew infection. Therefore, Margery Daughtrey decided to work solely with seedlings of *I. balsamina.* Balsam impatiens (*I. balsamina*) planted in the greenhouse from 5 internet seed sources was planted out adjacent to infected *I. walleriana* in a field plot in a shaded greenhouse in early September. Two weeks later (15 Sept) one balsam plant showed yellow lesions and sporulation on the undersurface of the leaf. When rated Oct 1, all of the balsam impatiens were showing symptoms of downy mildew, with no apparent difference in susceptibility according to the seed source.

In 2017-18, she repeatedly tried and failed to establish *I. capensis* and *I. pallida* from purchased seed, possibly due to unusual dormancy requirements. Experiments were thus limited to *I. balsamina*, which was easily grown from commercially-available seed.

However, during 2017, IDM appeared in field monitor plots of *I. walleriana* and *I. balsamina* in late summer, and infested plant material was left in place for observation of overwintering in spring 2018. IDM infected *I. walleriana* and *I. balsamina* examined in late July 2017 showed a few haustoria and oogonia, but no oospores. When a sample of balsam stems was examined for oospores at seed harvest in September 2017, there were still none found, presumably because of late onset of the downy mildew epidemic. Seed pods of *I. balsamina* collected from diseased plants were stored in the refrigerator at 7.2° C for later evaluation of seeds and their germination similar to the experiment reported under 15-8130-0274-CA. When 50 of these balsam seeds were sown in soilless mix in the greenhouse in January 2018, they germinated and grew, but plants did not develop any symptoms of downy mildew. The question raised by these observations is whether plentiful oospore formation in plant tissues during the growing season is essential for overwintering of IDM in or on balsam seed. If so, a season in which foliar disease is seen early (in June) might be more likely to lead to overwintering than a season in which disease symptoms are not seen until early fall, as this would provide more time for oospore formation. Maturation of oospores also requires chilling, as evidenced by research at ARSFD, so variation in winter temperatures along the east coast of the United States could affect the ability of the IDM to overwinter under natural conditions—and storage of seed at 7.2° C might have prevented the maturation of oospore inoculum on seed gathered in the fall.

A time course of oospore development in *I. balsamina*, *I. capensis* and *I. pallida* in contrast to *I. walleriana* (which they had hoped to supply) is still needed to improve our understanding of how IDM operates in the additional hosts outside of the control of the bedding plant industry.

During this research, Margery Daughtrey observed abundant oospores in impatiens stems and was able to recover them by grinding with sand and filtering through several sieves onto a 39 micron sieve. Observed under the microscope, these spores have very thick walls. Observations made on the wet preparations of oospores for two weeks after sieving (allowed to dry down over time) have shown no clear indications of zoospore

production (such as empty oospores or sporangia being produced from zoospores). Currently dried-down preparations of oospores (including some sand and organic debris) (either stored at room temperature after harvest or given a 24 hr freezing event) are being frozen and thawed repeatedly prior to another round of experiments, testing the ability of freezing/thawing to stimulate sporangial production or direct germination of oospores. The methods used to develop inoculum for laboratory/growth chamber tests can be used to develop inoculum for field studies to test oospores as a source of infection for impatiens planted into pots or directly into the ground. Larger quantities of inoculum might make infection via oospores possible if oospores characteristically break dormancy at different rates which possibly may depend upon the width of their walls. Amounts used thus far have been conservative (roughly 100 oospores per inoculation). Ten times this amount may be necessary to get the effect if oospores are not all fairly equivalent in their germination potential. Further tests in the growth chamber and in the field are needed.

Oospores: length of survival (Bio-ARSFD-7-Bio-HC-7)

One important factor in determining if oospores overwinter is to determine what triggers oospore germination. In previous work, Nina Shishkoff was able to get oospores to germinate after incubation at 0 C for 1-2 months. She completed formal experiments incubating oospores at -10 C, 0 C, 10 C and 20 C for 4 months, taking monthly samples to detect germination. In three trials, she took inoculated detached stems of *I. balsamina* with isolate OW, left the stems to incubate for 1 month at 20 C to allow the development and maturation of oospores, and then placed the stem segments in moist chambers held at the 4 temperatures. Samples were taken at 0, 1, 2, 3 and 4 months, the stem tissue dissected in water and stored at 10 C and examined ever 2-3 days for oospore germination (which generally took place after 5-10 days in water). Results showed that oospores germinated at 0 C after 1-2 months and thereafter, but no germination was observed at any other temperature even after 4 months.

Oospores: temperature requirements for germination (Bio-ARSFD-8, Bio-HC-8)

In work started in 2016, Nina Shishkoff undertook to understand the life cycle of *P. obducens* and whether the pathogen overwinters in the landscape by studying whether temperature triggers oospore germination. Previously, she had determined that oospores of IDM require at least 1-2 months of 0 C temperature to germinate, but it was important to better understand the cold requirement by conducting experiments over a longer term and where temperature was variable. Oospores of *P. obducens* were produced in detached stem culture by inoculating stem segments and incubating at 20 C for one month to allow maturation. These stem segments were then incubated at different temperatures and transferred to water to observe germination of the oospores. In one set of experiments, stem segments were incubated at -10, 0, 10, or 20 C and samples (2-4 stem segments per temperature) taken monthly. Stem segments were dissected in Petri dishes containing sterile water and allowed to incubate at 10 C for three weeks, observed under a dissecting microscope ever 2-6 days. If any germinating oospores were observed, that stem segment was considered positive. Below (Table 7) are the results for 4 different isolates of *P. obducens* (collected from plants in NY, MD, and NJ) over 7 months. Results after 7 months indicated that at least one month at 0 C stimulated oospore germination, although occasional germination was observed at 10 C. For many of these samples, viability of the oospores were tested using plasmolysis in 4 Molar NaCl solution and this demonstrated that some oospores were alive in each sample, even if they did not germinate. After 3-4 months, it was observed that the oospore wall had become thick in samples kept at -10 C. Oospore wall thickness after oospores were given a month to mature was 1.03-1.19 um. After 3-4 months of incubation at the different temperatures oospores at 0, 10, or 20 C had walls that were 1.06-2.03 um thick, while oospores held at -10 C had thicker walls, 1.98-2.94 um thick.

After the completion of this experiment, additional samples at 0 and -10 C still remaining at 7 months were split into groups: one group remained at -10 C, one group remained at 0 C, one group was moved from -10 C to 0 C, and one group was moved from 0 C to -10 C. Samples were taken every month to look at germination of oospores and viability of oospores. Germination results are given in Table 8. Oospores continued to germinate at O C (except in one trial where no germination was observed), and oospores moved from 0 C to -10 C were

observed to germinate for 1-2 months in some trials, with no germination observed in subsequent samples. Viability of oospores were tested in each sample by taking a subsample of oospores and immersing them in 4 molar NaCl to look for plasmolysis, and oospores were alive in each sample, even if germination was not observed.

Table 7. Oospore germination in infected stems incubated at -10, 0, 10, or 20 C (stems with germinating oospores/total stems) over seven months

		Months							
Isolate	**Temp**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
OW	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		0/4	*2/4	*1/4	*3/4	3/4	*4/4	*3/4
	10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
OW	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		*1/4	*4/4	*4/4	*4/4	3/4	*4/4	*2/4
	10		0/4	0/4	0/4	0/4	0/4	1/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
MC	-10		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0		0/2	*1/2	*1/2	*1/2	*1/2	*1/2	*1/2
	10		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	20	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
MC	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		0/4	*1/4	*1/4	*1/4	*2/4	0/4	0/4
	10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Utaz	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		0/4	0/4	0/4	*1/4	0/4	1/4	*1/4
	10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Utaz	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		0/4	0/4	1/4	0/4	*1/4	*1/4	*1/4
	10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
7b Trial 1	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		0/4	*2/4	*4/4	*3/4	*2/4	*1/4	*2/4
	10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
7b Trial 2	-10		0/4	0/4	0/4	0/4	0/4	0/4	0/4
	0		*1/4	*2/4	*4/4	*3/4	*2/4	*4/4	*2/4
	10		0/4	0/4	0/4	0/4	0/4	*1/4	0/4
	20	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Positive readings are highlighted in orange

Table 8. Oospore germination in samples kept at 0 C or -10 C for 7 months, then held at the same temperature for up to seven additional months, or moved from one temperature to another.

Isolate	Temp	Months							
		0	1	2	3	4	5	6	7
OW Trial 1	-10 C	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 C	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2
	-10 C to 0 C		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 to -10 C		*1/2	*2/2	0/2	0/2	0/2	0/2	0/2
OW Trial 2	-10 C	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 C	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2	*2/2
	-10 C to 0 C		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 to -10 C		*2/2	*2/2	0/2	0/2	0/2	0/2	0/2
7b Trial 1	-10 C	0/4	ND	0/4	0/4	0/4			
	0 C	*2/4	ND	0/4	0/4	0/4			
	-10 C to 0 C		ND	0/4	0/4	0/4			
	0 to -10 C		ND	0/4	0/4	0/4			
7b Trial 2	-10 C	0/4	0/4	0/4	0/4	0/4			
	0 C	*2/4	*3/4	*1/4	*2/4	*1/4			
	-10 C to 0 C		0/4	0/4	0/4	0/4			
	0 to -10 C		*2/4	0/4	0/4	0/4			

For each sample, stems with germinating oospores are shown over total stems, and positive samples are highlighted in orange

Another experiment took stems containing oospores and incubated them at 0 C, 10 C, or moved samples at 0 C to 10 C for 3 days each week and moved 10 C samples to 0 C for 3 days each week. Samples were then taken monthly for 4 months. Results are given in Table 9. Samples held at 0 C showed good oospore germination, and one sample held at 10 C showed germination; samples subjected to temperatures varying from 0 C to 10 C also showed germination of oospores.

Table 9. Oospore germination in samples kept at 0 C or 10 C or switched from one temperature to the other for 4 months.

Isolate	Temp	0 months	1 month	2 months	3 months	4 months
OW Trial 1	0 C		*1/4	*3/4	*1/4	*4/4
	10 C		*1/4	0/4	0/4	0/4
	10 C to 0 C		0/4	*3/4	*3/4	0/4
	0 to 10 C	0/4	*1/4	*1/4	*1/4	0/4
OW Trial 2	0 C		*3/4	*4/4	0/4	*4/4
	10 C		0/4	0/4	0/4	0/4
	10 C to 0 C		*3/4	*1/4	0/4	*1/4
	0 to 10 C	0/4	0/4	*2/4	*1/4	*1/4

The results of these experiments demonstrate that a period of cold (optimally 0 C) is necessary for oospore germination, but that samples kept at -10 C for more than two months would not germinate, although they were still alive. Moving those samples from -10 to 0 C did not, over 7 months, stimulate them to germinate. Oospores kept at 10 C sometimes germinated, but germination was never seen in oospores kept at 20 C. This suggests that oospores can survive cold winter temperatures but it isn't clear what happens if temperatures remain at -10 for long periods of time.

Cold treatment at 0 C for at least a month induced oospores to germinate and produce primary sporangia. Inoculation of plant tissue with germinating oospores led in infection. Other incubation temperatures (-10, 10, and 20 C) did not induce germination, but fluctuating temperatures (between -10 and 0 C, or 0 and 10 C)

induced some germination. Spores incubated at -10 C had significantly thicker walls than spores incubated at the other temperatures.

In one experiment, stem segments that had been incubating for 7 months at -10 C or 0 C were used to determine if a change in temperature would alter their ability to germinate. Some samples continued to be kept at -10 or 0 C for the entire study period; others were switched, so that 0 C samples were placed at -10 C, and -10 C samples were placed at 0 C. Samples were taken monthly from each treatment. During the first week of incubation of each stem segment, a subsample of tissue was taken and approx. 20 oospores in the tissue were measured for wall diameter and then treated with 4 M NaCl solution to estimate viability. This experiment was run using isolate OW for 7 mo and isolate 7b for 4 mo, with two trials of each isolate. When oospores that had been incubating at 0 or -10 C for seven months continued to be incubated at those temperatures or were switched (0 C samples moved to -10 C, or -10 C samples moved to 0 C), spores that remained at 0 C continued to germinate for an additional 7 months (14 months total). Oospores moved from -10 C to 0 C did not germinate, even after 7 months at 0 C. Oospores moved from 0 C to -10 sometimes germinated for the first 1-2 months at the new temperature, but then ceased to germinate (Table 10). Oospore wall thickness differed significantly by a Least Squares Means Test for spores incubated at -10 C and 0 C at each sampling time; spores switched from one temperature to another generally differed significantly from both the -10 C spores and the 0 C. When the -10 spores from isolate OW were moved to 0 C they did not over the course of 7 months develop dramatically thinner walls and the spores moved from 0 C to -10 C did not, until the 7 month sample, develop a thicker wall (Table 11).

In a second set of experiments, samples of stems containing mature oospores (grown in stem tissue for one month at 20 C) were placed in 4 plastic bags, two in an incubator set at 0 C and two in one set at -10 C. One bag in each incubator was left at that temperature continuously, the other two bags were removed from the incubators on Fridays and placed at the opposite temperature, then switched back on Mondays, so that bags initially placed at 0 C spent 3 days each week at -10 C and bags initially at -10 C spent 3 days each week at 0 C. Samples were taken at time zero and monthly for 4 months as described above for uniform temperatures. Each month wall diameter of oospores was measured and plasmolysis after immersion in a sodium chloride solution was performed as described above. Two trials were run on isolate OW. When samples were kept at 0 or -10 or switched between the two temperatures every 3-4 days, germination was most commonly seen at 0 C, but also seen in samples switched from 0 C to -10 C (Table 12). Wall thickness did not significantly differ among temperature treatments at one month, but by four months all treatments differed significantly in wall thickness (Table 14). There were plasmolyzing oospores in each treatment at each time (Table 14), suggesting that living oospores were present in each treatment.

Table 10. Oospore germination in samples kept at 0 C or -10 C for 7 months, and then held at those same temperatures or moved from one temperature to the other.

For each sample, stem segments with germinating oospores are shown over total stems, and positive samples are highlighted in gray.

Isolate	Temp	Observed germination of oospores (stems/total) over 4-7 months of incubation							
		0 mon	1 mon	2 mon	3 mon	4 mon	5 mon	6 mon	7 mon
OW Trial 1	-10 C	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 C	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
	-10 C to 0 C		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 to -10 C		1/2	2/2	0/2	0/2	0/2	0/2	0/2
OW Trial 2	-10 C	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 C	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
	-10 C to 0 C		0/2	0/2	0/2	0/2	0/2	0/2	0/2
	0 to -10 C		2/2	2/2	0/2	0/2	0/2	0/2	0/2
7b Trial 1	-10 C	0/4	ND	0/4	0/4	0/4			
	0 C	2/4	ND	0/4	0/4	0/4			
	-10 C to 0 C		ND	0/4	0/4	0/4			
	0 to -10 C		ND	0/4	0/4	0/4			
7b Trial 2	-10 C	0/4	0/4	0/4	0/4	0/4			
	0 C	2/4	3/4	1/4	2/4	1/4			
	-10 C to 0 C		0/4	0/4	0/4	0/4			
	0 to -10 C		2/4	0/4	0/4	0/4			

a= samples at each temperature consisted of 2-4 infested stem segments. Due to the low germination rate of oospores, each stem was rated as "+" or "-" and germination overall was rated as stem segments with any germinating oospores/total stems.

Table 11. Wall thickness of oospores in samples kept at 0 C or -10 C for seven months, held at the same temperatures or switched from one temperature to another and sampled for an additional seven months (isolate OW) or four months (isolate 7b).

Isolate OW	Oospore wall thickness (um ± standard deviation) [a]			
Incubation time [b]	**-10 C**	**0 C**	**-10 to 0 C**	**0 to -10 C**
time 0	2.50 ± 0.48a	0.96 ± 0.25b		
1 month	2.38 ± 0.45a	1.05 ± 0.18c	2.07 ± 0.37b	1.15 ± 0.31c
2 month	2.02 + 0.36a	1.11 + 0.26d	2.14 + 0.41b	1.45 + 0.32c
3 month	2.23 ± 0.37a	1.04 ± 0.17d	1.94 ± 0.41b	1.33 ± 0.53c
4 month	2.49 ± 0.45a	1.04 ± 0.24c	2.47 ± 0.42a	1.64 ± 0.63b
5 month	2.59 + 0.38a	1.14 + 0.20c	2.50 + 0.38a	1.48 + 0.33b
6 month	2.22 + 0.31a	1.08 + 0.18d	1.96 + 0.39b	1.49 + 0.40c
7 month	2.18 + 0.42a	1.15 + 0.16c	1.95 + 0.39b	2.18 + 0.47a
Isolate 7b	**Oospore wall thickness (um ± standard deviation) [a]**			
Incubation time [b]	**-10 C**	**0 C**	**-10 to 0 C**	**0 to -10 C**
time 0	2.61 ± 0.45a	1.19 ± 0.28b		
1 month	2.57 ± 0.41a	1.40 ± 0.40c	2.37 ± 0.45a	1.56 ± 0.53b
2 month	2.58 ± 0.35a	1.17 ± 0.23c	2.56 ± 0.42a	1.32 ± 0.28b
3 month	2.58 ± 0.37a	1.30 ± 0.32c	2.60 ± 0.41a	1.67 ± 0.40b
4 month	2.47 ± 0.36a	1.27 ± 0.28c	2.41 ± 0.36a	1.79 ± 0.43b

a=Sets of *I. balsamina* stems stored at 0 or -10 C for 7 months were subsequently used in germination studies where stems were divided into groups experiencing different temperature regimes (stems kept at continuous 0 C, at continuous -10 C, moved from -10 to 0 C, or from 0 C to -10 C) and sampled for up to seven months (see Table 10). Concurrent with germination samples, samples of oospores (approximately 40 oospores per stem from 2-4 stems) were measured for oospore wall diameter. Each experiment was performed twice, although a two month sample was not taken for isolate 7b in one of the trials. A General Linear Model analysis showed that temperature significantly affected wall thickness at each sample time ($P < 0.0001$). Results of Least Square Means analyses are shown on the table, with means ± standard deviation followed by letters indicating significant differences within each row at $P \leq 0.05$.

b= After the conclusion of 4 experiments shown in TABLE 6 (two for isolate 7b and two for isolate OW) samples remaining at 0 or -10 were used for this experiment. Therefore, "time 0" here is 8 months after inoculation of stem tissue (1 month at 20 C for maturation of oospores and seven months of incubation at 0 or -10 C). At the conclusion of this experiment, the stem tissue was therefore 15 months old.

Table 12. Oospore germination in samples kept at 0 C or -10 C or switched from one temperature to the other every 3-4 days for 4 months.

For each sample, stems with germinating oospores are shown over total stems, and positive samples are highlighted in gray.

Isolate	Temp[b]	Observed germination of oospores (stems/total) over 4 months of incubation[a]			
		1 month	**2 months**	**3 months**	**4 months**
OW Trial 1	0 C	3/4	2/4	4/4	3/4
	-10 C	0/4	0/4	0/4	0/4
	-10 C to 0 C	0/4	0/4	0/4	0/4
	0 to -10 C	0/4	0/4	0/4	1/4
OW Trial 2	0 C	0/4	2/4	4/4	2/4
	-10 C	0/4	0/4	0/4	0/4
	-10 C to 0 C	0/4	0/4	0/4	0/4
	0 to -10 C	0/4	0/4	2/4	0/4

a= samples at each temperature consisted of 4 infested stem segments. Due to the low germination rate of oospores, each stem was rated as "+" or "-" and germination overall was rated as stem segments with germinating oospores/total stem segments.

b= Samples at -10 or 0 C remained at these temperatures except for short periods at room temperature for sample removal. Samples termed “-10 to 0 C” were initially placed at -10 C and moved to 0 C, alternating every 3-4 days for four months. Samples termed “0 to -10 C” were first placed at 0 C and then moved to -10 C, alternating every 3-4 days for four months

Table 13. Percent plasmolysis of oospore cytoplasm in samples kept at 0 C or -10 C for four months or switched every 3-4 days between 0 and -10 C for four months.

		Percent plasmolysis			
		Incubation time [c]			
Time 0[b]	Temperature	1 month	2 month	3 month	4 month
63.30%	-10 C	66.40%	59.20%	62.80%	62.80%
	0 C	73.50%	72.20%	69.70%	69.70%
	-10 to 0 C	70.00%	55.50%	62.70%	62.70%
	0 to -10 C	69.60%	71.00%	70.30%	70.30%

b= Infected stem segments of *Impatiens balsamina* were incubated for 1 month at 20 C in a growth chamber until oospores had formed in tissue. This was considered "0 months".
c= Samples at -10 or 0 C remained at these temperatures except for short periods at room temperature for sample removal. Samples termed “-10 to 0 C” were initially placed at -10 C and moved to 0 C, alternating every 3-4 days for four months. Samples termed “0 to -10 C” were first placed at 0 C and then moved to -10 C, alternating every 3-4 days for four months.
d= Subsamples of stem tissue were immersed in a 4 Molar sodium chloride solution and observed microscopically. Approximately 50 oospores were assessed for plasmolysis in each sample. Plasmolysis was assumed to occur only in living spores.

Table 14. Wall thickness of oospores in samples kept at 0 C or -10 C for four months or switched every 3-4 days between 0 and -10 C for four months.

		Oospore wall thickness (um $\pm$ standard deviation)[a]			
		Incubation time [c]			
Time 0[b]	Temperature	1 month	2 month	3 month	4 month
1.12 $\pm$ 0.23	-10 C	1.08 $\pm$ 0.25a	1.53 $\pm$ 0.43d	1.88 $\pm$ 0.37c	2.17 $\pm$ 0.44d
	0 C	1.09 $\pm$ 0.21a	1.02 $\pm$ 0.18a	1.20 $\pm$ 0.21a	1.18 $\pm$ 0.20a
	-10 to 0 C	1.07 $\pm$ 0.18a	1.23 $\pm$ 0.26b	1.25 $\pm$ 0.20a	2.05 $\pm$ 0.48c
	0 to -10 C	1.11 $\pm$ 0.20a	1.36 $\pm$ 0.35c	1.37 $\pm$ 0.27b	1.55 $\pm$ 0.33b

a=Oospore wall thickness was measured each month for the four temperature treatments, in two trials. Each temperature treatment sample consisted of approx. 400 spores from four stems. A General Linear Model analysis showed that temperature significantly affected wall thickness ($P < 0.0001$). Results of Least Square Means analyses are shown on the table, with means $\pm$ standard deviation followed by letters indicating significant differences within each column at $P \leq 0.05$.
b= Infected stem segments of *Impatiens balsamina* were incubated for 1 month at 20 C in a growth chamber until oospores had formed in tissue. This was considered "0 months".
c= Samples at -10 or 0 C remained at these temperatures except for short periods at room temperature for sample removal. Samples termed “-10 to 0 C” were initially placed at -10 C and moved to 0 C, alternating every 3-4 days for four months. Samples termed “0 to -10 C” were first placed at 0 C and then moved to -10 C, alternating every 3-4 days for four months.

After oospore germination was shown to be enhanced by exposure to 0 C, conditioned oospores were used in experiments to try to infect plants through the root system. Oospores of *P. obducens* that had been cold-conditioned by exposure to 0 C for one month were added to potting media, and then germinated seed were placed on top, in three experiments for each plant species. Both *I. balsamina* (in 19-44 plants out of 50) and I. walleriana (8-18 plants out of 38) became systemically infected.

In late 2017 samples of oospores produced in stem culture were placed at 0 C, -10 C and -80 C and sampled monthly to measure oospore wall thickness, viability and germination. The experiment is on-going, but after 6 months of incubation, we’ve determined that oospores stored at 0 C for six months are mostly alive and can germinate after at least a month of cold conditioning. Oospores stored at -10 C are mostly alive but have developed a thick wall and have not been observed to germinate. Oospores stored at -80 C do not develop a thick wall but are alive and can germinate. This suggests that severe cold does not kill oospores, but quick freezing prevents a thick wall from forming. At -10 C, formation of a thick wall may be inducing dormancy in oospores. Further work needs to be done.

Sporangia and oospore inoculation of roots and systemic infection (Bio-ARSFD-9)

Plants of *I. balsamina* or *I. walleriana* inoculated with sporangia on roots developed systemic infections and eventual sporulation from leaves. If *I. walleriana* were inoculated on roots when in the 4-leaf stage (two cotyledons and four true leaves), it took 8-12 days for infection to travel up roots through the stem and into leaves, where sporulation occurred. These results indicate that inoculum may not only spread in air from leaf to leaf but may also spread from air-borne inoculum to soil-borne inoculum to roots. Additional studies used conditioned oospores (oospores kept at 0 C for at least one month) to inoculate roots of *I. balsamina*, causing systemic infection. This shows that infection of plants by oospores is possible.

Figure 9. Macroscopic infection symptoms of systemic infection of *Impatiens balsamina* by oospores applied to the root system.

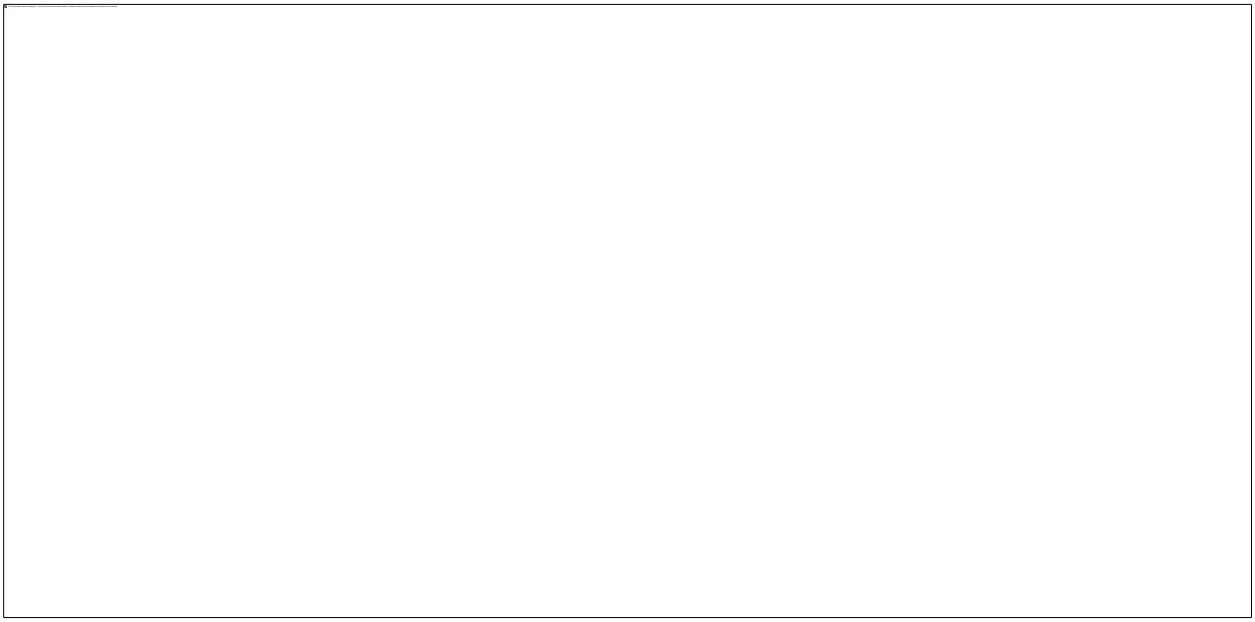
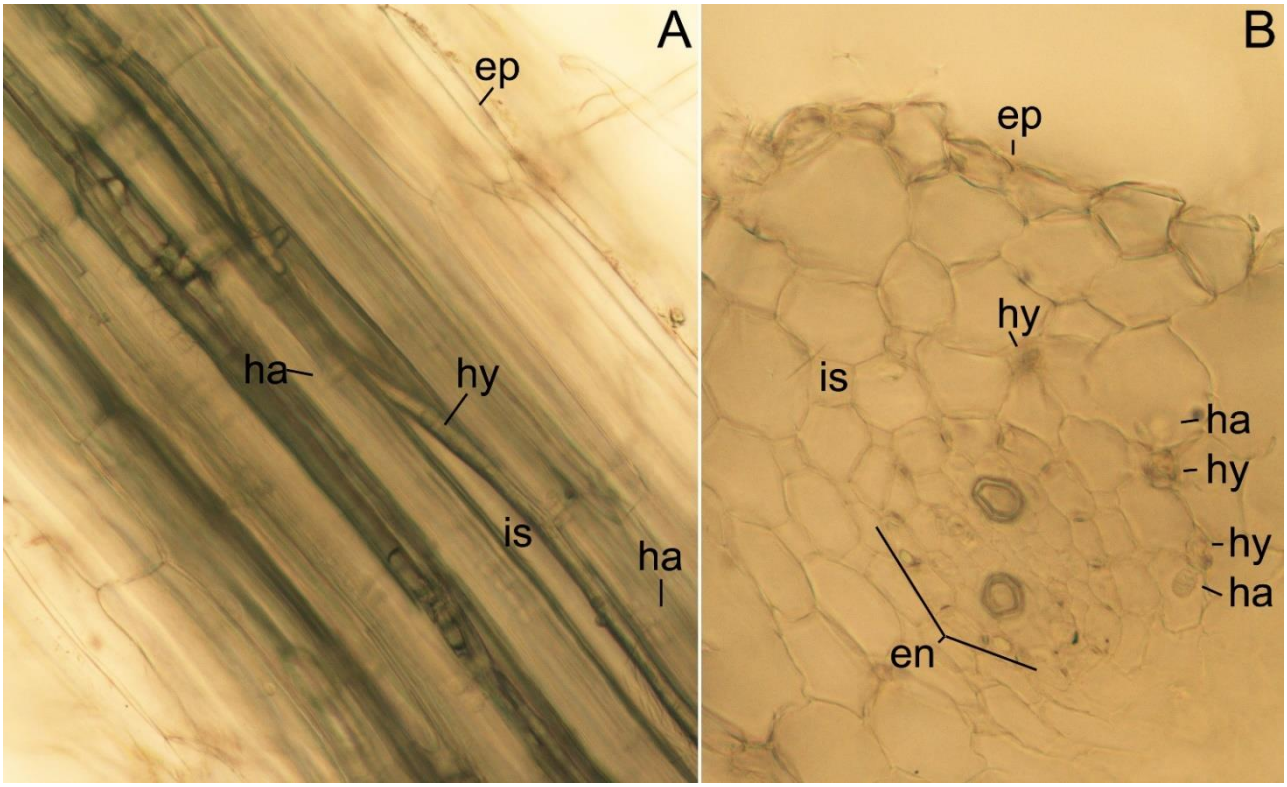


Figure 10. Systemic root infection of *Impatiens walleriana*



A. Transverse view of a root, showing a downy mildew hypha growing inside an intercellular space between cortical cells. e= epidermis, is = intercellular space, hy= hypha, ha = haustorium (penetrating cortical cell).

B. Cross-section of a root, showing hyphae in intercellular spaces, producing haustoria in cortical cella. e = epidermis, is = intercellular space (without mycelium), hy = hypha (within intercellular space), ha = haustorium (penetrating cortical cell), en = endodermis with Casparian strip.

Additional experiments were performed to examine upward spread of *P. obducens* within the plant when roots were inoculated. Three cultivars were compared: *I. walleriana* 'Elfin White"- a cultivar developed in the 1980s (considered an "early" cultivar), *I. walleriana* 'Elfin orange'- more recent cultivar, and New Guinea Impatiens (*I. hawkeri* 'Divine Lipstick'), which has been reported to get little or no infection. Seeds of each cultivar were germinated and raised to the cotyledon stage, then a sporangial suspension (2000 sporangia/mL) was carefully poured into the pots of treated plants. A water control was poured over control plants. Treated plants were removed at 1, 4, 8, 12 and 16 days after inoculation (3 plants each of the *I. walleriana* cultivars, two of *I. hawkeri*); controls were removed on day 16. Sampled plants were placed in 70% ethanol to clear them, and then examined microscopically for mycelium and oospores of the pathogen.

One day after inoculation, no symptoms were seen on any plant. After four days, mycelium with characteristic *Plasmopara* haustoria were observed in roots of all cultivars. After eight days, oogonia were visible in roots of all plants (Figure 11), and in *I. walleriana* plants, mycelium was seen in the hypocotyl (Figure 12).

After 12 days, stem tissue of infected *I. walleriana* contained oospores and downy mildew mycelium was present in cotyledons and true leaves (Figure 13). Sporangiophores were beginning to emerge from stomates and form sporangia (Figure 14). *Impatiens hawkeri* plants had mildew sporulatiing on exposed root tissue and exhibited dark stripes of necrotic hypocotyl tissue (Figure 15), associated with mycelium in the stems (Figure 16).

After 16 days, infected *I walleriana* plants were observed to have oogonia in leaf tissue and abundant sporulation. In *I. hawkeri*, the necrosis was never seen to extend beyond the hypocotyl. Sporulation was seen on adventitious roots.

Figure 11. Oospores in the roots of *I. walleriana* plants 8 days after inoculation.

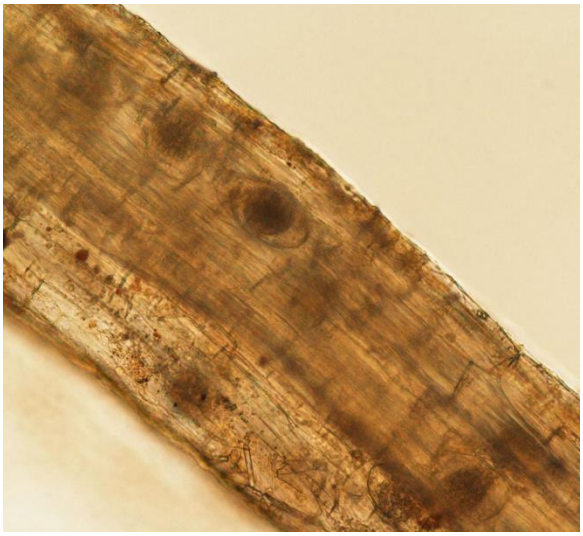


Figure 12. Mycelium with dumbbell-shaped haustorium in the stem of I. walleriana 8 days after infection.

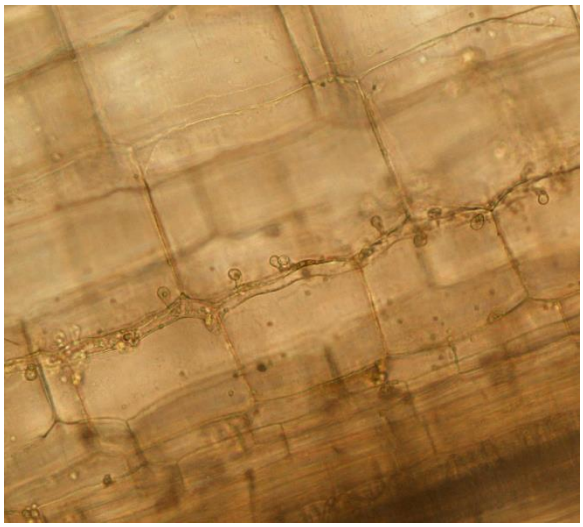


Figure 13. Downy mildew mycelium in *I. walleriana* cotyledon tissue 12 days after roots were inoculated.

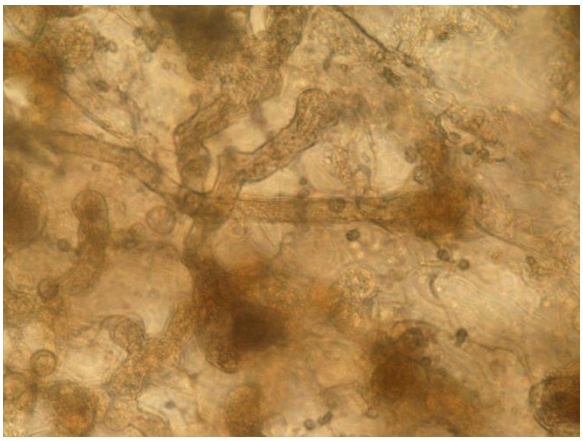


Figure 14. Sporangiophore emerging from a stomate on the underside of an *I. walleriana* leaf at 12 days after inoculation of roots.

Figure 15. Necrotic tissue in the hypocotyls of infected *I. hawkeri* plants at 12 days.

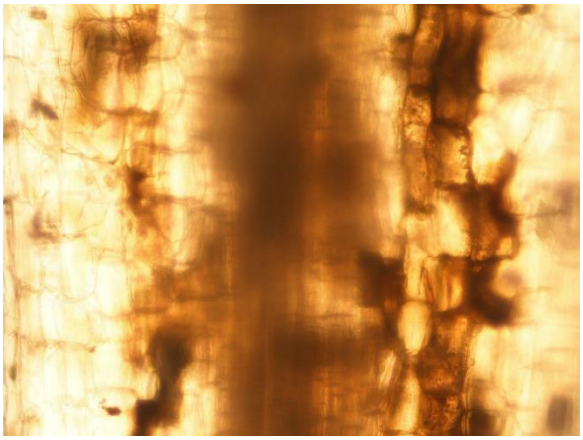
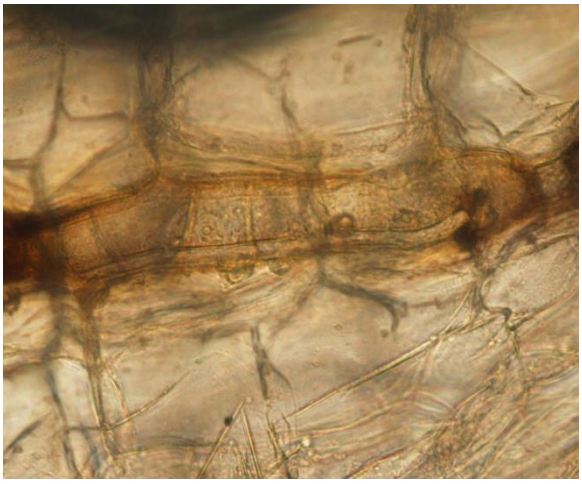


Figure 16. Necrotic stem tissue in *I. hawkeri* associated with downy mildew mycelium and haustoria.



Objective 1e: Biology and Epidemiology of IDM and CDM: Overwintering

IDM Survival in Monitor Plots with Natural Infections or Inoculations

Maryland (Bio-ARSFD-10, Bio-HC-10)

2013. Two monitor plots were set up to look for natural infection of Impatiens plants in Maryland and two other sites in Maryland were identified. One monitor plot at Fort Detrick contained 3 species of Impatiens (*I. walleriana, I. balsamina, I. capensis*); the other at Mount Airy contained *I. walleriana and I. balsamina*. A site in Fairplay MD contained *I. balsamina* and one in Silver Spring MD contained *I. walleriana*. These plots were checked daily for signs of infection. At Fort Detrick, MD, no disease was observed. At Fairplay, MD, disease was observed Aug 9; Mount Airy, MD August 14; Silver Spring, MD: September 17. Isolates were collected at Mount Airy and Fairplay for genetic analysis at ARS Beltsville. Debris at these sites was left *in situ* for overwintering experiments in the spring.

2014. Monitor plots were maintained at 3 sites in Maryland with two or three species of impatiens, and two of the species *(I. walleriana* and *I. balsamina*) became infected with IDM in late summer 2014. The third, *I. capensis*, was artificially infected in the laboratory using isolates derived from *I. balsamina*. Infected impatiens from the monitor plots in Frederick MD and Mount Airy, MD were left *in situ*.

Figure 17. A monitor plot set up in MD during the fall of 2014 around debris from infected impatiens.

Impatiens seeds and seedlings were planted in this soil in spring 2015.

In November of 2014, debris from infected *I. walleriana* plants (known from microscopic examination to contain oospores) was scattered in two 1m x 1m plots in Frederick MD; debris from other garden plants were scattered in two control plots. Two plots (one infested, one control) were set up in Mount Airy, MD.

2015. After danger of frost was over, plots were planted with seeds of *I. balsamina* (May 15 in Frederick, May 18 in Mount Airy). Seeds were slow to germinate in the unseasonably cool weather. Seedlings in the infested plot in Mount Airy emerged small and chlorotic and the cotyledons were found to be infected with IDM on June 9 (Figure 18). The control plot, 4 m away, was not infected as of July 21. Infected *I. balsamina* continue to grow weakly, and *I. walleriana* plants transplanted to the site on June 18 became infected, while I. capensis planted at the site continue to be unaffected. All Frederick plots have healthy plants as of July 21. While infection at the Mount Airy site may have been due to airborne inoculum, the same plot in 2014 became infected much later in the season, in the first week of August, and inoculum was assumed to have been airborne, since impatiens had not been grown on the site for at least 13 years (before the emergence of the disease in the US). Because the infected cotyledons were barely above the debris layer of the plot, circumstantial evidence suggests that they emerged already infected.

In spring 2015, seeds of *I. balsamina* were planted in three sites in Frederick and Mount Airy MD that had been treated with infected litter the previous autumn. In early June the emerging seedlings at the Mount Airy site were found to be infected with IDM, suggesting that they had been infected by the overwintering inoculum. No infection of plants was seen at the other two sites and no IDM was reported in Frederick until September. The three sites are being prepared for 2016; Infected *I. balsamina* litter at one site is being left *in situ* and clean seeds will be planted next spring; at the other two sites infested litter is being collected and laid down for overwintering.

Figure 18. *Impatiens balsamina* plants on June 9, 2015 in Mount Airy monitor plot that had been covered with oospore-infected debris the previous fall.

Seedlings were stunted and chlorotic and the undersides of the cotyledons were covered with sporangiophores of the pathogen.

2016. During spring 2016, no infection was observed in seedlings of *I. capensis* or *I. balsamina* planted in the site where infection was seen during 2015.

2017. In 2017 monitor plots (one meter square) were established at several locations (Fort Detrick MD, Walkersville MD, Mount Airy, MD). The plots consisted of four areas where infested debris from diseased plants had been left in place in fall 2017 and three where only non-diseased plants had been present. Plots were seeded with *I. capensis* and *I. pallida* (in fall) and *I. balsamina* (in spring as soon as the danger of frost was past). Seedlings in the seven monitor plots were monitored as they emerged and sampled (5-15 per plot). Sampled plants were cleared, dissected and microscopically examined for mycelium and oospores of *P. obducens*. Some seedlings from all the plots with infested debris emerged infected with *P. obducens*, in either the cotyledons, stems or roots. Infected plants left in the plots infected other plants. *Impatiens walleriana* was planted in some plots in June and became infected. Some plots contained *I. capensis* and *I. pallida* (wild or naturalized Impatiens); these never showed signs of infection in leaves, stems or roots. Infested debris has been left in place for next year, when the process will be repeated.

In previous work, systemic infection of *I. balsamina* was observed after exposure to vegetative sporangia of IDM. Now systemic infection has been observed when *I. balsamina* seeds were planted in pots containing oospores of *P. obducens* that had been conditioned for 1 month at 0 C. Infected seedlings were stunted and chlorotic and displayed sporulation when tissue was placed in moist chambers for observation. This suggests that overwintering oospores can infect plants through roots and systemically infected plants might be early sources of inoculum as in the case in other downy mildew disease systems, such as in sunflower downy mildew.

Heavy infestation of *I. walleriana* and *I. balsamina* was seen in September in two of our monitor plots; infected plants were left in place to allow overwintering of debris for next year's research. Pods from infected *I. balsamina* were collected from three sites to use seeds for histological studies and germination studies to determine if the pathogen is seed-borne.

Maryland Citizen Science Project (Bio-ARSFD-11)
Dozens of impatiens plants were given out at the 2017 and 2018 Fort Detrick Spring Research Festival and as part of the Fort Detrick Take-Your-Child-to-Work Day activities. "Citizen Scientists" were encouraged to report to our website (https://www.ars.usda.gov/northeast-area/frederick-md/foreign-disease-weed-science-research/docs/citizen-science-project/) when and if their plants developed downy mildew. Results in 2017 demonstrated a peak of IDM in June at a few Maryland locations, then another peak in late September over a wide area in West Virginia, Maryland, New Jersey, Pennsylvania and Delaware. In 2018 there were fewer respondents but also cool rainy weather all summer that caused widespread disease.

New York (Bio-CU-3)
2015. The sentinel plot on Long Island did not have any overwintering of balsam impatiens in 2015. It was suspected that Master Gardeners weeding the garden mistook the balsam as weeds. Neither balsams nor double impatiens showed any downy mildew at the Buffalo Botanical Garden as of the end of August 2015.

Monitor plots for observing oospore transmission were established in a field of Riverhead sandy loam soil at the Long Island Horticultural Research Lab in Riverhead, NY. Three rows of 22 impatiens at 2-foot spacing within and between rows were established in August 2015. These were naturally infested with *P. obducens* from a nearby plot during September, and plants developed severe symptoms and defoliated. These plants were allowed to collapse and decay naturally.

2016. In June 2016, plots of *Impatiens walleriana* 'Super Elfin White' were established by transplanting healthy seedlings into two rows, directly into the soil in an outdoor shaded hoop house. One row of 85 plants was planted into an area where impatiens had been killed by downy mildew (*P. obducens*) the previous summer, while a second row of 85 was positioned next to the edge of the house where impatiens had not been grown. Plants were examined weekly for any signs of downy mildew. Temperatures were in the 90s following transplant. Plants were set on trickle irrigation. Branches of stunted plants were brought into the lab every 14 days and sectioned to look for mycelium and haustoria of downy mildew, plated to isolate other pathogens, and held in moist chambers for 48 hours to encourage development of sporangia. Only some *Colletotrichum* sp. and *Rhizoctonia solani* were associated with plants showing stunting, and no *Verticillium* cultures or downy mildew sporulation were observed from these stunted plants. Overhead irrigation was supplied 3 times per week beginning mid-August. On 31 Aug one impatiens plant developed downy mildew, in the row that was 5 feet away from the row thought to be more at risk. No sporulation was found in any other plant at that time. The delay in sporulation is easily attributable to the high summer temperatures after transplanting to the field, and the lack of rain events. Two weeks later, every plant in the plot was showing sporulation of *P. obducens*. Our conclusion from this study is that oospores in the soil in this area were responsible for the initial infection. Whether the first plant to show symptoms was the first one to become infected they cannot know, but there were no other sources of downy mildew in the area, so overwintered oospore inoculum was likely to have been responsible for the symptoms that appeared on one plant out of 170. The epiphytotic progressed very quickly once sporulation was formed on that first plant that was presumably infected via oospores.
The infected plants were allowed to die in place.

2017. The overwintering monitor plot established in a high tunnel shade house at the Cornell University LIHREC was planted with 30 healthy greenhouse-grown transplants on May 30, 2017, in a row in the same location where plants were allowed to collapse naturally following IDM in 2016. Although plants were chlorotic and grew slowly, no IDM sporulation was observed in June, but ratings made 18 July indicated 16/30 plants were sporulating, with 1-6 branches per plant affected, and some leaf drop (Figure 22). Sporulating plants were found most often in the center of the row, where there would have been less air movement and thus probably higher humidity for disease development. Samples from each of these outbreaks and one from Litchfield Co., CT, were forwarded to Nicholas LeBlanc at JoAnne Crouch's USDA-ARS lab in Beltsville for genetic analysis. Because sporulation appeared at two other locations on the east end of Long Island prior to it being seen in the overwintering plot, aerial inoculum cannot be entirely ruled out for the LIHREC monitor plot—but the poor growth of the plants suggests that they might have been infected from the soil several weeks before showing sporulation.

A natural outbreak of *P. obducens* was found on Long Island in a home landscape in Cutchogue, NY on June 17, 2017 on *Impatiens walleriana* purchased and transplanted as plugs on May 27. The greenhouse grower who supplied these plants had not observed any symptoms during production, and impatiens purchased by the gardener's mother-in-law the same day at the same greenhouse source and planted 5 miles away grew normally (
Figure 19 - Figure 21). The gardener had not observed impatiens downy mildew disease in her yard since 2015 and had not used impatiens during 2016. She reported that mulch was not removed following the outbreak, so it appears likely that oospores produced in 2015 or earlier were still present and were activated by the frequent rains and cool temperatures in spring 2017. Rainfall of greater than 0.1 inch was recorded on 5/29, 6/5, 6/6, 6/16 and 6/20 and temperatures exceeded 85°F only June 11-14. Nighttime temperatures ranged from 56°F – 71°F in the two weeks preceding the day when the disease was noticed and were in the 50s prior to the short heat spell June 11-14—thus providing conducive conditions for *P. obducens*. Additional sightings of the disease in 2017 were made in Saratoga Springs, NY on July 3 and in Riverhead, NY on July 8, on *I. walleriana* planted where impatiens had been known to be diseased with downy mildew in earlier years. Balsam impatiens were seen infected on July 13, 2017 in Lockport, NY, in a garden in which plants had shown symptoms in several earlier years, but not 2016.

Figure 19. Healthy impatiens purchased from same retail grower as in Figure 20 growing in location 5 miles away

Figure 20. Impatiens declining due to overwintering IDM inoculum in Cutchogue, NY

Figure 21. Closeup of impatiens from Cutchogue landscape showing sporulation on undersurface of leaves on June 20

Figure 22. Impatiens plant with IDM in overwintering plot at Cornell University LIHREC

Determine whether oospores survive over winter (Bio-CU-4)

In the monitor plots established in Maryland and New York, there was evidence of early *P. obuscens* infections, well before infections from aerial sporangia could occur. Experiments were undertaken to observe similar early infections in more controlled environment using infected plant stems and soil collected during 2012 and 2013 in Suffolk, Monroe and Tompkins Counties, NY. In two experiments, a tablespoon of soil collected from the vicinity of DM infected impatiens was added to the soilless growing medium around 20 transplanted *I. walleriana* seedlings. The medium was flooded for 24 hours immediately after inoculum was added. The impatiens plants were bagged individually along with individual non-inoculated controls. No sporulation developed on any of the plants in one month, but a number of these impatiens were lost to *Rhizoctonia solani* stem cankers or foliar blight. However, Dr. Shishkoff was successful at observing oospore germination to produce zoospores in one sample from Mt. Sinai, Long Island, NY. they have had more success with stems of infected plants from the previous growing season used as inoculum.

To more rigorously examine the potential for different sources of *P. obducens* inoculum to infect *I. walleriana*, they conducted series of experiments utilizing oospores in soil, in dried impatiens stems or in water, and zoospores from sporangia.

Oospores in soil.
On 2 Feb 15, dried stems of diseased impatiens (1.1 g) held from the 2014 growing season were used to make an oospore inoculum preparation. Stems were ground with a mortar and pestle with 1T of sterile sand added. One T of sterile deionized water was added and the material was allowed to hydrate for 48 hrs. On 5 Feb, the stem material was rubbed through 150 micron and 105 micron sieves, and collected on a 38 micron sieve. The collection was rinsed into a beaker with 40 ml water. Oospores were observed in the slurry using the microscope; none were seen in the water that ran through the 38 micron sieve. The collected oospore material was ground again and frozen overnight.

On 5 Feb 15, four impatiens seedlings, raised in a disease-free growth chamber in 804 packs, were transplanted to 4-in. pots in soilless mix and double-bagged to serve as controls. Four additional plants were lifted after transplanting, and 1/8 tsp. of the frozen ground oospore inoculum was added to each root ball, adding some to all sides of the root ball, before replanting. Each pot was given 400 ml water after double-bagging, to saturate the soil. Excess water was poured off 24 h later. Plants were maintained in a growth chamber with 12 h light:12 h darkness, at 70°F.

Plants were observed for sporulation biweekly. No sporulation was evident 22 April; plant (still double bagged) were moved outside to a plastic covered hoop house.

On 2 June 15, stunting and yellowing of one of the inoculated plants was noted relative to the controls. Closer examination showed *P. obducens* sporulation on many of the leaves. The plant showed symptoms of systemic infection with downy mildew. Symptoms were not seen in other inoculated plants or in the controls. Low inoculum levels may have been responsible for the low 20% infection rate observed in this initial trial.

Figure 23. Comparison of non-inoculated (left) and soil inoculated (right) impatiens plants.

Oospores in dried impatiens stems.
An experiment was initiated 20 May 2015 to test the ability of oospores in dried impatiens stems (that had received freezing treatments in the lab) to serve as inoculum for infection of 4 wk old seedlings of *I.* 'Super Elfin White' and *I. balsamina* 'Camellia flowered'. Clear plastic boxes (6 qt capacity) were filled with 2L peat-lite germination mix moistened with 1 L tap water, and 4 impatiens or 4 balsam seedlings (1st true leaf stage) were transplanted from a clean growth chamber into each of 8 boxes (4/species). Control boxes (1 for each species) received no inoculum. Inoculum for Treatment 1 was the same as that used for Experiment 1 above (frozen & sieved ground dry stems). Inoculum for Treatment 2 was made using 1.9 g dried stems, ground in a

mortar and pestle to break up the tissue prior to sieving and collection on a 38-micron sieve and then frozen and thawed alternately over a 7 day period after drying down. Inoculum for Treatment 3 was made from dry stems that were ground but not sieved or frozen.

For Treatments 1, 2 and 3, 1/8 tsp of the respective inoculum was added to the wet germination mix next to each plant. Boxes were incubated in a growth chamber at 70F with 12 hours light and 12 hours darkness, adding water as necessary to maintain a flooded soil condition. On 26 May 15, 2 seeds of impatiens or balsam were added next to each original transplanted seedling to provide additional, very young, trap plants to detect zoospores formed from oospores in the inoculum.

None of the treatments resulted in downy mildew infection after plants were held for 2 months.

Oospores in water, petri dishes
Complementing the above experiment, individual seedlings were laid individually into petri dishes with water and 1/8 tsp. of one of the 3 inoculum preps on 20 May 15 (frozen & sieved ground dry stems; repeatedly frozen & sieved ground dry stems; ground dry stems without freezing or sieving), and these were examined microscopically for 1 week in hopes of detecting swimming zoospores moving to the roots. Occasional small swimming cells were observed in these petri dishes, but their identity could not be determined visually and there was no "swarm" of potential zoospores. None of these seedlings appeared to become infected during the observation period.

Zoospores from sporangia.
On 1 May 2015, individual impatiens leaves covered with sporangia were added to beakers of water, and then impatiens were placed into these beakers, and held in the laboratory under fluorescent lighting. Plants in noninoculated beakers served as controls. One month later, 26 May, yellowing and stunting (typical of systemic downy mildew infection) was apparent in the inoculated plants relative to the controls. The plants were bagged, with the addition of a wet paper towel to increase humidity, and moved to the growth chamber with 12 h light: 12 h darkness at a constant 70F.

Downy mildew sporulation was evident on the inoculated plants, but not the controls, 24 h later. This is evidence that impatiens roots in flooded soil are susceptible to zoospore infection originating from sporangia. In order to demonstrate oospores as a source of effective inoculum in flower beds, it will be necessary to have a sufficient quantity of oospores in inoculum to develop enough sporangia that will develop enough zoospores after flooding for successful infection. Different methods of handling the tissue will be tried in 2015-2016 and further results will be reported under 15-8130-0274-CA.

Figure 24. Close up of infected impatiens plant after root inoculation with zoospores.

Oospores in water, plastic bags

Because our earlier results suggested that oospore inoculum quantity may have been insufficient in trials to see uniform infection, 30 garden impatiens were planted midsummer 2015 to use as a source of more concentrated inoculum in winter growth chamber trials. These plants were grown in a greenhouse free from *P. obducens*, transplanted to 1 gal nursery containers, and taken to a shade-covered plastic hoop house during August 2015. Plants were exposed to inoculum from nearby downy mildew infected *I. walleriana*, and developed symptoms that progressed to nearly complete defoliation (Figure 25). These infected plants were kept outdoors exposed to natural conditions until after the first frost, at which time stems were harvested and stored in paper bags.

Figure 25. Downy mildew infected impatiens whose stems will be used for oospore inoculum

To prepare the harvested stems as an inoculum source, the stems were placed in a freezer for 2 weeks prior to grinding with a mortar and pestle until fine-textured. One teaspoon of this ground material was added to each of 4 plastic bags containing 50 mls water and a young impatiens plant ('Super Elfin White' grown in peat-lite mix) on February 11. Plants were given 12 hrs light and 12 hrs dark in the lab, at room temperature, and bags were opened weekly to check for symptoms or signs. There were 4 control bags and 4 with inoculated plants (given ground stems). 1 of 4 inoculated impatiens developed downy mildew sporulation, which was visible on March 4th. Control plants remained healthy. This adds to the evidence for oospores being effective as overwintering inoculum for impatiens downy mildew.

In 2015, gardeners generally reported very late (October) onset of impatiens downy mildew in plantings made into previously affected flowerbeds. Late onset indicates that the quantity of inoculum is not the only important factor in downy mildew development: environmental conditions determine when soil inoculum will be triggered to produce zoospores and initiate infections, just as they affect foliar infection.

Seed infection in naturally infected plants of I. balsamina (Bio-CU-5, Bio-ARSFD-12, Bio-HC-12)

Balsam impatiens seed harvested from Long Island garden plants infected with downy mildew in 2013 was planted in 10 pots that were kept in sealed plastic bags in the greenhouse. Of 20 seed, 11 germinated, and of these 2 plants developed a coating of downy mildew sporulation on their first pair of true leaves. As this trial was conducted in closed bags in a mildew-free greenhouse in January, the observation of *P. obducens* sporangiophores and oospores was convincing evidence that balsam impatiens can have a role in overwintering of the pathogen. Whether this is from surface contamination of the seed with oospores or from contamination with downy mildew inside the seed is not yet clear.

Seeds planted in the greenhouse from balsam impatiens infected in nursery containers in 2014 yielded only healthy plants in 2015.

Subsequently, seed of infected *I. balsamina* from Long Island, NY was collected and sent to Ft. Detrick for testing.

At Fort Detrick, litter from infected *I. balsamina* was collected, seeds were given a cold treatment and then germinated. However, germination trials in 2015/2016 did not yield any infected plants. Efforts continued in summer 2016 to obtain infected seed.

Preliminary experiments were then conducted to determine if systemic infection of plants from infested seed can be detected. *Impatiens balsamina* seedlings were exposed through roots only to a suspension of IDM sporangia, and some plants grew poorly, with sporulation of IDM along the petioles of leaves, suggesting systemic infection.

Subsequently, seeds planted from balsam plants infected in 2016 were planted in the greenhouse in packs grown within plastic bags (5 bags containing 4 seedlings each) to enclose any resulting sporulation. After two and a half months of examination (April – June) the plants were discarded after examination for stunting or sporulation indicative of downy mildew. No plants exhibited symptoms or signs, which is unusual considering that oospores were detected adhering to seed from the same collection.

In the fall of 2017, seed pods of infected *Impatiens balsamina* from 4 MD locations were collected and labeled. Some of the pods were placed in 70% ethanol for at least 6 months to clear the tissue. Other pods were opened, the seeds removed and stored at 4 C for future planting and the pods placed in ethanol.

One hundred and four of these pods have been examined for the presence of oospores and mycelium of downy mildew. For each pod, the pedicel was split and examined microscopically for oospores, then the pod was opened and rated for presence of oospores in the ovary wall, locule partitions and columella, as well as each funiculus. If seeds were present, each seed was rated for oospores present on the hilum and the outer and inner layers of the seed coat. The Embryo was removed, checked for signs of discoloration, surface hyphae or irregular growth. If any such signs were present, the embryo was crushed and examined.

Many pods contained oospores in some part of the ovule tissue. Although pedicels were often internally infected with oospores, it was clear that pods could be infected on the outer surface, with mycelium spreading inward to the locule paritions and the central columella. In the case of severe infection, the locule cavities themselves became filled with downy mildew mycelium and oospores. Some of the 1328 seeds present inside the pods had oospores present on the outer seed coat or hilum. In one case an oospore was observed in embryonic tissue in a young seed that looked nonviable.

Of the 19 infected *I. balsamina* seeds examined from the 2015 New York collections, 16 of them had oospores present on adhering tissue, the hilum or the outer cell wall (Table 15). In two instances, haustoria were seen entering seed cells, but only in the outer layer of the seed. No oospores were seen on the inner aril layers or in the seed embryo. This association of oospores with the outer portions of the balsam seed indicated that *P. obducens* was well positioned to be carried over to the following year on seed from infected balsam impatiens.

Table 15. Presence of oospores on seeds of *Impatiens balsamina*.

seed	in ovary tissue adhering to seed	on hilum	on outer layer of aril	in inner layer of aril	in scleroid layer	in embryo
1	**y**	**y**	n	n	n	n
2	n	n	n	n	n	n
3	**y**	n	n	n	n	n
4	**y**	n	n	n	n	n
5	n	n	n	n	n	n
6	n	**y**	n	n	n	n
7	**y**	**y**	**y***	n	n	n
8	**y**	n	n	n	n	n
9	**y**	n	n	n	n	n
10	n	**y***	n	n	n	n
11	**y**	n	n	n	n	n
12	**y**	n	n	n	n	n
13	n	**y**	n	n	n	n
14	**y**	n	**y**	n	n	n
15	**y**	n	**y**	n	n	n
16	n	n	**y**	n	n	n
17	n	n	n	n	n	n
18	**y**	n	n	n	n	n
19	**y**	n	n	n	n	n

A "Y" indicates oospores were seen in conjunction with a tissue type (ovary tissue, hilum, outer aril layer, inner aril layer, scleroid layer or embryo tissue. An asterisk indicates that mycelium with haustoria were observed.

Some infested debris from outdoor plots was taken indoors after overwintering outdoors and was spread on the surface of trays of sterile potting mix, which was then sown with clean *I. balsamina* seed in two trials (a total of 960 seeds). In each trial, some infected plants emerged, suggesting that infested debris could infect germinating seedlings. In 2018 hundreds of pods were collected from infected and uninfected Impatiens plants for additional study.

An additional 2000 seeds from infected plants and uninfected controls were cold-conditioned and planted in sterile potting mix (in six trials) to look for seed-borne infection. Germination of seeds from infested pods was low, but one seedling emerged infected with impatiens downy mildew. In 2018, additional seeds from infested pods were being cold-conditioned for trials later that spring.

Objective 1f: Biology and Epidemiology of IDM and CDM: Alternative Hosts

Role of Alternate Hosts for IDM (Bio-CU-6)

A study in summer/fall 2015 exposed 23 *Impatiens* species or hybrids to inoculum of *P. obducens* in a shaded hoop house provided with overhead irrigation twice daily. A number of new hosts for the downy mildew were identified, using molecular sequencing of the rDNA ITS region (provided by collaborator Catalina Salgado at USDA-Beltsville) to verify the identity of *P. obducens* on plants showing downy mildew symptoms or sporulation. All of the samples showed 100 percent nucleotide identity with *P. obducens* from *I. walleriana*. The downy mildew that is so destructive to *I. walleriana* caused scattered leaf lesions and occasional stem lesions on *I. briartii*, *I. cinnabarina*, *I. grandis*, *I. irvingii*, *I. laurentii*, *I. repens* and *I. sodenii* var. *uguensis*, none of which had previously been reported anywhere in the world as hosts of *P. obducens*. *Impatiens briartii* and *I. cinnabarina* were two of the plants susceptible to *P. obducens* during this trial (Figure 26 through Figure 28). No *Impatiens capensis* plants developed symptoms or signs of downy mildew during these 2015 trials. Thus far

no other species of impatiens has been identified that is as susceptible as *I. walleriana* to *P. obducens*. Other impatiens species may hold genetic solutions to the downy mildew problem to which *I. walleriana* is so susceptible.

Figure 26. ***Impatiens cinnabarina*****, a new host for downy mildew caused by** ***P. obducens***

Figure 27. Downy mildew caused chlorosis, blackening or reddening on infected leaves of ***I. cinnabarina***

Figure 28. Closeup of downy mildew on *I. cinnabarina*

Impatiens walleriana Cultivar Screening (Bio-CU-7, Bio MSU-3)

New York.

A range of white and colored-flower cultivars of the common impatiens, *I. walleriana*, were compared to see if there is a correlation between flower color and downy mildew susceptibility. Seeds of 28 colored- and white-flowered cultivars were received from Syngenta and Benary and seeded 4 Aug in 128-cell trays. Plugs of a red and a white cultivar were supplied from Ball Seed. All were transplanted on 29 Aug into 4.5" pots in Promix BX. At planting, 0.3 g Osmocote was added to each pot. Fertilizer (15-5-15) was also used on a constant-liquid-feed basis while plants were in the greenhouse. The impatiens were moved outdoors on 28 Sept, which put them immediately into contact with downy mildew inoculum available from adjacent experiments. Plants were watered 15 min each day at 5:40 am. The impatiens plants were rated on disease incidence on 9 October by counting the number of leaves showing downy mildew sporulation on each plant. On 12 October, plants were rated on a 5-point quality scale, with 1=defoliated, 2=some leaf drop, 3=advanced chlorosis, 4=one yellow leaf and 5=healthy plant.

There was a surprising similarity in susceptibility to downy mildew across all cultivars. It is quite apparent that *I.* from a diversity of genetic backgrounds are extremely susceptible to *P. obducens*, the cause of downy mildew, regardless of flower color. The various white flowered cultivars showed some of the most and some of the least sporulation on 9 Oct. There was some statistical separation when the sporulation of the leaves was rated: Super Elfin White was distinguished by having the highest number of sporulating leaves, a greater number than 24 of the other plants tested. Lollipop Raspberry Violet had more sporulating leaves than Accent Premium Salmon and Accent Premium Red on 9 Oct. The two cultivars with the lowest number of sporulating leaves were Accent Salmon and Accent Premium Red. Most of the cultivars were statistically similar. It is possible that some plants with lower counts were actually advanced in disease development (defoliated leaves could not be counted). Most importantly, the plant quality rating on 12 October indicated that all the plants were similar, with the mean score rating varying only from 3.0 to 4.2. None were unaffected by the disease, and plants continued to defoliate after this rating on 12 October. It appears that inter-specific crosses will be needed to provide significant resistance to impatiens bedding plants.

In the summer of 2017, they also assisted in the evaluation of the F1 generation of impatiens (interspecific hybrids) being developed by Dr. Mark Bridgen, Cornell Sections of Horticulture and Plant Breeding. Breeding will continue on these lines, and plants will be evaluated each year for performance in the presence of downy mildew disease.

Table 16. Downy Mildew Rating on Impatiens Cultivars

Series	Color	No. leaves w/ DM sporulation* 9 October 17	Plant Quality Rating** 12 October 17
SuperElfin	White	50.8 a	3.6
Lollipop	Raspberry Violet	36.6 ab	3.6
Accent Premium	Lilac	31.6 abc	3.4
Xtreme	Orange	31.2 abc	3.6
SuperElfin	Red	29.4 abc	3.4
Tumbler	Violet	27.4 abc	3.0
Lollipop	Fruit Punch Rose	25.4 bc	3.6
Lollipop	Pomegranate	25.2 bc	3.8
Xtreme	Bright Eye	23.2 bc	4.2
Accent Premium	Rose	22.2 bc	4.0
Tumbler	Rose Star	22.2 bc	3.0
Lollipop	Peach Salmon	21.8 bc	3.8
Xtreme	White	21.6 bc	3.4
Lollipop	Bubblegum Pink	21.0 bc	3.0
Lollipop	Cherry Red	20.8 bc	3.8
Xtreme	Salmon	20.6 bc	3.6
Xtreme	Violet	20.2 bc	3.0
Lollipop	Coconut White	19.6 bc	3.0
Accent Premium	Pink	18.4 bc	3.4
Accent	Deep Orange	18.0 bc	3.8
Tumbler	White	17.6 bc	4.0
Xtreme	Pink	17.2 bc	4.0
Tumbler	Scarlet	17.2 bc	3.6
Accent Premium	Bright Eyes	17.0 bc	3.8
Xtreme	Lilac	16.8 bc	3.8
Xtreme	Bright Rose	15.4 bc	3.2
Accent Premium	Violet	15.2 bc	3.2
Accent Premium	White	14.6 bc	3.8
Accent Premium	Salmon	11.0 c	3.6
Accent Premium	Red	9.0 c	3.4
			NS

* Values represent means of 5 single-plant replications of each cultivar, counts made of the number of leaves with downy mildew sporulation on each plant. Values followed by the same letter are not significantly different (Tukey's Protected LSD, P=0.05).

** Values represent means of 5 single-plant replications of eeach cultivar, rating plants on a 5-point scale with 5=healthy. NS indicates there were no statistically significant differences.

Michigan

Dr. Mary Hausbeck et al completed two cultivar screens in 2013, one of which was conducted in the greenhouse (25 varieties), the second in the landscape (28 varieties), to determine if a particular series or cultivar showed any resistance to the pathogen.

Role of alternate hosts in CDM outbreaks by using monitor plots (Bio-NCSU-1, Bio-MSU-3)

To determine if wild cucurbit hosts could be serving as natural reservoir of CDM in the US they planted duplicated field plots of both commercial and wild cucurbit hosts, as well as possible alternative hosts (Table 17). In total they established three field plots located in the western (Waynesville), central (Cleveland) and eastern (Kinston) areas of NC that have significant cucurbit production. Plots were scouted weekly and once a CDM natural infection was confirmed in the field by visual inspection, leaves were be collected from all hosts with foliar lesions, and isolates stored for later experiments.

Koch's postulates were completed in the lab for any wild or alternative host that presented a CDM infection based on visual inspection and a molecular confirmation of CDM as the cause of infections observed in the field. Molecular confirmation of a CDM infection was performed by amplifying and sequencing the internal transcribed spacer (ITS) region.

Table 17. Non-commercial or alternative hosts planted and infected by CDM in NC

Host	Common name	Infected by CDM	New report
Momordica charantia	Bitter melon	Yes	Yes, published (1)
Momordica balsamina	Balsam apple	Yes	Yes, published (1)
Cucurbita foetidissima	Buffalo gourd	Yes	Yes, published (2)
Luffa cylindrica	Luffa	Another DM detected	Yes, report of new *Plasmopara* spp. published (2)
Melothria pendula	Creeping cucumber	Yes	Yes, Koch's postulates in progress
Lagenaria siceraria	Bottle gourd	Yes	Yes, Koch's postulates in progress
Humulus lupulus	Hop	No	

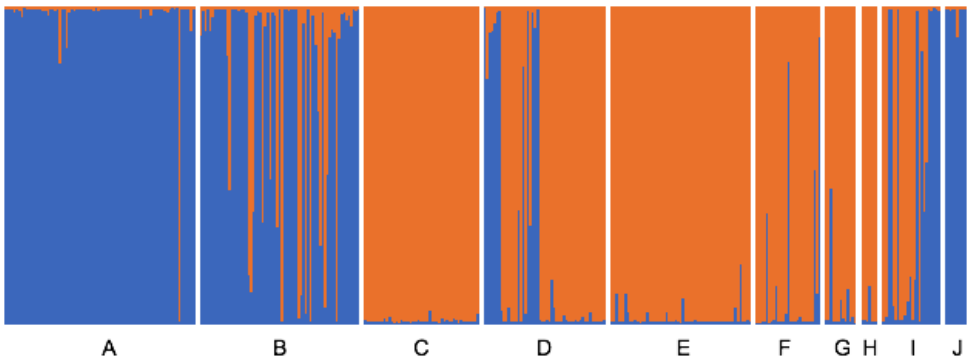
(1) Wallace E., Adams M., Ivors K., Ojiambo P., and Quesada-Ocampo L. M. (2014) First report of *Pseudoperonospora cubensis* causing downy mildew on *Momordica balsamina* and *M. charantia* in North Carolina. Plant Disease 98: 1279.
(1) Wallace E., Adams M., and Quesada-Ocampo L. M. (2015) First report of downy mildew on buffalo gourd (*Cucurbita foetidissima*) caused by *Pseudoperonospora cubensis* in North Carolina. Plant Disease: in press.
(2) Wallace E., Choi Y. J., Thines M., and Quesada-Ocampo L. M. (201X) First report of *Plasmopara* aff. *australis* on *Luffa cylindrica* in the United States. Plant Disease: in press.

Ten simple sequence repeat (SSR) or microsatellite markers were applied to 385 isolates from six commercial and three non-commercial cucurbits from the three locations representing different growing regions across NC. Population analyses revealed the greatest genetic differentiation by host species, which supports the concept of host-driven differentiation (Table 18). Two host adapted subpopulations in *P. cubensis* were discovered with one clade occurring more frequently in *Cucumis* species and the other in *Citrullus*, *Momordica* and *Cucurbita* species, except for *Cucurbita foetidissima* (Figure 29, Wallace et al., submitted). The lowest genetic differentiation occurred between isolates from *Momordica balsamina* and *Cucurbita pepo* and *Cucurbita moschata*. Furthermore, the highest genetic differentiation occurred between isolates from *Cucumis* versus *Citrullus* and commercial *Cucurbita* hosts. Surprisingly, the isolates from *Cucurbita foetidissima*, a wild perennial cucurbit, are less differentiated from isolates from *Cucumis* than they are differentiated from isolates from commercial *Cucurbita* species. These findings suggest that wild and non-commercial cucurbits are playing a role in *P. cubensis* diversification, and possibly contributing inoculum for different genera of commercial cucurbit hosts.

Table 18. Pairwise Population Matrix of Nei Unbiased Genetic Distance of *P. cubensis* isolates grouped by host species

	Cucumis sativus	*Cucumis melo*	*Cucurbita pepo*	*Cucurbita maxima*	*Cucurbita moschata*	*Citrullus lanatus*	*Cucurbita foetidissima*	*Momordica charantia*	*Momordica balsamina*
Cucumis sativus	0.000								
Cucumis melo	0.011	0.000							
Cucurbita pepo	0.330	0.237	0.000						
Cucurbita maxima	0.188	0.115	0.018	0.000					
Cucurbita moschata	0.324	0.226	0.000	0.013	0.000				
Citrullus lanatus	0.309	0.208	0.036	0.035	0.033	0.000			
Cucurbita foetidissima	0.050	0.049	0.216	0.131	0.215	0.157	0.000		
Momordica charantia	0.133	0.082	0.148	0.087	0.146	0.087	0.090	0.000	
Momordica balsamina	0.263	0.178	0.000	0.003	0.000	0.016	0.170	0.097	0.000

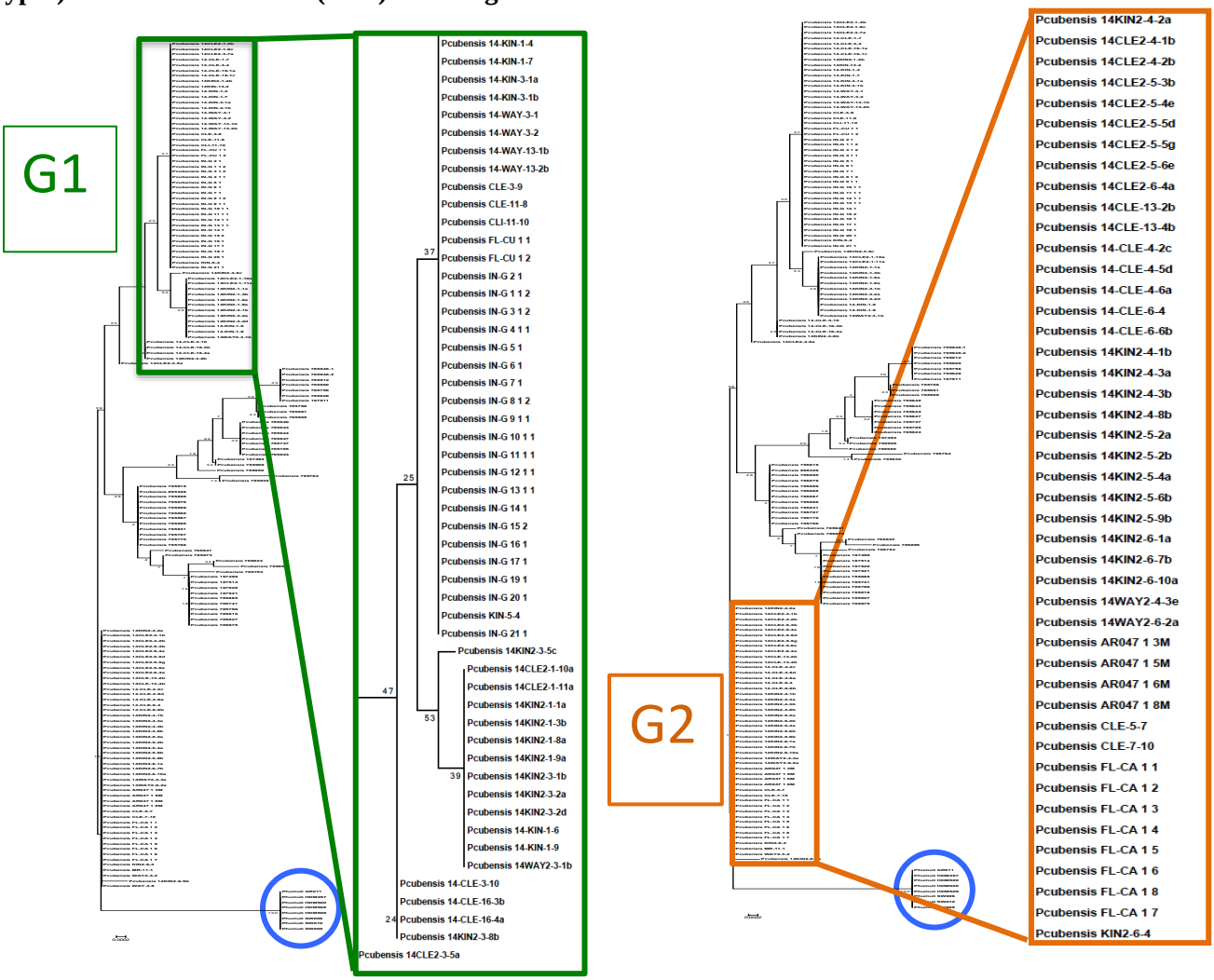
Figure 29. Population structure analysis of 385 *P. cubensis* isolates using 10 microsatellites revealed the presence of two host-adapted subpopulations (blue and orange).



Isolates are sorted by host of origin: A, *Cucumis sativus* (cucumber); B, *Cucumis melo* (cantaloupe); C, *Cucurbita pepo* (acorn squash); D, *Cucurbita maxima* (pumpkin); E, *Cucurbita moschata* (butternut squash); F, *Citrullus lanatus* (watermelon); G, *Momordica charantia* (bitter melon); H, *Momordica balsamina* (balsam apple); I, *Cucurbita foetidissima* (buffalo gourd); J, *Lagenaria siceraria* (bottle gourd) (Wallace et al., submitted).

Based on our previous findings that isolates from *Cucurbita foetidissima*, a wild perennial cucurbit, are less differentiated from isolates from *Cucumis* than they are differentiated from isolates from commercial *Cucurbita* species, molecular phylogenetic analyses of *P. cubensis* isolates from *Cucumis* and *Cucurbita* species were performed. Using nuclear (*ITS* and *ypt1*) and mitochondrial (*cox2*) genetic regions a multilocus sequence analyses (MLSA) tree was generated to study each populations genetic diversity and similarities (Figure 30). Not surprisingly, isolates from *Cucumis* and wild hosts *Cucurbita foetidissima* and *Lagenaria siceraria* separated in the same group (G1) whereas all isolates from *Cucurbita* species separated in another group (G2). All 8 *P. humuli* isolates, which were used as outgroup, separated in a different group. This data further consolidates our hypothesis that wild and non-commercial cucurbits could play an important role in *P. cubensis* diversification, and possible source of inoculum.

Figure 30. Phylogenetic tree based on MLSA analysis of 162 *P. cubensis* isolates using 2 nuclear (ITS and ypt1) and 1 mitochondrial (cox2) DNA region.



Isolates in the MLSA tree by host of origin: G1 (green box): *Cucumis sativus* (cucumber), *Cucumis melo* (cantaloupe), *Cucurbita foetidissima* (buffalo gourd) and *Lagenaria siceraria* (bottle gourd); G2 (orange box): *Cucurbita pepo* (acorn squash), *Cucurbita maxima* (pumpkin), *Cucurbita moschata* (butternut squash) and *Citrullus lanatus* (watermelon). Blue circle: 8 *P. humuli* isolate.

Additional research, outside the scope of this project, is underway investigating phylogenetic relationship of *P. cubensis* isolates from several different hosts including *Cucumis* sp. *Cucurbita* sp. and other wild cucurbit plants using 3 additional mitochondrial genetic regions, grand total of 6 genetic regions (2 nuclear and 4 mitochondrial).

Objective 2a: Genomic Resources & Diagnostics: Sampling

Collect and process CDM and IDM (Gen-CU-1, Gen-MSU-1, Gen-NCSU-1, Gen-UF-1)

Sampling of CDM and IDM during calendar years 2013 and 2014 was completed. Specimens of IDM were collected from across NY state and soil samples were gathered from gardens where the disease occurred. Samples of infected impatiens during 2013 from MI and NJ have been sent to Dr. Joanne Crouch at USDA-ARS, and infected cucumbers from MI sent to Dr. Lina Quesada at NCSU.

For 2014, trap plants of healthy *Impatiens walleriana* were distributed to Erie, Saratoga, Niagara, Tompkins and Suffolk Counties, NY in spring 2014, and monitored by Master Gardeners and other collaborators. Leaf samples of IDM occurring in the retail trade in spring 2014 were collected from two NY garden centers. IDM also was collected from trap plants of *I. walleriana* in Saratoga and Niagara Counties, NY and from Newark, DE on balsam impatiens and trap plants. All 2014 isolates were sent to the Crouch Lab at Beltsville and the Warfield Lab at Ball Horticultural Company for genotyping, NJ isolates were also sent to the Shishkoff Lab at Fort Detrick. In addition, soil core samples were collected in Ontario, Saratoga and Suffolk Counties and sent to the Crouch Lab for analysis.

In addition to samples collected by this research team, Crouch et al prepared a "Wanted" poster and mailed it to 150 diagnostic clinics, master gardeners, state agricultural agents, and plant pathologists, requesting samples of IDM. A website connected to the posters was developed (www.OrnamentalPathology.com) to facilitate sample submission.

Objective 2b: Genomic Resources & Diagnostic: Genetic Resources

Genomic Resources for CDM (Gen-NCSU-2)

Identify species-specific genes in *P. cubensis* by using comparative genomics with *P. humuli*.

Dr. Quesada'a lab extracted and sequenced single-end RNA libraries from eight *P. humuli* (hop DM) isolates after performing a sporangia-increase on detached hop leaves (Table 19). They mapped all the reads from *P. humuli* isolates to the *P. cubensis* genome, which allowed identification of species-specific exons (1,099 exons) that are only present in *P. cubensis* and not in *P. humuli* (Figure 31).

In addition, they obtained DNA and RNA from one *P. humuli* (hop DM) sample from 'Centennial' hop in Oregon for DNA and RNA sequencing after performing a sporangia-increase on detached hops leaves (Table 3). Paired-end (PE) DNA and RNA libraries were made, assessed for quality and sequenced at a length of 100 bp. A total of 36,599,100 and 21,778,230 high-quality (>30) reads were obtained from the DNA and RNA samples, respectively. Even though, obtaining a genome for *P. humuli* was not an explicit objective in this project, due to the high-quality data obtained, they assembled the data to determine if it was possible to generate an annotated reference genome for *P. humuli*.

Determine the intra-specific genetic variation of *P. cubensis* by sequencing transcriptomes of strains representative of the pathogen's diversity in the US.

Quesada et al extracted and sequenced single-end RNA libraries from seven *P. cubensis* (cucurbit DM) isolates after performing a sporangia increase on detached cucurbit leaves (Table 19). They obtained high-quality sequencing data for all isolates, and for most isolates they obtained more than 10 million reads. The goal was to obtain at least 10-15 million reads per isolate.

They mapped all the reads from diverse *P. cubensis* isolates to the *P. cubensis* genome, which allowed identification of exons that are always expressed in *P. cubensis* (2,250 exons) as well as exons that are isolate-specific (Figure 31). For developing a diagnostics tool, the goal was to identify genomic regions (genes, exons, or SNPs) that are conserved among different *P. cubensis* isolates.

Establish target genomic regions for diagnostics and marker development by using comparative genomics and bioinformatics approaches.
Seventy nine (79) exons that are consistently present in different *P. cubensis* isolates but not in *P. humuli* isolates were selected as candidates for cucurbit DM diagnostics (Figure 31). The parameters set to identify these candidates were very strict to increase the likelihood of *P. cubensis* selective markers, however, these parameters could be relaxed to cast a wider net of candidates.

To identify possible SSR markers for *P. cubensis*, they performed a whole-genome search (23,522 genes) and identified 2,738 SSRs contained in 2,398 genes. This means that 12% of the *P. cubensis* genome contains SSRs, and the most abundant type were trimmers, which corresponded to 61% of SSRs identified. They were able to design primers for 2,263 of the total SSRs,

For additional research and validation on these markers, please see Objective 2c: Genomic Resources & Diagnostics: Primer/Probe Development

Figure 31. Candidate diagnostic markers for *P. cubensis* identified through comparative genomics with *P. humuli*.

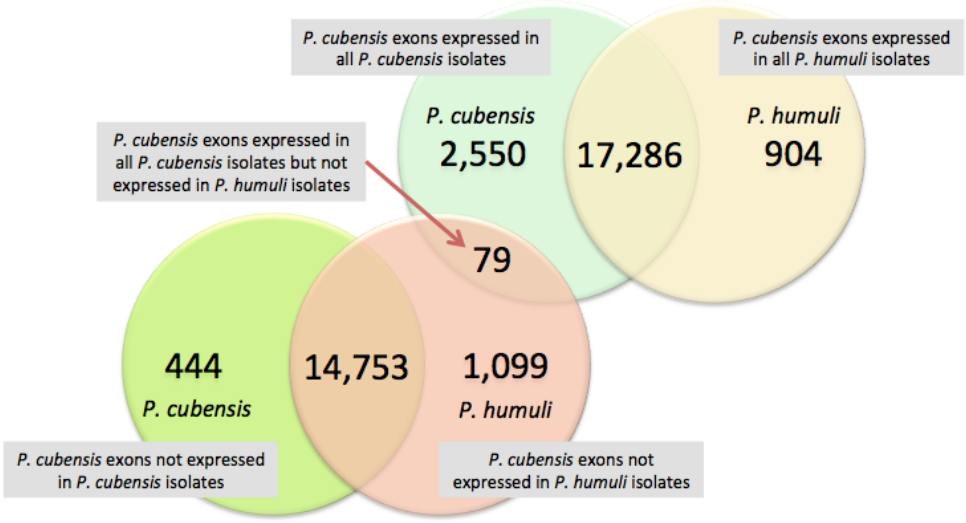


Table 19. Downy mildew isolates sequenced to date and quality assessments.

Code	Species	Host	Region	Total reads (all with >30 quality)	Reads mapped to *P. cubensis* reference	Reads mapped to cucumber reference
DNA seq PE						
HDM502AA	*P. humuli*	Hop (Centennial)	OR	36,599,100	95.99%	13.20%
RNA seq PE						
HDM502AA	*P. humuli*	Hop (Centennial)	OR	21,778,230	97.46%	12.02%
RNA seq SE						
CUCA11	*P. cubensis*	Cucumber	NC	24,982,992	85.5%	11.15%
CUC2013C3	*P. cubensis*	Pumpkin	NC	8,131,394	87.66%	6.51%
CUCWAY2-1	*P. cubensis*	Cucumber	NC	21,038,800	94.58%	6.70%
CUC1982	*P. cubensis*	Cantaloupe	SC	22,117,725	93.6%	6.24%
CUC08A1	*P. cubensis*	Cucumber	CA	20,500,146	89.89%	15.23%
CUC2013F2	*P. cubensis*	Acorn squash	SC	16,956,357	79.89%	5.8%
CUCDM_D3	*P. cubensis*	Butternut squash	SC	29,304,711	95.08%	4.26%
HDM502AA	*P. humuli*	Hop (Centennial)	OR	26,116,982	91.56%	19.16%
HDM510-1	*P. humuli*	Hop	WI	6,324,605	86.64%	8.9%
HDM501BA	*P. humuli*	Hop (Mt. Hood)	OR	14,429,953	88.53%	5.55%
HDM490	*P. humuli*	Hop	Japan	11,942,671	66.91%	6.45%
HDM498SA	*P. humuli*	Hop	Japan	12,421,813	85.25%	8.43%
HDM503A3	*P. humuli*	Hop (Horizon)	VT	14,576,962	91.75%	5.69%
HDM482CA	*P. humuli*	Hop	NY	15,906,769	91.45%	7.54%
HDM490-5	*P. humuli*	Hop	Japan	20,211,068	92.12%	4.58%

Genomic Resources for IDM (Gen-ARSB-1)

Three different strategies were employed to develop genetic resources for understanding the diversity of *P. obduscens* and used to analyze new and herbarium accessions of this organism.

Ribosomal/ITS DNA Marker Genotypes

Ribosomal/ITS DNA markers are the first choice for genotypic analysis of organisms without any existing genetic resources, as "universal" primers can be utilized. As a result, the rDNA ITS is considered the official "barcode" marker for many organisms, including the fungal kingdom. The rDNA/ITS is multi-copy, so it can be easier to PCR amplify, and concerted evolutionary processes typically prevent the accumulation of mutations in individual genes. But the rDNA/ITS may be insufficiently variable to fully reflect genetic variation, especially at a population level, and, for diploid organisms, the analytical tools must be capable of identifying two alleles. Therefore, standard Sanger sequencing can only be used after the cloning of PCR amplification product, which is a time consuming approach.

Droplet digital PCR (ddPCR™) technology was used to compare pre-epidemic and modern populations of IDM. Dr. Crouch's lab identified five single nucleotide polymorphisms (SNP) by performing Sanger sequencing of the nuclear ribosomal DNA of 134 *P. obducens* samples. Taqman® hydrolysis probes were designed for four SNPs and used to genotype 830 *P. obducens* samples, including 87 herbarium specimens collected between 1881 and 2005. The ddPCR™ analyses revealed allelic ratios departing from 1:1 for multiple heterozygote samples, with 100 of the 449 samples analyzed to date exhibiting a 3:1 allelic skew. For two of the SNPs analyzed, this 3:1 allelic ratio was observed in 30 to 38% of all the modern samples and only in 4 to 6% of herbarium specimens. This observed 3:1 allelic ratio departs from the expected 1:1 ratio in diploid organisms.

Single sporangium genotyping through ddPCR™ of a modern isolate confirmed the presence of aneuploidy in a modern specimen. An increase in ploidy levels in modern samples could have an impact on the genetic diversity of the pathogen and thus contributed to the recent outbreaks of IDM. Further analyses are underway to confirm this potential increase in ploidy in modern samples of IDM.

Simple Sequence Repeat (SSR) Marker Development

SSRs evolve rapidly, and they are co-dominant, which means they are capable of identifying allelic variants, so they are suitable for studying populations of diploid organisms such as *P. obducens*. SSRs are distributed across genomes, so multiple loci can be examined. Species- and/or population-specific primers make them suitable for DNA mixtures as only the target organism is amplified.

To develop specific *P. obducens* SSR markers, the draft genome of H1214 was mined for SSR motifs using PrimerPro v1.0, an integrated pipeline in Python for automatically searching microsatellites (MISA), associated PCR primer pairs (Primer3) and performing BLAST to ensure unique priming sites. Motifs ranging from tri- to trideca-nucleotides were identified, with the minimum repeat units set to five for tri-, tetra-, penta- and hexa-nucleotides, and four for the remaining motifs. Mono- and di-nucleotide motifs were not included in the search as it has been shown that routine PCR amplification alters mono- and di-nucleotide repeat lengths, changes that could be mistaken as polymorphisms or mutation. Because the genome assemblies generated for *P. obducens* were from environmental samples (non-axenic), sequences derived from plant or other microbes were identified using the software Kraken, which classifies data by mapping *k*-mer sequences to the lowest common ancestor. After removal of non-oomycete sequences, a total of 189 candidate SSR loci were selected *in silico*, based on the heterozygosity identified for *P. obducens* isolate H12.11-14 (Figure 32). From this total set of candidates, 62 were selected for trial in the lab, and PCR primers were pre-tested for positive amplification on genomic DNA of *P. obducens*. Of this set of 62 screened SSR markers, 37 SSR markers have shown reproducible results.

Figure 32. Example of microsatellite showing heterozygosity evolving under a step-wise model.

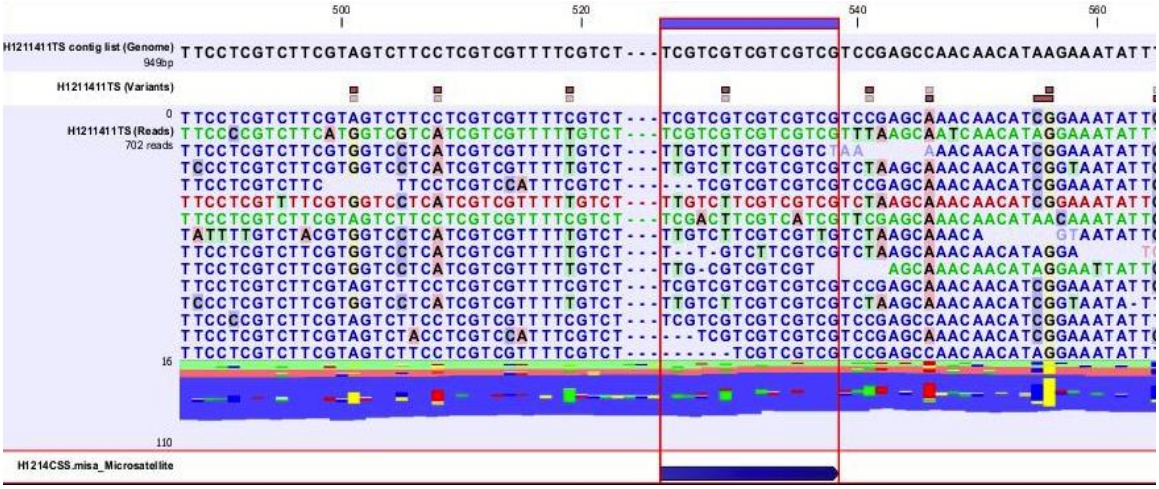


Table 20. Summary of SSRs identified in *Plasmopara obducens* de novo assembly

Total number of sequences examined	45,679
Total size of examined sequences (bp)	150,937,199
Total number of identified SSRs	33,157
Contigs with SSR motifs	15,798
Number of contigs containing >1 SSR	7,165
Number of SSRs present in compound formation	1,114
Tri-nucleotide	2,766
Tetra-nucleotide	148
Penta-nucleotide	65
Hexa-nucleotide	62
Hepta-nucleotide	7
Octa-nucleotide	3
Nona-nucleotide	4
Undeca-nucleotide	1
Dodeca-nucleotide	2
Trideca-nucleotide	2

Survey of Microsatellite Polymorphism

PCR amplifications were performed in a 10 µl total volume containing 2-10 ng of the sample DNA from 96 isolates of *P. obducens*. Reactions were performed on a Bio-Rad Thermal Cycler (Bio-Rad Laboratories, USA) using the following amplification program: 94°C for 5 minutes; 30 cycles at 94°C for 30 seconds, required annealing temperature for 45 seconds, and 72°C for 45 seconds; 8 cycles at 94°C for 30 seconds, 53°C for 45 seconds, and 72°C for 45 seconds; and a final extension step at 72°C for 10 minutes. PCR products with different amplicon sizes were labeled with one out of four different fluorescent dyes (FAM, PET, VIC, NED) and multiplex groups were created (Table 21). One microliter of PCR product was then added to 9µl of Hi-Di™ Formamide (Applied Biosystems, USA) supplemented with GeneScan™500 Liz ® size standard and denatured at 95°C for 2 minutes. Denatured products were injected on an Applied Biosystems automated Sequencher. Results were analyzed using GeneMarker®v2.6.3 (SoftGenetics, USA) using the following settings: intensity for peak detection threshold > 1000, percentage > 5% Max, Local Region > 25 Local Max, stutter peak filter 90 Left-30 Right. The characteristics of the 37 microsatellite loci tested so far is summarized in Table 22.

GenAlEx v.6 was used to generate summary statistics for all loci tested. Allele frequency for each loci was used to calculate the polymorphism information content (PIC), according to the formula $PIC = 1- \Sigma pi^2$ (where pi is the frequency of i^{th} allele for each locus (Table 22).

With the exception of two microsatellite markers that to date have shown to be monomorphic (Pob7989, Pob10169), the selected markers show from 2 to 6 alleles. Independent from the number of alleles, polymorphic markers, are the most useful for the study of biological populations.

Table 21. Characteristics of 37 novel genomic microsatellite loci primers develop for *Plasmopara obducens*.

Locus	Dye	Multiplex group	Repeat motif	Observed range size(bp)	Tm (°C)
Pob52	PET	6	TTA	222-234	65
Pob1601	FAM	6	TTC	142-148	65
Pob1861	VIC	11	CTA	266-275	65
Pob2171	VIC	7	GAC	250-262	65
Pob2497	FAM	12	GAA	260-272	65
Pob2739	VIC	13	GGA	286-289	65
Pob2910	NED	11	GTAT	165-169	65
Pob2933	VIC	8	AGA	219-228	65
Pob3024	NED	7	TTC	292-295	65
Pob3075	VIC	6	CCG	269-275	65
Pob3197	NED	8	TTC	266	58
Pob3896	FAM	5	CGA	280-295	64
Pob4176	PET	11	AAT	210-222	65
Pob4357	NED	*	TGG	266-272	63
Pob4700	PET	*	TTC	257-269	65
Pob5097	PET	13	GCA	237-249	65
Pob5487	FAM	2	CTT	272-284	59
Pob5494	FAM	3	TAT	285-303	65
Pob5875	VIC	2	CTT	221-230	65
Pob6030	VIC	3	TTC	220-229	65
Pob7328	PET	1	AGA	137-155	64
Pob7989	NED	4	AAG	202	63
Pob8649	NED	1	TCG	159-174	65
Pob10169	NED	6	GAC	293	65
Pob11069	NED	5	CTT	188-200	65
Pob11700	PET	4	AAT	272-299	65
Pob11993	FAM	1	TTA	208-217	65
Pob12309	VIC	1	AGT	270-282	65
Pob14678	PET	7	GTC	208-220	65
Pob21005	FAM	7	TCTTGTCTCCAGC	134-163	65
Pob29057	NED	9	GTT	251-260	65
Pob33638	PET	8	CTT	166-196	65
Pob36128	VIC	9	ATTTA	198-214	65
Pob47245	FAM	10	GAAA	262-274	62
Pob48178	VIC	10	CGA	214-226	65
Pob52381	NED	10	AAG	173-179	65
Pob60359	PET	12	ATA	200-203	65

* No multiplexing.

Figure 33. Electropherogram of heterozygous microsatellite. Observe two alleles, one at ~222 and one at ~234. Homozygous isolates for this marker would have only one of either allele.

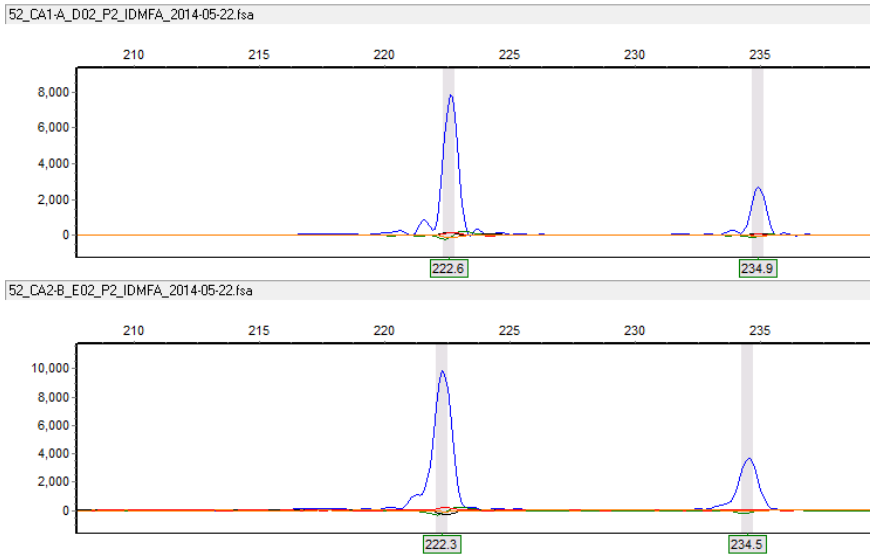


Table 22. Genetic characteristics of the 37 microsatellite markers

Locus	N	Na	Ho	He	PIC
Pob52	59	2	0.695	0.486	0.485
Pob1601	87	3	0.023	0.023	0.022
Pob1861	48	2	0.042	0.080	0.079
Pob2171	89	3	0.640	0.508	0.508
Pob2497	75	3	0.013	0.114	0.114
Pob2739	47	2	0.000	0.081	0.081
Pob2910	71	2	0.718	0.475	0.475
Pob2933	77	2	0.403	0.322	0.322
Pob3024	71	2	0.549	0.443	0.443
Pob3075	79	2	0.570	0.418	0.418
Pob3197	35	1			
Pob3896	72	3	0.069	0.167	0.167
Pob4176	49	2	0.041	0.040	0.040
Pob4357	81	3	0.741	0.483	0.483
Pob4700	82	4	0.073	0.503	0.503
Pob5097	74	4	0.270	0.569	0.569
Pob5487	70	5	0.100	0.376	0.376
Pob5494	78	5	0.103	0.225	0.225
Pob5875	68	3	0.074	0.099	0.099
Pob6030	82	2	0.341	0.283	0.283
Pob7328	74	4	0.892	0.507	0.507
Pob7989	8	1			
Pob8649	78	3	0.603	0.425	0.425
Pob10169	64	1			
Pob11069	80	5	0.563	0.501	0.501
Pob11700	74	5	0.541	0.746	0.746
Pob11993	69	2	0.072	0.070	0.070
Pob12309	74	4	0.257	0.605	0.605
Pob14678	78	2	0.397	0.318	0.318
Pob21005	88	2	0.670	0.489	0.489
Pob29057	83	2	0.325	0.272	0.272
Pob33638	50	6	0.280	0.486	0.486
Pob36128	47	3	0.021	0.102	0.102
Pob47245	58	2	0.431	0.338	0.338
Pob48178	77	3	0.831	0.553	0.553
Pob52381	70	2	0.714	0.459	0.459
Pob60359	70	2	0.000	0.459	0.459

N: number of isolates that positively amplified, Na: number of alleles,
Ho: observed heterozygosity, He: expected heterozygosity,
PIC: polymorphic information content

Genome sequencing

Comparison of the full genome sequence of *P. obducens* from different genetic groups can provide information about the genetic factors that contributed to the emergence of IDM disease in *I. walleriana*. A draft genome assembly was generated for two *P. obducens* samples: H1214 (Michigan, collected 2012 and PA 1-1 (Pennsylvania, collected 2014). The *P. obducens* genome characterization in progress. Crouch et al generated genome sequences of related species for comparative analyses: *P. halstedii* (five isolates), *Peronospora belbarii* (basil), and an isolate of *Peronospora* from agastache. These genomes and those of other downy mildews were mined for conserved, single copy orthologous genes for use in phylogenetic analysis.

Development/refinement of qPCR

From genome sequences of *P. obducens* and other downy mildews sequenced in the Crouch lab, mitochondrial genome assemblies were constructed and compared to a database of mtDNA from other oomycetes (F. Martin, USDA-ARS) to identify a high-copy number DNA region distinct for *P. obducens* that can be used for a sensitive diagnostic assay. Two regions were identified (see figures below). Region1 is situated in the cox1-atp1 region, which is organized in a distinct order in *Plasmopara* and *Bremia*. In *Plasmopara*, the cox1-atp1 region is further distinguished from *Bremia*, as the space between the genes is ca. 200 bp (*Plasmopara)*, versus 2 kbp (*Bremia*). The cox1-atp1 region is also variable between different species of *Plasmopara* based on individual nucleotide sequence differences. Region 2 includes the Rps10 locus, which also has utility for discriminating *P. obducens* from other *Plasmopara*. PCR primers were designed and used to amplify the regions across a representative set of *P. obducens* samples representing the major populations (identified through our previous Farm-Bill-funded SSR-based research) and other *Plasmopara* sp. Sanger sequencing of the marker regions showed no intra-specific variation in the probe region, except between *P. obducens* population A (*i.e.* pre-2004 populations) and the remaining *P. obducens* populations. This indicates that the target region will be useful for detecting and quantifying the presence of modern, epidemic populations of *P. obducens* from the environment.

Primers and probes for a real time real-time PCR detection assay were designed and synthesized for the mtDNA regions. A universal plant marker was used to develop an internal control for the assay. The internal control marker has been tested and is working well. Preliminary assessment of the cox2 and rps10 probes showed that the rps10 probe provided detection during earlier cycles of the qPCR reaction and with less background. Therefore the rps10 probe was utilized for all further testing. A second rps10 probe, capable of detecting *P. obducens* population A, was designed and tested. Assays were tested and optimized to ensure optimal conditions and specificity. Annealing temperature was empirically optimized using the rps10 probes. Amplification with 1, 2, 3, and 4 mM Mg was evaluated to minimize background amplification, as the concentration of this reagent can have a big impact on template amplification as well as if there is background, nonspecific amplification. This was done first with standard PCR and the master mix spiked as needed to provide the best amplification. Since PCR master mix can also have a significant impact on final results, they compared ABI with 5 Prime and found the 5 Prime reduced the Ct by 4 when run at the same time with the exact same DNA. Final assay conditions were formulated form these experiments, and used to screen a representative sample of IDM material; a manuscript on this research is currently being prepared for peer-reviewed publication.

Figure 34. Partial sequence alignment of the mtDNA cox1-atp1 locus, showing variation (red highlighted areas) between *P. obducens* and *P. viticola, P. halstedii* and *Plasmopara* sp. from rudbeckia.

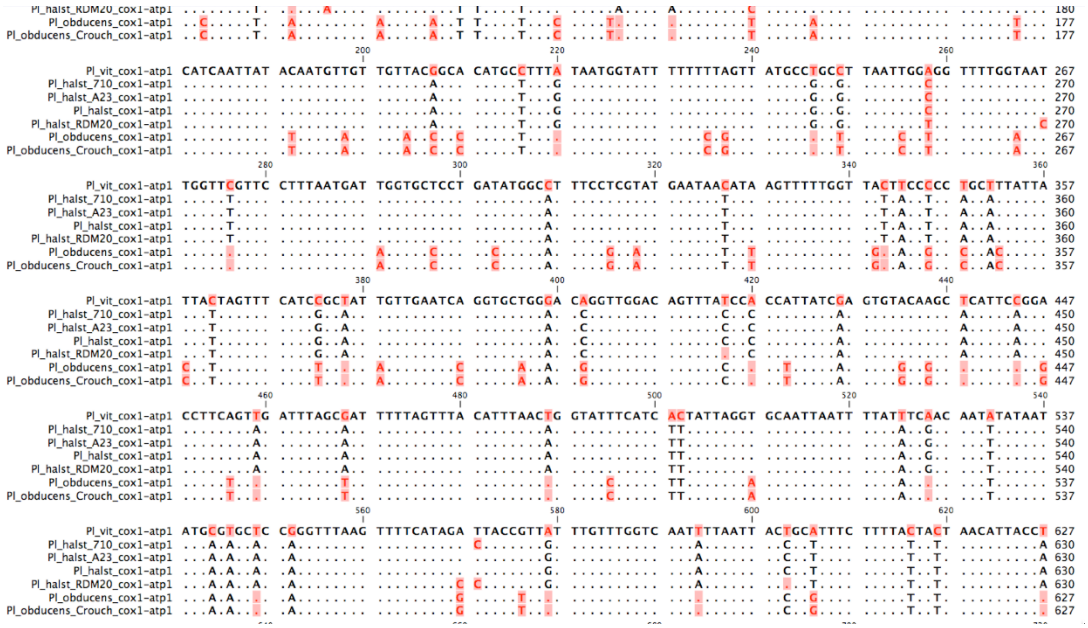
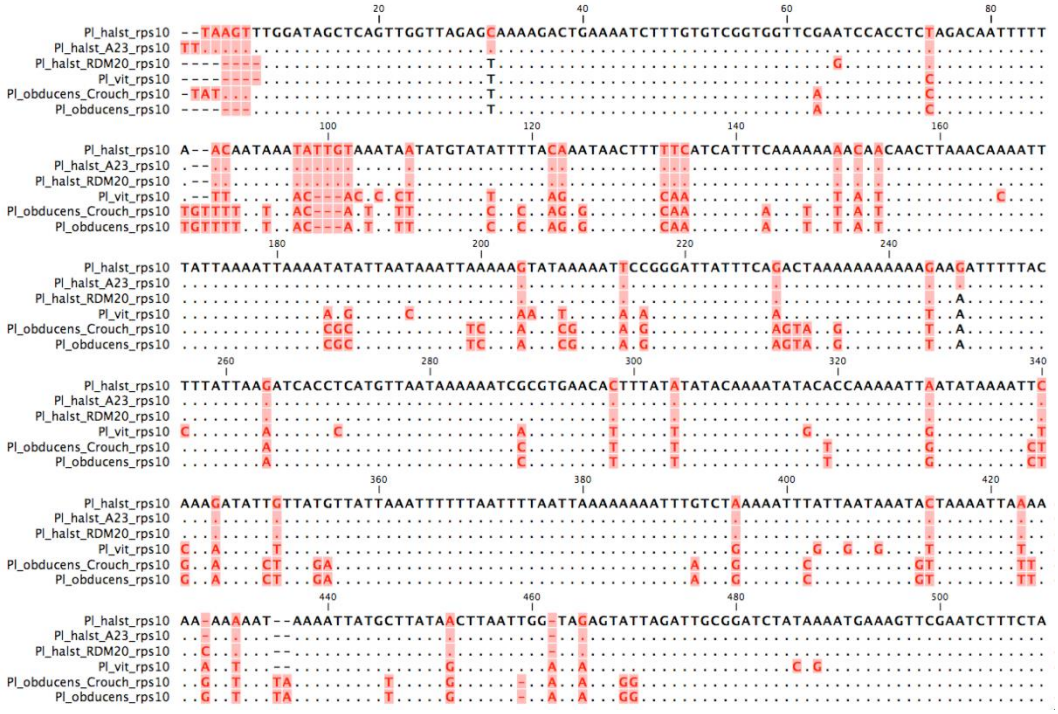


Figure 35. Partial sequence alignment of the mtDNA Rps10 locus, showing variation (red highlighted areas) between *P. obducens* and *P. viticola, P. halstedii* and *Plasmopara* sp. from rudbeckia.



Genomic resources for P. belbahri and P. obducens (Gen-NCSU-3)

Dr. Quesada's team extracted and sequenced paired-end DNA libraries from two *P. belbahri* (basil DM) and one *P. obducens* (impatiens DM) isolates and paired-end RNA libraries from five *P. belbahri* and four *P. obducens* isolates after performing a sporangia increase on detached cucurbit leaves (Table 23). For all isolates, they obtained high-quality DNA, RNA, whole genome, and whole transcriptome sequencing data. The goal was to obtain at least 10-15 million high quality reads per isolate, which was accomplished.

Table 23. Downy mildew isolates sequenced.

Code	Species	Host	Region
DNA seq PE			
bdmHI	*P. belbahrii*	Basil	HI
bdmsw021gen	*P. belbahrii*	Basil	NC
Idmsw031	*P. obducens*	Impatiens	NY
RNA seq PE			
bdmHI	*P. belbahrii*	Basil	HI
bdmsw021gen	*P. belbahrii*	Basil	NC
bdm19563	*P. belbahrii*	Basil	NC
bdmJ&Bgen	*P. belbahrii*	Basil	NC
bdmJ&Bswt	*P. belbahrii*	Basil	NC
idmMD-nina	*P. obducens*	Impatiens	MD
idmmsu003	*P. obducens*	Impatiens	MI
idmmsu001	*P. obducens*	Impatiens	MI

Identify mating type determinants (Gen-ARSB-2, Gen-Rut-2)

According to mating tests conducted by Shishkoff, *P. obducens* appeared to be homothallic. Therefore, genomic inquiries into mating type determinants was not warranted.

Objective 2c: Genomic Resources & Diagnostics: Primer/Probe Development

Test candidate genes for CDM diagnostics (Gen-NCSU-4, Gen-MSU-2)

North Carolina. In the first year of the project Dr. Quesada's group generated genome-wide datasets for cucurbit and hop downy mildew to identify genomic regions that are species-specific. Through that effort they were able to identify 70 genomic regions that were specific to CDM according to transcriptome data. In this objective, they aimed to develop and validate a molecular diagnostic test for CDM using those candidate genes (Figure 36). As a first step, they generated genome-sequencing data for one isolate of *P. humuli* to ensure that the *P. cubensis*-specific regions were not due to differentia expression only, which would be a confounding factor for a DNA-based molecular test. After comparing the 70 candidates with the *P. humuli* genomic data, they retained 41 candidates for PCR screening in the lab. An initial screen of six diverse *P. cubensis* and six *P. humuli* isolates eliminated another 15 candidates from a subset of 26 candidates selected for validation. Evaluations of the remaining 11 candidate diagnostic regions with diverse isolates including 40 *P. cubensis*, 34 *P. humuli*, five *P. belbahrii*, three *P. obducens*, six *Phytopthora capsici*, eight *Phytophthora infestans* and eight uninfected cucurbit, basil and hop plant samples, revealed that 10 diagnostic candidates in seven genes would be specific and robust enough for CDM-specific diagnostics (Figure 37).

Figure 36. Gel electrophoresis images showing PCR products of one example *P. cubensis* diagnostic candidate for diverse cucurbit downy mildew (C6 - C37) and hop downy mildew isolates (H1 - H34).

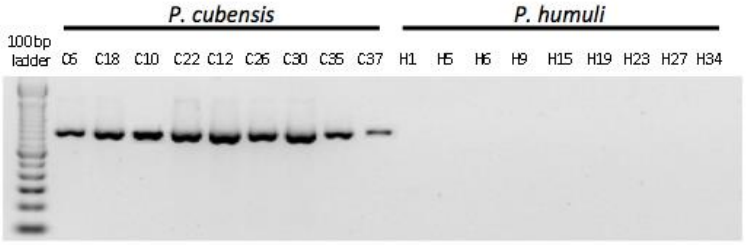
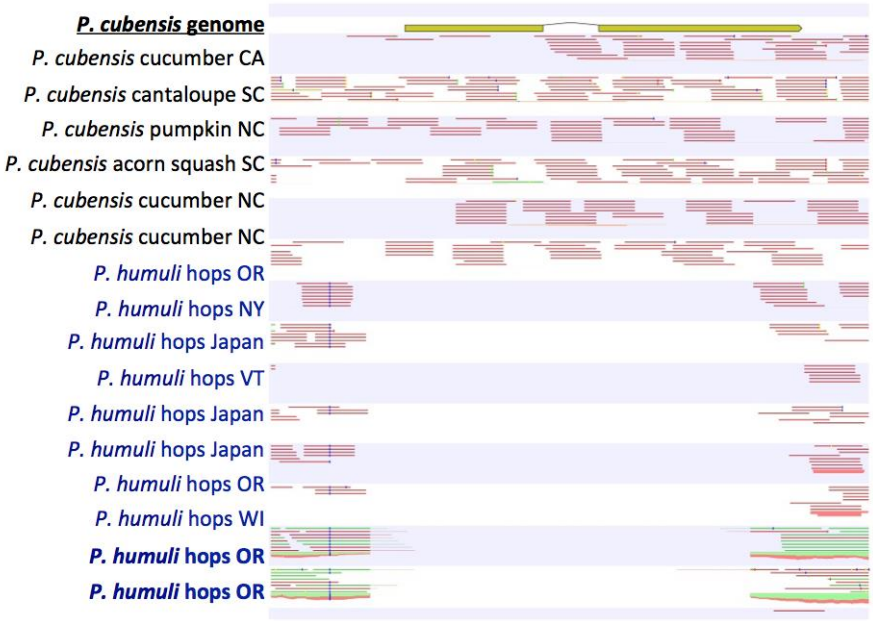


Figure 37. Example candidate diagnostic marker for CDM.



The first row shows the gene model for that region according to the published *P. cubensis* genome (black, bold, underlined font), the next six rows show transcriptome data generated in this project from *P. cubensis* isolates and mapped to the *P. cubensis* genome indicating that this gene is consistently expressed in *P. cubensis* isolates (black font), the following eight rows show transcriptome data generated in this project from *P. humuli* isolates and mapped to the *P. cubensis* genome indicating that this gene is never expressed in *P. humuli* isolates (blue font), the last two rows show genome data generated in this project from *P. humuli* isolates and mapped to the *P. cubensis* genome indicating that this gene is consistently missing in the genome of *P. humuli* isolates (blue, bold font).

Michigan. Next-generation sequencing (NGS) methods allow generating genomic resources required to identify candidate genomic markers. Being closely related sister species, hop downy mildew pathogen *P. humuli* and cucurbit downy mildew pathogen *P. cubensis* could be used through a comparative genomics analyses to identify species-specific diagnostic markers. Dr. Hausbeck's team propagated several isolates of *P. humuli* and *P. cubensis* on detached leaves of hop cv. Pacific Gem and cucumber cv. Straight 8, respectively, to obtain high quality RNA and DNA for sequencing. Following propagation, sporangia from both species were harvested and genomic materials (RNA and DNA) were extracted using BeadRuptor and Qiagen miniprep kits. High quality total RNA and DNA were used for sequencing using Illumina HiSeq 2500 (Illumina) at Michigan State University Research Technology Support Facility (MSU-RTSF). RNA-seq and DNA-seq reads were then analyzed for quality using FastQC (v. 0.10.1), and Cutadapt (v. 1.8.1) and FASTX-Toolkit (v. 0.0.13) were used to remove adaptors and low quality sequences.

Figure 38. Electrophoresis results of high quality RNA extracted from *P. humuli* (HDM) and *P. cubensis* (CucDM) isolates.

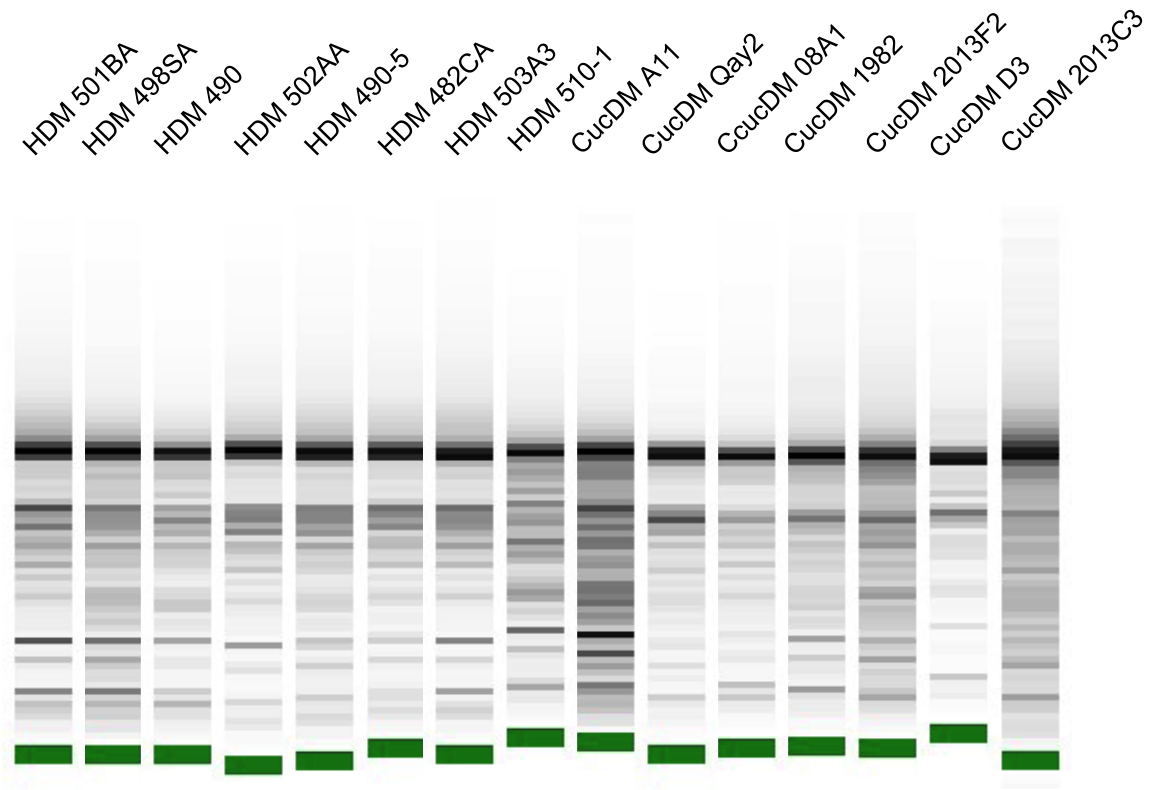


Figure 39. FastQC (v. 0.10.1) quality score of RNA-seq data from OR502AA, a representative isolate of *P. humuli*.

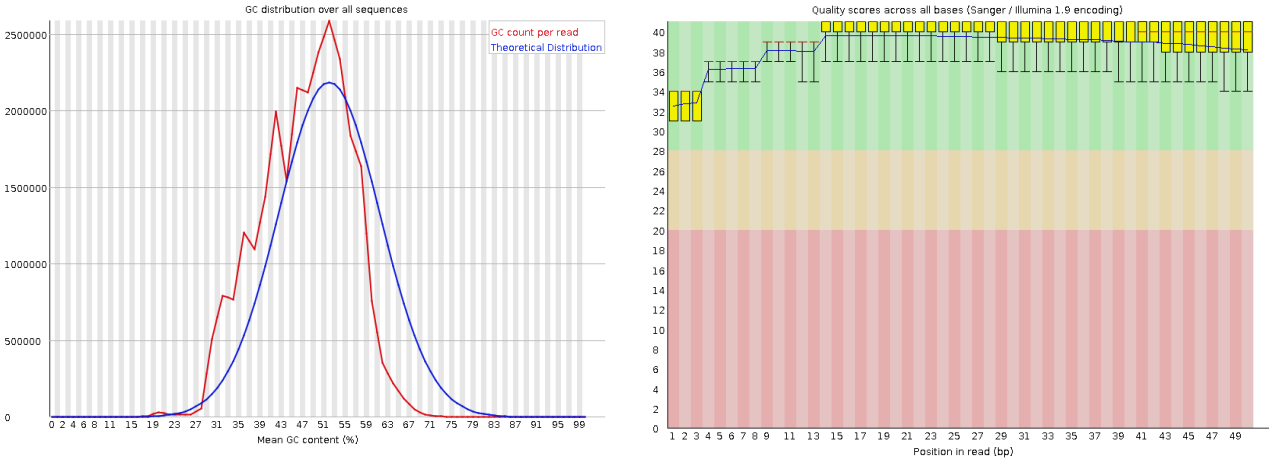


Table 24. Summary of quality control analysis for *Pseudoperonospora humuli* and *Pseudoperonospora cubensis* samples used for Illumina 50bp single-end RNAseq and 100bp paired-end DNAseq.

Pathogen species	Isolate	Molecule	Sequencing mode	Number of total reads	Number of high quality reads
Pseudoperonospora cubensis	CA08A1	RNA	SE	23,691,206	23,576,348
	SC1982	RNA	SE	22,117,725	22,049,442
	NC2013C3	RNA	SE	28,035,867	27,888,243
	SC2013F2	RNA	SE	16,956,357	16,886,934
	NCA11	RNA	SE	28,101,878	27,962,061
	SCD3	RNA	SE	29,304,711	29,218,196
	NCWAY2-1	RNA	SE	21,038,800	20,974,369
Pseudoperonospora humuli	NY482CA	RNA	SE	15,906,769	15,860,532
	JP490-5	RNA	SE	20,211,068	20,149,698
	JP490	RNA	SE	11,942,671	11,913,949
	JP498SA	RNA	SE	26,977,356	26,866,989
	OR501BA	RNA	SE	14,429,953	14,399,612
	OR502AA	RNA	SE	26,193,148	26,086,672
	VT503A3	RNA	SE	14,576,962	14,543,704
	WI510-1	RNA	SE	29,264,282	29,150,378
	OR502AA_R1	DNA	PE	36,599,100	21,778,230
	OR502AA_R2	DNA	PE	36,599,100	21,778,230

The next steps identified diagnostic candidates for *P. humuli* using RNA-seq reads from *P. humuli* and *P. cubensis* through bioinformatics pipeline, which involves use of Bowtie2 short read aligner, HTSeq and selected Perl and Bash programming. See below.

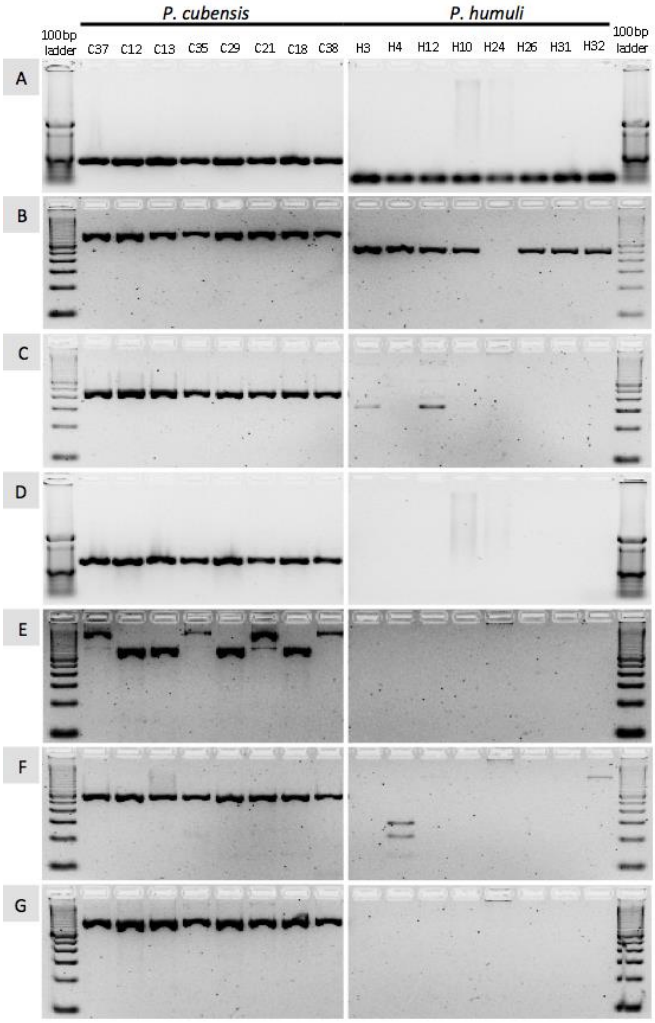
Objective 2d: Genomic Resources & Diagnostics: Detection

Field-friendly molecular diagnostics of P. cubensis for early warning systems (Gen-NCSU-5, Gen-MSU-3)

North Carolina

Dr. Quesada's team identified candidate diagnostic markers for *P. cubensis* by comparing next generation sequencing data from a diverse panel of *P. cubensis* and hop downy mildew (HDM), *P. humuli*, isolates, two very closely related oomycete species. *P. cubensis* isolates from diverse hosts and geographical regions in the United States were selected for sequencing to ensure that candidates were conserved in *P. cubensis* isolates infecting different cucurbit hosts. Genomic regions unique to and conserved in *P. cubensis* isolates were identified through bioinformatics pipeline. These candidate regions were then validated using end-point PCR against a larger collection of isolates from *P. cubensis*, *P. humuli*, and other oomycetes. Overall seven diagnostic candidate markers were found to be uniquely specific to *P. cubensis* (Figure 40). These markers showed the potential to be used in pathogen diagnostics and are currently being adapted for monitoring airborne inoculum of *P. cubensis* sporangia with real-time quantitative PCR and spore traps.

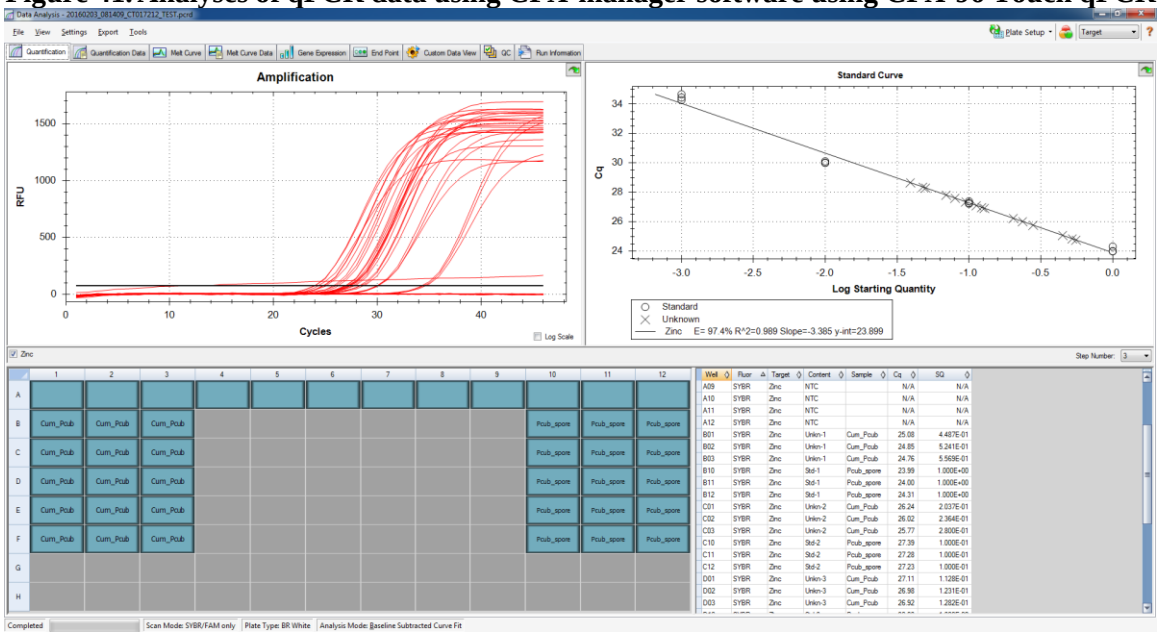
Figure 40. *Gel electrophoresis images showing 7 P. cubensis diagnostic candidate PCR products for diverse CDM and HDM samples.*



CDM samples are C37: watermelon NC, C12: cucumber NC, C13: cantaloupe NC, C35: pumpkin NC, C29: cucumber MI, C21: butternut squash NC, C18: buffalo gourd NC, and C38: watermelon NC. HDM samples are H3: NC, H4: OR, H12: NC, H10: Japan, H24: NC, H26: OR, H31: WA, and H32: NY. Primers for candidates A, B, C, and F are being re-designed to ensure that they are differentiated by presence in *P. cubensis* and absence in *P. humuli* instead of product size differences for using markers in *P. cubensis* quantification. Once primers are optimized, they will begin development of real-time PCR quantification assays.

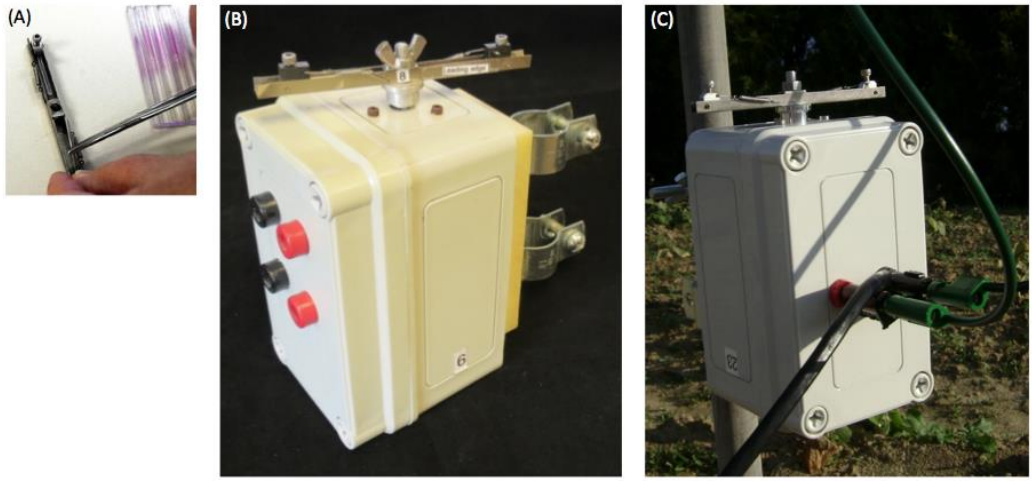
They obtained spore traps to use for sampling airborne inoculum in North Carolina, purchased a quantitative PCR machine, and developed real-time PCR assays for *P. cubensis* spore DNA quantification. Preliminary qPCR testing using a gene encoding zinc finger C3HC4/FHA domain-containing protein and a SYBR green-based qPCR method, determined an initial threshold detection level of target pathogen DNA from *P. cubensis* sporangia and infected plant tissue (Figure 41). The qPCR assay successfully detected up to 5.3 pg/µl of sporangia DNA, indicating the possibility of developing a qPCR assay for quantitative detection *P. cubensis* sporangia DNA.

Figure 41. Analyses of qPCR data using CFX-manager software using CFX-96 Touch qPCR machine.



Amplification and Standard curves show detection of *P. cubensis* in samples using a gene encoding zinc finger C3HC4/FHA domain-containing protein and a SYBR green-based qPCR method.

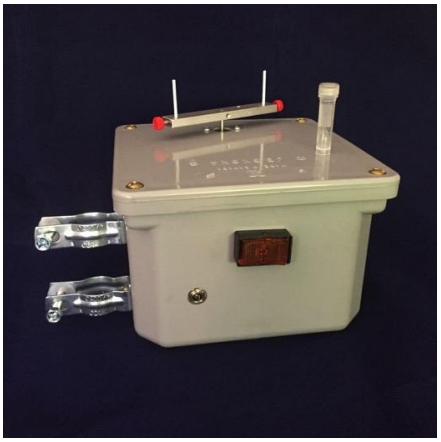
Figure 42. Roto-rod trap for P. cubensis.



(A) Sampling rod on rotorod arms; (B) Rotorod sampler unit with arms attached; (C) Rotorod sampler unit with power cable in the field.

In order to monitor aerial inoculum of *P. cubensis* sporangia, roto-rod type spore traps as the ones shown in Figure 42, have been deployed in two regions of North Carolina. Three traps were placed in the cucumber, squash, and watermelon plots at the research station in Clayton, NC and at the research station in Kinston, NC. Rods are run from every Tuesday through Friday and changed every day. A roto-rod trap shown in Figure 43 was developed to allow growers to perform their own monitoring in the future. The trap is easy to use and inexpensive. This year NCSU has sent 3 traps to MSU for initial testing, if successful, a small number of traps will be given to grower cooperators in future years for validation.

Figure 43. Grower-friendly roto-rod trap developed for monitoring *P. cubensis* airborne inoculum.



For all seven candidate genomic markers identified, qPCR primers were developed and to determine the sensitivity of each set of primers, DNA was extracted from different dilutions of *P. cubensis* sporangia ranging from 10^6 to 1. Using end-point PCR and SYBR-green based qPCR method, candidate markers c52.12e13, c2555.2e1, and c3155.4e9 exhibited consistent amplification across all isolates, dilutions, and replicated experiments. Our next step was to determine the *P. cubensis* sporangia detection limit using sporangia dilutions (10^6 to 1 sporangia, Table 25 and Table 26), sporangia on leaf disks (5 mm diameter, Table 25 and Table 26) and sporangia on plastic sampling rods (rotorod sampler, Table 25 and Table 26) using these three genomic markers with end-point PCR and qPCR methods (SYBR green). Using the SYBR green qPCR assay, they successfully detected up to 1 pg/µl of *P. cubensis* sporangia DNA (Figure 12). Based on our end-point PCR and qPCR (SYBR green) results, assay developed for marker c3155.4e9 consistently amplified sporangia DNA from *P. cubensis* across isolates and dilutions, therefore, was chosen for developing a probe-based qPCR assay. Sensitivity of *P. cubensis* DNA detection on leaf disks (LD), sampling rods (ROD), and sporangia suspension (SPR) based on the genomic marker c3155.4e9 exhibited significant improvement of sporangia DNA detection using LNA (locked-nucleic acid) probe-based qPCR (Table 27). Using the probe-based qPCR assay, they successfully detected up to 10 pg/µl of *P. cubensis* sporangia DNA (Figure 45). Specificity of PCR and qPCR (SYBR green and probe based) detection of *P. cubensis* sporangia DNA based genomic marker c3155.4e9 was also determined by using DNA from closely related *P. humuli* and other oomycetes such as *Pythium* and *Phytophthora* species (Table 28). While PCR and SYBR green-based qPCR method exhibited non-specificity, our probe based qPCR assay for c3155.4e9 exhibited complete specificity only towards the target DNA from *P. cubensis* sporangia.

Table 25. Detection limit of *P. cubensis* sporangia by seven candidate genomic markers

Diagnostic marker	*P. cubensis* sporangia detection limit
c52.12e13	10^2
c572.6e19	10^3
c2183.6e2	10^4
c2555.2e1	10^2
c2555.3e7	10^3
c3155.4e9	10^3
c10851.1e0	10^3

Table 26. Detection limit of *P. cubensis* sporangia on leaf disk, sampling rod and sporangia suspension by the candidate genomic markers using end-point and quantitative PCR.

Diagnostic marker	*P. cubensis* sporangia detection limit					
	Leaf disk (5 mm)		Sampling rod		Sporangia	
	PCR	qPCR	PCR	qPCR	PCR	qPCR
c52.12e13	1	1	10^4	50	10^2	1
c2555.2e1	10	1	10^5	50	10^2	1
c3155.4e9	10	1	10^6	10	10^3	1

Figure 44. Amplification and Standard curves using the c3155.4e9 genomic marker showing detection of *P. cubensis* in samples.

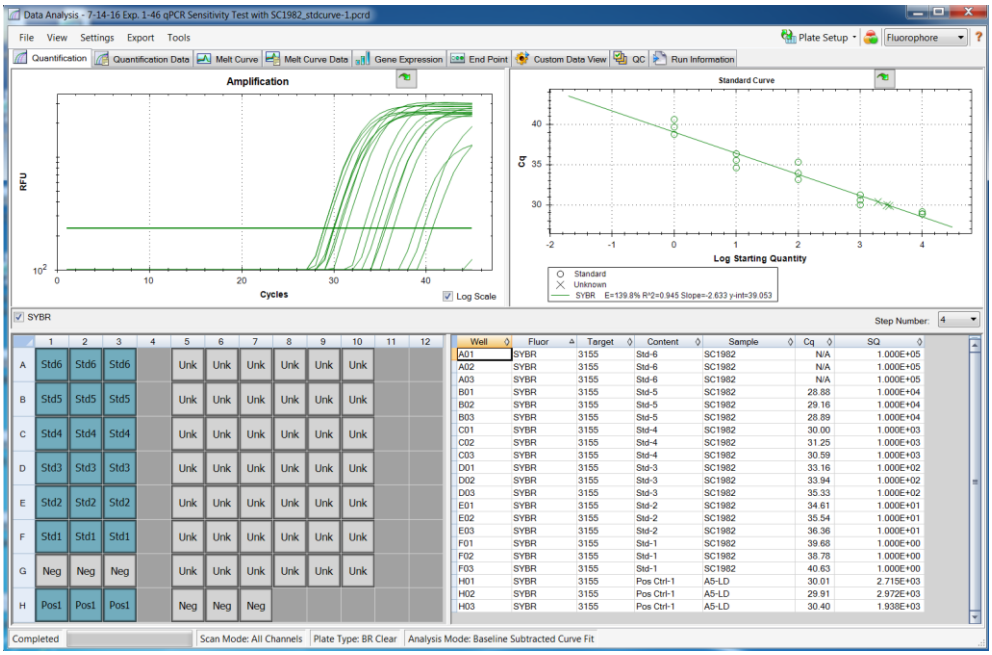


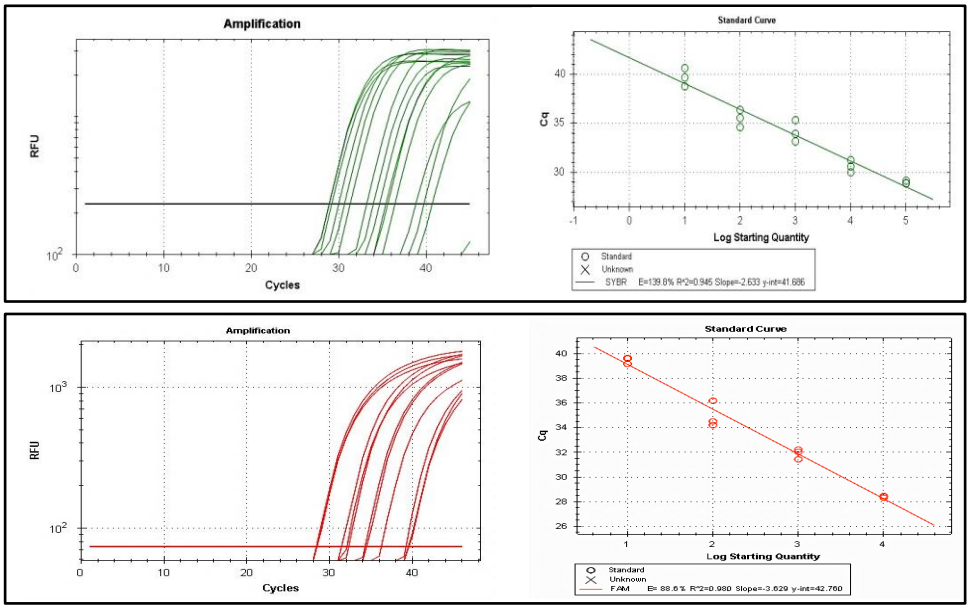
Table 27. Detection limit of *P. cubensis* sporangia by candidate genomic marker c3155.4e9.

Sample name	PCR	Cq value	
		SYBR	Probe
LD-50	+	34.46	34.34
LD-10	+	34.52	36.21
LD-1	+	36.38	38.44
SPR-50	-	31.35	34.75
SPR-10	-	33.73	36.15
SPR-1	-	33.99	37.33
RD-50	-	37.60	38.73
RD-10	-	37.33	38.52
RD-1	-	36.36	38.07

Table 28. Specificity of *P. cubensis* sporangia DNA detection based c3155.4e9 genomic marker using end-point and quantitative PCR.

Sample name	PCR	qPCR	
		SYBR	Probe
Pythium ultimum	+	+	-
Pythium aphanidermatum	+	+	-
Pythium myriotylum	+	+	-
Pythium Aphanidermatum	+	+	-
Pythium catenulatum	+	+	-
Pythium dissotocum	+	+	-
Phytophthora cinnamomi	+	-	-
Phytophthora citrophthora	+	+	-
Phytophthora cryptogea	+	+	-
Phytophthora dreschleri	+	+	-
Phytophthora nicotiana	+	+	-
Phytophthora capsici	+	+	-
Pseudoperonospora humuli 224	+	+	-
Pseudoperonospora humuli SW007	+	+	-

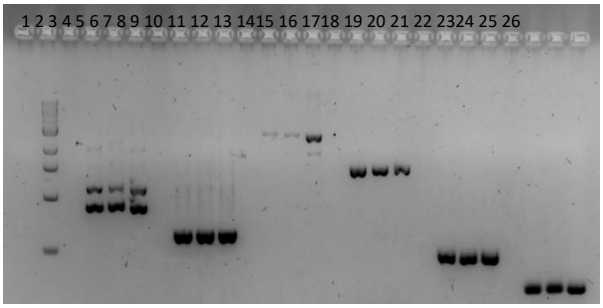
Figure 45. Amplification and standard curves of probe-based qPCR (top – SYBR green; bottom – probe) using the c3155.4e9 genomic marker showing detection of *P. cubensis* in samples.



Although they successfully developed a single copy nuclear marker c3155.4e9 for detection and identification of *P. cubensis* spores trapped by impaction type roto-rod spore trap, they continued to test suitability of other markers that were identified by our Bioinformatics pipeline (Figure 46). These marker genes encode virulence or pathogenicity related proteins, which could be useful for separating virulent isolates on certain hosts from the rest. During initial screening with end-point PCR, none of these marker genes exhibited any cross-amplification of other oomycete organisms tested. However, initial screening using SYBR green qPCR method did exhibit some non-specific amplification but that could be due to primer dimer which would be eliminated when using Taqman probe based qPCR assays. The efficiency of other potential genomic markers were also evaluated. Except for c27478.0e0, the rest of the markers exhibited a single amplicon during end-point PCR evaluation.

These genomic markers will be further evaluated by designing primers for SYBR green and Taqman probe detection for higher efficiency (lower Cq value) compared to c3155.4e9.

Figure 46. PCR amplification of candidate diagnostic genomic markers of *P. cubensis* identified from NGS data using bioinformatics pipeline.



*1kb ladder

Description	Notes
c27478.0e0	RxLR effector candidate protein (lane 4-6)
c33204.0e0	Expressed gene of unknown function (lane 8-10)
c5641.0e0	Conserved hypothetical protein (lane 12-14)
c12575.0e0	Conserved hypothetical protein (lane 16-18)
c30239.0e0	Putative aldose 1-epimerase (lane 20-22)
c3294.1e3	Expressed gene of unknown function (lane 24-26)

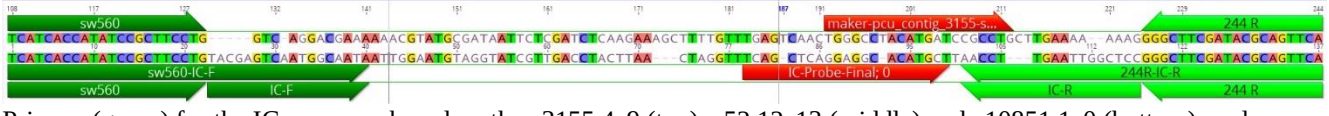
Table 29. Gene ID and functional annotation of the marker genes in *P. cubensis* to be tested.

Gene ID	Functional annotation
c52.12e13	Crinkler family protein (CRN)
c10851.1e0	Necrosis-inducing *Phytophthora* protein 1 (NPP1)
c27478.0e0	RxLR effector candidate protein (lane 4-6)
c30239.0e0	Putative aldose 1-epimerase

In these experiments, presence of silicon grease showed variable inhibitory effect on PCR amplification. Other research groups also noted similar inhibition when using DNA extracted from grease-coated rotating sampling rods. Therefore, they used three different DNA extraction techniques including Phenol-choloroform (modified and developed in the Quesada lab), Qiagen (QIAmp DNA mini kit), and MO-BIO Power-soil DNA extraction kit (used by several other researchers) to identify the most efficient technique for extracting DNA from *P. cubensis* sporangia on leaf disk and sampling rods. Modified Phenol-choloroform and MO-BIO kit performed similarly both of which were better than QIAmp DNA mini kit in extracting DNA from artificially inoculated sampling rods. Data suggests that DNA extraction buffer containing both SDS and Triton X-100 performs better with phenol-chlorofom extraction method providing maximum DNA yield and minimum PCR inhibitory silicon grease contamination.

A second approach investigated was the use of IC to determine presence of PCR inhibitors in extracted DNA from *P. cubensis* sporangia on silicon grease-coated sampling rods. Having the same primer as the genomic marker but a different Taqman probe, the IC DNA sequence can be multiplexed with a target DNA master mix. Any change in the Cq value of IC multiplexed with pathogen DNA compared to the control Cq value of IC alone will provide an indication of PCR inhibitor presence. They designed an IC (Figure 47) for investigating any qPCR inhibition with the already developed genomic marker c3155.4e9..

Figure 47. Illustration of *P. cubensis* genomic markers and IC probe.



Primers (green) for the IC assay are based on the c3155.4e9 (top), c52.12e13 (middle) and c10851.1e0 (bottom) nuclear genomic marker.

Furthermore, to determine effect of different qPCR mastermix reagents on the Cq value three different qPCR reagents were tested: Perfecta Fast Mix II, Perfecta qPCR ToughMix (Quantabio) and SsoAdvanced Universal Probes Supermix (BioRad). It has been documented that different qPCR mastermix may vary significantly with different sample types. This helped to determine a probe based qPCR mastermix most suitable for *P. cubensis* sporangia DNA detection assays that they have developed.

Since it is necessary to have a robust DNA extraction procedure that will effectively release any and all DNA from *P. cubensis* sporangia trapped by silicon grease on sampling rods. Although, the Quesada lab modified phenol-chloroform extraction protocol performed best, they continued to improve the protocol further since as low as 1 *P. cubensis* sporangium may be trapped on sampling rods and detection of such low level of sporangia count would help predict downy mildew disease progression to growers. They tested several different organic solvents that may be used to solubilize silicon grease and thereby help improve DNA extraction. They identified two different organic solvents – diisopropylamine and ethyl acetate, that were able to disperse silicon grease and neither solvents was known to have any detrimental effect on DNA molecules.

Following QC check of RNA-seq reads from *P. humuli* and *P. cubensis*, they used Bowtie2 to align the reads to the draft genome assembly of *P. cubensis* and *P. humuli* generated in the Quesdad lab as well as with the published genome assembly of *Cucumis sativus* (cucumber) and *Humulus lupulus* (hop) (Table 30, Table 31, Table 32, Table 33). Since both *P. humuli* and *P. cubensis* are obligate pathogens, it is not unusual to have plant cell contamination while harvesting pathogen sporangia from leaf surface. Bowtie2 alignment thus served two purposes, first to identify reads that are unique to *P. humuli* and second, to determine contaminating plant RNA in the RNA-seq reads.

Table 30. Summary of Bowtie2 alignment of *P. humuli* (HDM) and *P. cubensis* (CUC) RNA-seq reads with *P. humuli* draft genome.

	Bowtie2 *P. humuli* genome (OR502AA)							
LIBRARY	TOTAL READS	UNIQUELY MAPPED READS	% UNIQUELY	MULTIPLE MAPPED READS	% MULTIPLE	% MAPPED	UNMAPPED READS	% UNMAPPED
HDM482CA	15,906,769	10,008,660	62.92	1,800,841	11.32	74.24	4,097,268	25.76
HDM490-5	20,211,068	14,174,555	70.13	2,613,284	12.93	83.06	3,423,229	16.94
HDM490	11,942,671	5,073,019	42.48	1,036,510	8.68	51.16	5,833,142	48.84
hdm498SA	26,977,356	20,548,239	76.17	3,886,691	14.41	90.58	2,542,426	9.42
HDM501BA	14,429,953	9,199,981	63.76	1,711,803	11.86	75.62	3,518,169	24.38
hdm502AA	26,193,148	20,677,750	78.94	3,733,820	14.25	93.20	1,781,578	6.80
HDM503A3	14,576,962	9,745,144	66.85	1,771,842	12.16	79.01	3,059,976	20.99
hdm510-1	29,264,282	20,586,298	70.35	4,563,084	15.59	85.94	4,114,900	14.06
cdm08A1	23,691,206	16,792,544	70.88	3,842,232	16.22	87.10	3,056,430	12.90
cdm2013C3	28,035,867	18,552,807	66.18	4,250,206	15.16	81.34	5,232,854	18.66
cdmA11	28,101,878	19,254,329	68.52	3,722,885	13.25	81.76	5,124,664	18.24
CUC1982	22,117,725	13,930,912	62.99	2,630,253	11.89	74.88	5,556,560	25.12
CUC2013F2	16,956,357	8,731,719	51.50	1,693,867	9.99	61.48	6,530,771	38.52
CUCDM	29,304,711	19,853,436	67.75	4,074,901	13.91	81.65	5,376,374	18.35
CUCWAY2-1	21,038,800	13,582,852	64.56	2,599,129	12.35	76.91	4,856,819	23.09

Table 31. Summary of Bowtie2 alignment of *P. humuli* (HDM) and *P. cubensis* (CUC) RNA-seq reads with *P. cubensis* draft genome.

	Bowtie2 *Pcubensis*_MSU1_v2_ genome							
LIBRARY	TOTAL READS	UNIQUELY MAPPED READS	% UNIQUELY	MULTIPLE MAPPED READS	% MULTIPLE	% MAPPED	UNMAPPED READS	% UNMAPPED
HDM482CA	15906769	10304979	64.78	2075464	13.05	77.83	3526326	22.17
HDM490-5	20211068	14206011	70.29	2558829	12.66	82.95	3446228	17.05
HDM490	11942671	5403244	45.24	1193511	9.99	55.24	5345916	44.76
hdm498SA	26977356	20164089	74.74	3165433	11.73	86.48	3647834	13.52
HDM501BA	14429953	9367506	64.92	1770134	12.27	77.18	3292313	22.82
hdm502AA	26193148	20263970	77.36	2907089	11.10	88.46	3022089	11.54
HDM503A3	14576962	9975043	68.43	1841878	12.64	81.07	2760041	18.93
hdm510-1	29264282	20804411	71.09	3484254	11.91	83.00	4975617	17.00
cdm08A1	23691206	17750381	74.92	3275690	13.83	88.75	2665135	11.25
cdm2013C3	28035867	19666188	70.15	3568213	12.73	82.87	4801466	17.13
cdmA11	28101878	20274930	72.15	3093563	11.01	83.16	4733385	16.84
CUC1982	22117725	14957692	67.63	2871597	12.98	80.61	4288436	19.39
CUC2013F2	16956357	9642733	56.87	1975076	11.65	68.52	5338548	31.48
CUCDM	29304711	20999487	71.66	4108366	14.02	85.68	4196858	14.32
CUCWAY2-1	21038800	14590546	69.35	2868843	13.64	82.99	3579411	17.01

Table 32. Summary of Bowtie2 alignment of *P. humuli* (HDM) and *P. cubensis* (CUC) RNA-seq reads with *H. lupulus var lupulus* genome.

	Bowtie2 Hops genome (*H. lupulus* var *lupulus* GCA_000831365.1)							
LIBRARY	TOTAL READS	UNIQUELY MAPPED READS	% UNIQUELY	MULTIPLE MAPPED READS	% MULTIPLE	% MAPPED	UNMAPPED READS	% UNMAPPED
HDM482CA	15,906,769	188,540	1.19	1,296,991	8.15	9.34	14,421,238	90.66
HDM490-5	20,211,068	133,898	0.66	913,502	4.52	5.18	19,163,668	94.82
HDM490	11,942,671	146,330	1.23	956,464	8.01	9.23	10,839,877	90.77
hdm498SA	26,977,356	18,631	0.07	103,913	0.39	0.45	26,854,812	99.55
HDM501BA	14,429,953	156,289	1.08	825,788	5.72	6.81	13,447,876	93.19
hdm502AA	26,193,148	15,002	0.06	77,691	0.3	0.35	26,100,455	99.65
HDM503A3	14,576,962	161,855	1.11	824,839	5.66	6.77	13,590,268	93.23
hdm510-1	29,264,282	48,644	0.17	219,002	0.75	0.91	28,996,636	99.09
cdm08A1	23691206	9226	0.04	390222	1.65	1.69	23291758	98.31
cdm2013C3	28035867	21803	0.08	517627	1.85	1.92	27496437	98.08
cdmA11	28101878	31299	0.11	604711	2.15	2.26	27465868	97.74
CUC1982	22117725	187420	0.85	1529335	6.91	7.76	20400970	92.24
CUC2013F2	16956357	239676	1.41	1282280	7.56	8.98	15434401	91.02
CUCDM	29304711	175845	0.6	1249580	4.26	4.86	27879286	95.14
CUCWAY2-1	21038800	156969	0.75	1349511	6.41	7.16	19532320	92.84

Table 33. Summary of Bowtie2 alignment of *P. humuli* (HDM) and *P. cubensis* (CUC) RNA-seq reads with *Cucumis sativus* genome.

	Bowtie2 Cucumber (*Cucumis sativus* var chinese long) genome							
LIBRARY	TOTAL READS	UNIQUELY MAPPED READS	% UNIQUELY	MULTIPLE MAPPED READS	% MULTIPLE	% MAPPED	UNMAPPED READS	% UNMAPPED
HDM482CA	15,867,481	253,438	1.60	942,327	5.938730918	7.54	14,671,716	92.46
HDM490-5	20,153,641	200,369	0.99	722,375	3.584339922	4.58	19,230,897	95.42
HDM490	11,914,466	198,327	1.66	570,500	4.788296849	6.45	11,145,639	93.55
hdm498SA	26,866,989	57,983	0.22%	88,294	0.33%	0.54%	26,720,712	99.46
HDM501BA	14,405,460	165,038	1.15	633,948	4.40074805	5.55	13,606,474	94.45
hdm502AA	26,086,672	54,346	0.21%	78,568	0.30%	0.51%	25,953,758	99.49
HDM503A3	14,549,813	182,579	1.25	645,240	4.434696171	5.69	13,721,994	94.31
hdm510-1	29,150,378	60,975	0.21%	137,419	0.47%	0.68%	28,951,984	99.32
cdm08A1	23,576,348	50,661	0.21%	64,912	0.28%	0.49%	23,460,775	99.51
cdm2013C3	22,050,773	297,119	1.35	1,078,337	4.890245798	6.24	20,675,317	93.76
cdmA11	27,888,243	61,109	0.22%	111,789	0.40%	0.62%	27,715,345	99.38
CUC1982	16,887,570	241,053	1.43	738,953	4.375721315	5.80	15,907,564	94.20
CUC2013F2	27,962,061	64,446	0.23%	109,215	0.39%	0.62%	27,788,400	99.38
CUCDM	29,219,510	288,893	0.99	956,935	3.274986473	4.26	27,973,682	95.74
CUCWAY2-1	20,976,739	433,343	2.07	971,308	4.630405136	6.70	19,572,088	93.30

Using bioinformatics pipeline that utilizes HTseq, Perl and bash programming, and EMBOSS (extracseq), 242 unique candidate genomic markers that are present in *P. humuli* genome but absent in *P. cubensis* were identified.

Following identification and extraction of gene sequences of the unique candidate markers, primers were designed for all 242 candidate marker genes within 200 bp of the 5′ and 3′ end of each gene with a diagnostic marker using Geneious 10.2.3 (Biomatters Ltd., Auckland, New Zealand). Primers were designed to produce product sizes between 500 and 1500 bp with an annealing temperature of 55 to 58˚C, and were ordered through IDT (Integrated DNA Technologies) or Eton Bioscience, Inc. The PCR reactions, completed according to manufacturer's guidelines, contained 1X Promega GoTaq Green Master Mix (Promega, Durham, North Carolina), 10μM of forward and reverse primers, and were amplified with a program starting with 94˚C for 3 minutes, followed by 35 cycles of 94˚C for 30 seconds, 55˚C for 30 seconds, 72˚C for 30 seconds, with a final elongation step of 72˚C for 5 minutes. Products were analyzed by gel electrophoresis on a 1.5% agarose gel containing 0.2μg/ml ethidium bromide, followed by detection with a Biorad Geldoc Imager using Quantity One software (Bio-Rad, Hercules, California). Product sizes were estimated by comparison to 100bp DNA ladder (New England Biolabs, Inc., Ipswich, MA).

This initial PCR screening with 10 *P. humuli* isolates (Table 34), resulted in single PCR amplicon for 39 candidate genomic markers that matched with their corresponding predicted amplicon sizes (Table 35). For the rest of the 202 candidate markers, 164 exhibit amplification for some or none of the 10 *P. humuli* isolates and 39 candidate markers showed multiple or variable PCR amplification products among the isolates.

Table 34. List of *P. humuli* isolates used for experimental validation of candidate genomic markers and concentration of extracted DNA from corresponding isolates.

External code	Source	Pathogen species	DNA conc. (ng/µl)
HDM501	Oregon State University	*Pseudoperonospora humuli*	386.55
HDM 508	Oregon State University	*Pseudoperonospora humuli*	446.4
HDM 509	Oregon State University	*Pseudoperonospora humuli*	392.1
HDM 500	Oregon State University	*Pseudoperonospora humuli*	296
HDM 457	Oregon State University	*Pseudoperonospora humuli*	317.2
OR502AA	Oregon State University	*Pseudoperonospora humuli*	665.8
SW006	NCSU	*Pseudoperonospora humuli*	585.6
SW009	NCSU	*Pseudoperonospora humuli*	408.5
SW010	NCSU	*Pseudoperonospora humuli*	568.2
AR011	NCSU	*Pseudoperonospora humuli*	404.3

Table 35. Results of laboratory PCR screen of 39 primer sets using 10 P. humuli isolates DNA as template.

#	EXON ID	GENE FUNCTIONAL ANNOTATION	Expected Product Size	OR502AA	HDM501	HDM508	ar011	HDM509	HDM500	HDM457	SW009	SW010	SW006
1	maker-contig_125015-augustus-gene-0.3-mRNA-1:1	Coenzyme Q-binding protein COQ10; homolog, mitochondrial	639										
2	maker-contig_123548-snap-gene-0.64-mRNA-1:2	Gene of unknown function	896										
3	snap_masked-contig_126244-processed-gene-0.3-mRNA-1:1	Gene of unknown function	563										
4	maker-contig_127529-snap-gene-0.164-mRNA-1:2	Gene of unknown function	614										
5	maker-contig_126854-snap-gene-0.17-mRNA-1:2	Gene of unknown function	894										
6	maker-contig_127446-snap-gene-0.1-mRNA-1:1	Gene of unknown function	632										
7	maker-contig_122365-snap-gene-0.7-mRNA-1:2	Conserved gene of unknown function	794										
8	maker-contig_123723-snap-gene-0.2-mRNA-1:3	Conserved gene of unknown function	671										
9	maker-contig_127566-snap-gene-0.179-mRNA-1:3	Glucose-methanol-choline oxidoreductase	1,149										
10	maker-contig_122878-snap-gene-0.0-mRNA-1:1	Gene of unknown function	796										
11	snap_masked-contig_127474-processed-gene-0.29-mRNA-1:2	Potassium channel beta	689										
12	maker-contig_126939-augustus-gene-0.3-mRNA-1:1	Conserved gene of unknown function	704										
13	maker-contig_122506-augustus-gene-0.36-mRNA-1:2	Gene of unknown function	722										
14	snap_masked-contig_122995-processed-gene-0.8-mRNA-1:1	60S ribosomal export protein NMD3	737										
15	maker-contig_123475-snap-gene-0.7-mRNA-1:3	Serine protease family S08A	868										
16	maker-contig_125400-snap-gene-0.18-mRNA-1:2	TBC1 domain family, member 19	973										
17	maker-contig_124066-snap-gene-0.26-mRNA-1:1	Inner-membrane translocator	913										
18	maker-contig_123543-snap-gene-0.12-mRNA-1:3	Gene of unknown function	1,050										
19	maker-contig_123704-snap-gene-0.1-mRNA-1:1	Protein phosphatase 2A scaffold subunit	997										
20	maker-contig_126160-snap-gene-0.25-mRNA-1:2	Gene of unknown function	734										
21	augustus_masked-contig_125861-processed-gene-0.16-mRNA-1:1	Inositol polyphosphate multikinase	719										
22	maker-contig_126448-augustus-gene-0.96-mRNA-1:5	NAD-dependent histone deacetylase sir2	1,070										
23	maker-contig_127679-snap-gene-0.12-mRNA-1:1	Titin isoform N2-B	693										

#	EXON ID	GENE FUNCTIONAL ANNOTATION	Expected Product Size	OR502AA	HDM501	HDM508	ar011	HDM509	HDM500	HDM457	SW009	SW010	SW006
24	maker-contig_127397-augustus-gene-0.68-mRNA-1:8	Cohesin subunit	427										
25	maker-contig_127233-augustus-gene-0.2-mRNA-1:1	Conserved gene of unknown function	1,213										
26	maker-contig_127233-snap-gene-0.5-mRNA-1:3	Gene of unknown function	860										
27	maker-contig_122872-snap-gene-0.14-mRNA-1:1	Gene of unknown function	1,099										
28	maker-contig_124188-augustus-gene-0.12-mRNA-1:1	Gene of unknown function	982										
29	snap_masked-contig_123802-processed-gene-0.2-mRNA-1:1	Titin isoform N2-B	779										
30	maker-contig_123612-snap-gene-0.51-mRNA-1:1	Gene of unknown function	860										
31	maker-contig_124726-snap-gene-0.1-mRNA-1:3	Gene of unknown function	796										
32	maker-contig_127618-snap-gene-0.57-mRNA-1:2	Gene of unknown function	760										
33	maker-contig_125767-snap-gene-0.108-mRNA-1:3	DNA binding protein	1,011										
34	augustus_masked-contig_127600-processed-gene-0.22-mRNA-1:2	Regulator of chromosome condensation (RCC1)	1,605										
35	maker-contig_126365-snap-gene-0.1-mRNA-1:5	Conserved gene of unknown function	1,425										
36	maker-contig_126726-snap-gene-0.26-mRNA-1:2	N-acetyltransferase	741										
37	maker-contig_126726-snap-gene-0.25-mRNA-1:3	ATP-binding Cassette (ABC) superfamily	1,077										
38	maker-contig_127169-snap-gene-0.21-mRNA-1:2	Pentatricopeptide repeat-containing protein	821										
39	maker-contig_124879-snap-gene-0.34-mRNA-1:3	Gene of unknown function	827										

[1] Light green highlight indicates candidate genomic markers with known function and light red highlights indicate candidate markers with unknown function.
[2] White = no PCR amplification; Green = positive PCR amplification with expected amplicon length (base pair); Red = PCR amplification but amplicon length (bp) is different than expected size (bp).

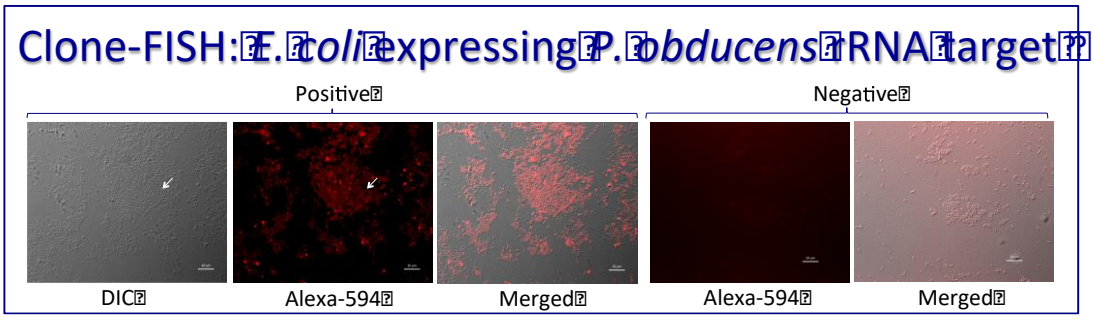
Michigan
Monitoring of airborne spore concentration has been used for several years in conjunction with forecasting of environmental conditions to provide early warnings of cucurbit downy mildew (CDM) occurrence. Burkard spore traps have been the gold standard instrument for this purpose and over the last five years spore estimations provide by these spore traps have been correlated with disease onset in Michigan. However, the high-cost and the extensive labor required for processing of Burkard spore traps have lead scientists to look for more versatile options like Rotorod spore traps. A protocol combining Burkard spore traps with qPCR has been implemented at Hausbeck lab to monitor the airborne concentration of *P. cubensis* (causal agent of CDM) in Michigan cucumber fields. The protocol reduced the labor and response time of the system and was able to detect spore counts as low as 20±5 every 6 hours.

Illumina sequencing and FISH assays for IDM (Gen-ARSB-3, Gen-Rut-3)
JoAnne Crouch's lab examined two new high-throughput tools to detect, visualize and quantify *P. obducens* from environmental samples: (1) a fluorescence in situ hybridization (FISH) method to visualize the presence of *P. obducens* using targeted oligonucleotide probes in undisturbed soil and plant tissue via fluorescence microscopy; and (2) a next generation sequencing (NGS) metagenomic amplicon sequencing approach, whereby specific DNA or RNA targets are PCR amplified from the *P. obducens* genome, then sequenced in parallel using Illumina technology for use in detection and quantification of *P. obducens*. The goal was to provide species-level identification of *P. obducens*.

They developed two FISH probes specifically targeting the DNA of *P. obducens* from multicopy regions of the genome: the rDNA-ITS and the mitochondria. After screening environmental components for autofluorescence (soil, impatiens leaves, soil, stems, and microorganisms commonly inhabiting the environment), probes were synthesized with either a red or blue fluorophore. A set of negative controls have been developed to ensure that the probes do not cross react with other microorganisms that may be encountered in the environment. Control organisms include species of the oomycetes *Phytopthora*, *Pythium*, plus a panel of endophytic symbionts and soil-inhabiting fungi isolated from the plant host and soil (*Colletotrichum*, *Fusarium*, *Trichoderma*, etc.). Since *P. obducens* cannot be cultured or easily increased to provide a positive control for these assays, they used recombinant DNA technology to develop a positive control by inserting either the *P. obducens* rDNA or mitochondrial target DNA sequence into an expression vector, then introducing that vector into the bacteria *E. coli*.

Fluorescence in situ hybridization (FISH) visualization of IDM pathogen.
In this study, (i) an oligonucleotide probe for the oomycete pathogen *Plasmopara obducens* was tested in laboratory conditions using the clone-FISH approach. Following validation, (ii) the probe was applied to *P. obducens* mycelia and infected *Impatiens walleriana* leaves for observation within plant tissues.

Figure 48. A region of the rDNA that is unique and diagnostic for *P. obducens* was designed from nucleotides sequence alignments of oomycete rDNA sequences.



The probe was synthesized with a flourescent dye label, Alex-594. Since *P. obducens* is unculturable, they generated an artificial positive control by cloning and expressing the *P. obducens* target sequence into *E. coli* (clone-FISH). The clone-FISH experiments demonstrate fluorescence in the presence of the target DNA sequence. **Alexa-594**: fluorescent dye filter; **DIC**: Differential interference contrast image; **GFP**: green-fluorescent protein filter; **Merged**: merged images DIC + fluorescent filter; **M**: *P. obducens* mycelia; **Sp**: sporangia, **Sph**: sporangiophore; **VT***: I. walleriana* vascular tissue.

Figure 49. *P. obducens* mycelia and spores mounted on an agar-coated microscope slide.

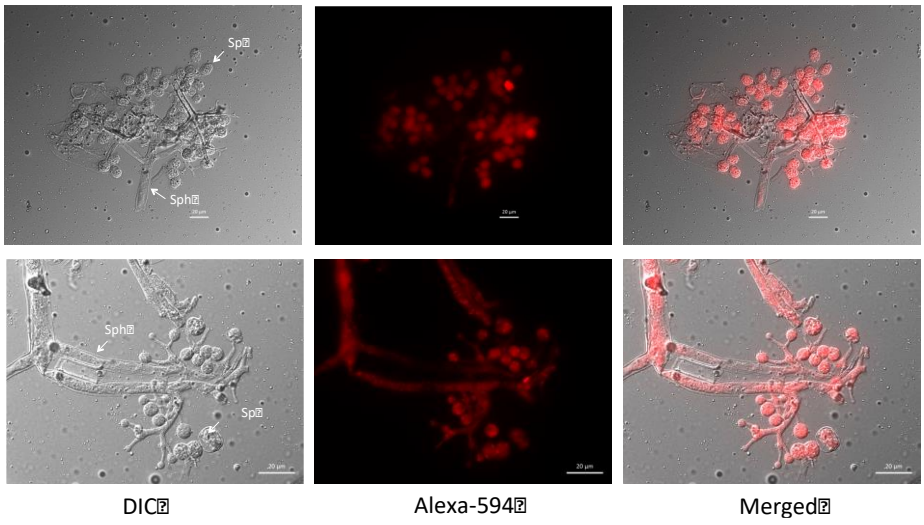
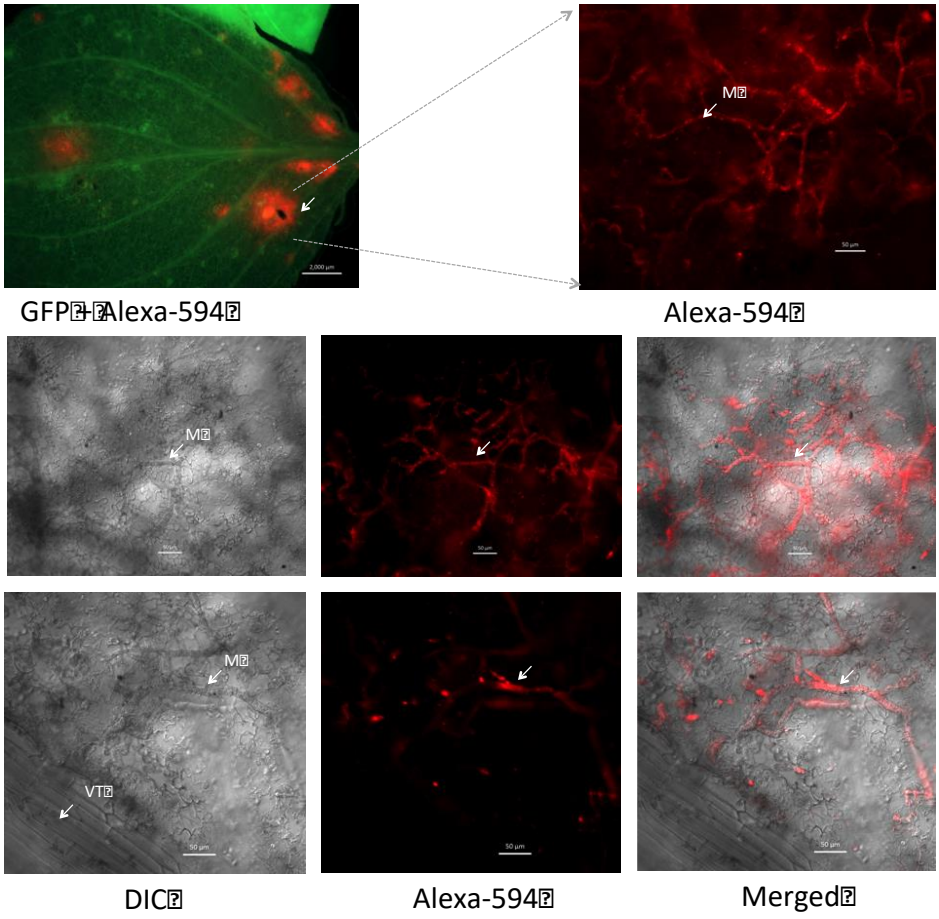


Figure 50. ***Impatiens walleriana*** **leaf tissue showing signs and symptoms of IDM disease.**

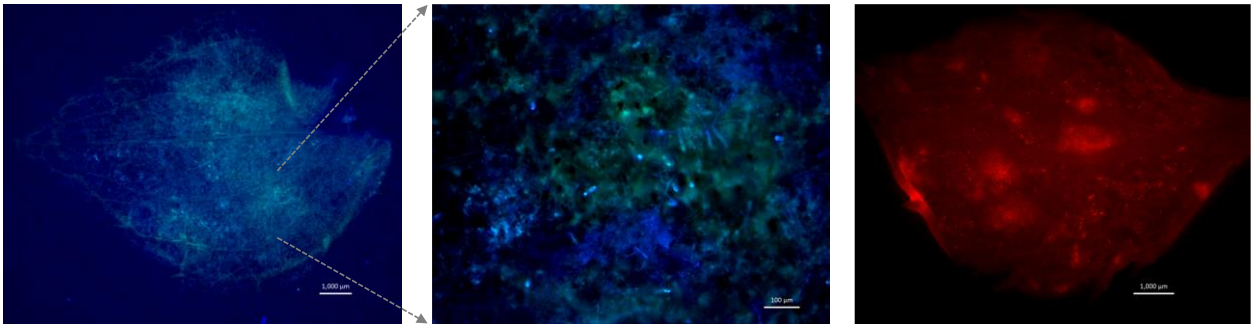


They then optimized our oligonucleotide probes that hybridize to the oomycete pathogen *P. obducens*, allowing fluorescent *in situ* hybridization (FISH) visualization and detection of the pathogen. Figure 51 through Figure 59 contain images from plant tissue and soil are from natural infections.

Because host substrate and other materials encountered from environmental samples may emit background fluorescence that could interfere with FISH probe labels, they synthesized and tested a new FISH probe labeled with an Alexa-350 (blue emission) probe, and compared it with the Alexa-594 labeled FISH probe developed in the previous two quarters of the grant cycle.

The Alex-350 labeled blue probe performs at almost the same level as the Alexa-594 labeled red probe (Figure 51) when used to take detailed images. Please see Figure 52 through Figure 59 for optimization and validation research.

Figure 51. Comparison of two FISH probe labels (Alexa 350 and Alexa 594)

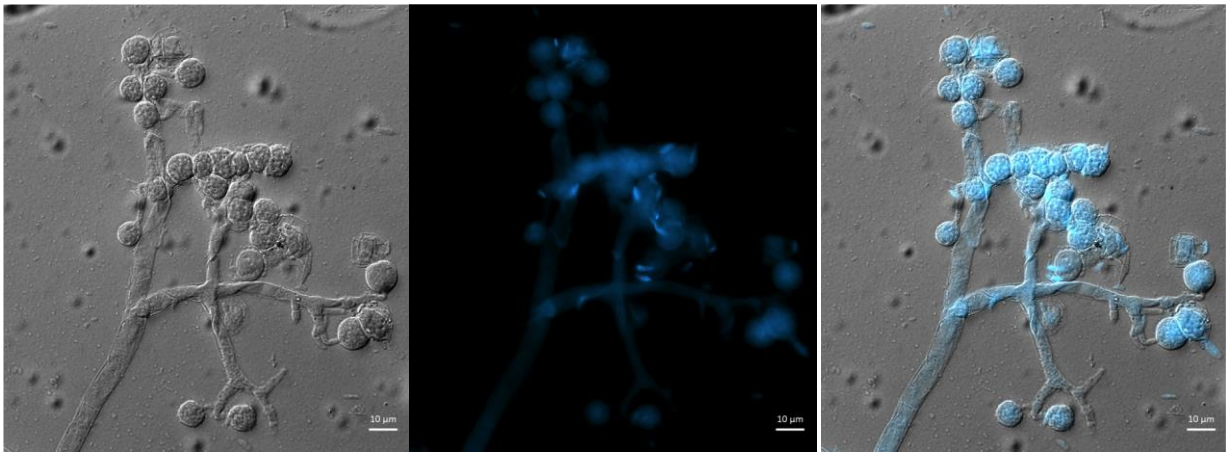


A）GFP）+）Alexa-350）　　**B**）GFP）+）Alexa-350）　　**C**）Alexa-594）

Above: Images of *I. walleriana* leaves infected by *P. obducens* (shown in blue in images A and B, and shown in red in image C). Images captured using a Zeiss Axio Zoom fluorescence stereo zoom microscope.

- The Alexa-594 labeled red probe (image C) performs better for imaging of *P. obducens* infecting whole specimens (whole leaves), when compared with the blue probe (Alexa-350; images A-B above). GFP=green fluorescent wavelength emission, due to plant leaf tissue autofluorescence.
- The Alex-350 labeled blue probe performs at almost the same level as the Alexa-594 labeled red probe when used to capture more detailed images, such as the structures shown in figure B above.
- In figure C above, areas where *P. obducens* infection is concentrated (red) in the leaf can be observed, as these areas emit greater levels of fluorescence than other areas.
- Very bright spots in figures B-C above show *P. obducens* mycelial tips (B=blue, C=red), where cells are actively growing (cells dividing).

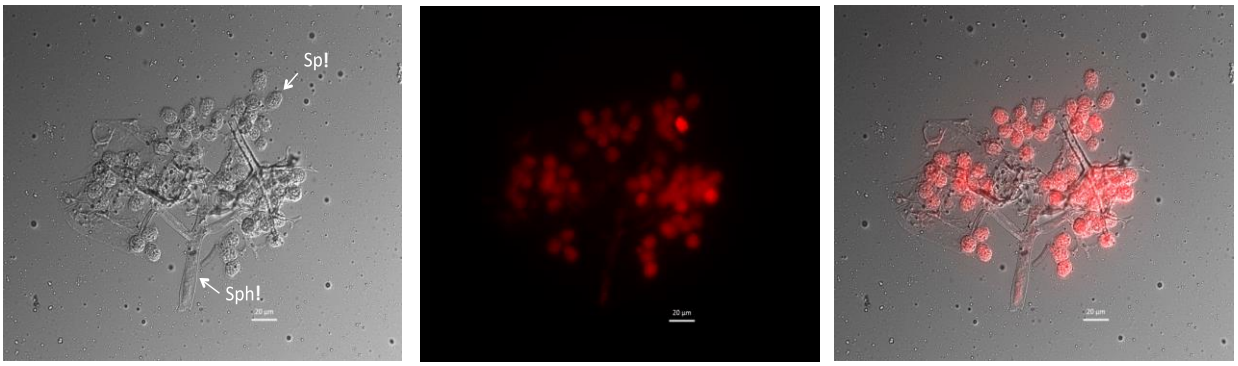
Figure 52. Visualization of P. obducens using the Alexa-350 labeled (blue) FISH probe



DIC）　　Alexa-350）　　Merged:）DIC）+）Alexa-350）

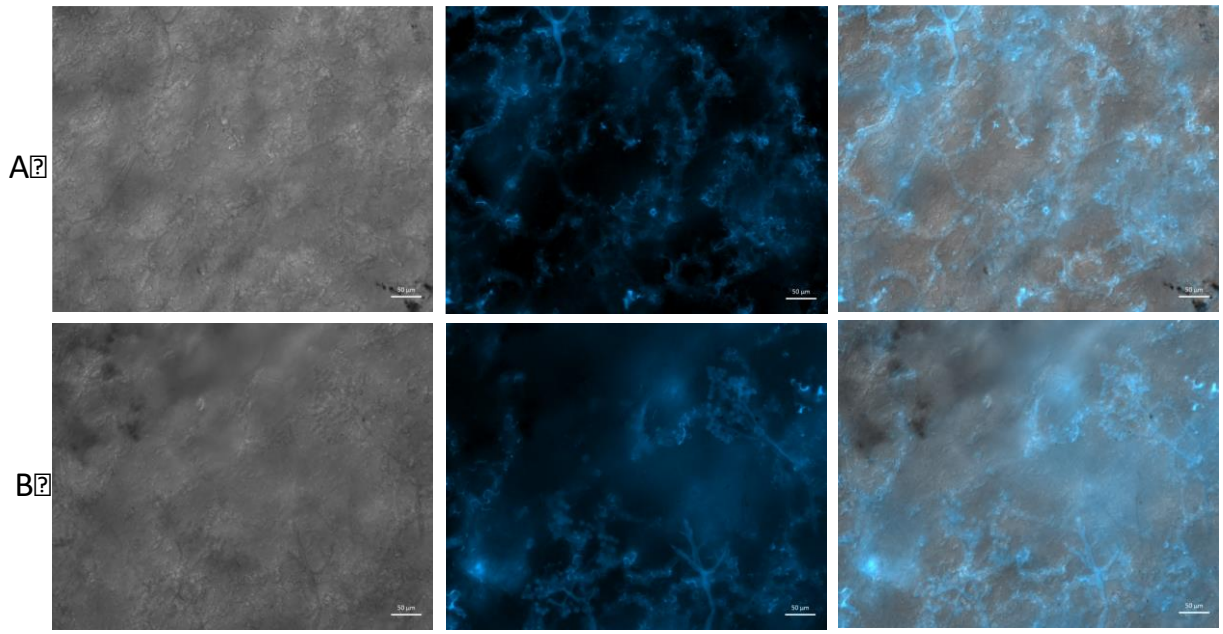
Above: Images of *P. obducens* mycelia, sporangiophores and sporangia hybridized with the Alexa-350 blue labeled probe, mounted on an agar-coated microscope slide. Images captured using a Zeiss Axio Imager 2 fluorescent microscope.

Figure 53. Visualization of *P. obducens* using the Alexa-594 labeled (red) FISH probe



DIC Alexa-594 Merged: DIC + Alexa-594

Figure 54. Visualization of *P. obducens* colonizing *I. walleriana* leaf tissue using FISH

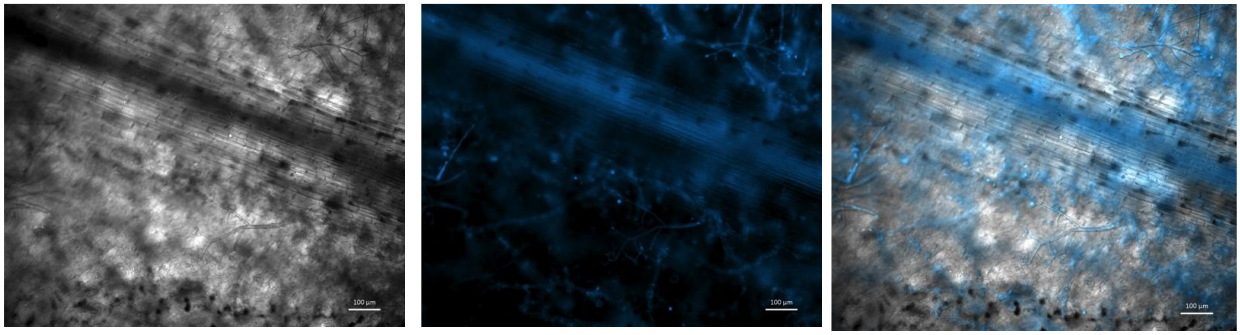


DIC Alexa-350 Merged: DIC + Alexa-350

Above: Images of *I. walleriana* leaves infected by *P. obducens* (shown in blue) taken using a Zeiss Axio Imager 2 fluorescent microscope. Leaf tissue has been cleared of chlorophyll using a solution of ethanol 95%, acetic acid, glycerol (75:15:10 v/v), and *P. obducens* hybridized with Alexa-350 blue labeled probe.

- **Images in Figure A**. Mycelia growing inside leaf. Mycelia grows thicker inside of the leaf tissue. No oospores or zoospores observed inside this tissue.
- **Images in Figure B**. This field shows mycelia that is growing outside of the leaf (underside). Sporangiophores and sporangia can be seen. Note the difference between mycelia inside and outside of leaf tissue.

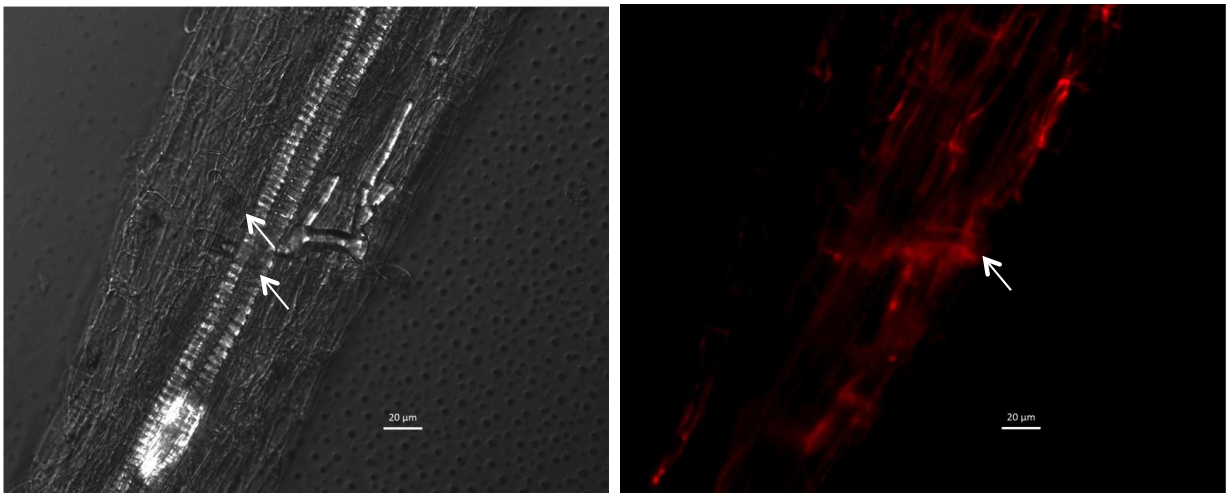
Figure 55. Visualization of *P. obducens* colonizing *I. walleriana* leaf tissue using FISH



DIC Alexa-350 Merged: DIC + Alexa-350

Above: Images of *Impatiens walleriana* leaves infected by *Plasmopara obducens* (shown in blue) taken using a Zeiss Axio Imager 2 fluorescent microscope. Leaf tissue has been cleared of chlorophyll using a solution of ethanol 95%, acetic acid, glycerol (75:15:10 v/v), and *P. obducens* hybridized with Alexa-350 blue labeled probe.

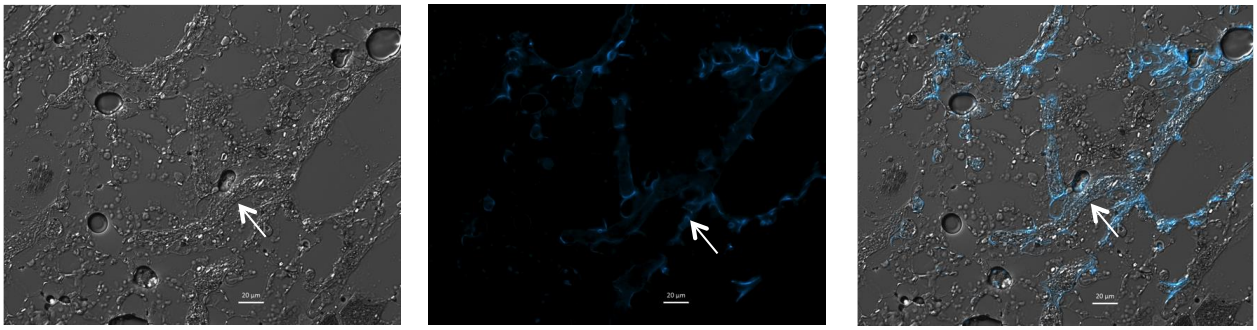
Figure 56. Visualization of *P. obducens* colonizing *I. walleriana* root tissue using FISH



DIC Alexa-594

Above: Images of *Impatiens walleriana* root infected by *Plasmopara obducens* (shown in red) taken using a Zeiss Axio Imager 2 fluorescent microscope. To prepare these images, roots were collected from *I. walleriana* plants with heavy infection of *P. obducens* on above ground tissue. Roots were fixed onto a glass microscope slide, and hybrized with the Alexa-594 red labeled probe. No sections of roots were made. No oospores or zoospores were observed. In the root shown above, in the area indicated by the arrow, a structure was observed that my show the entry of a hyphae into the root.

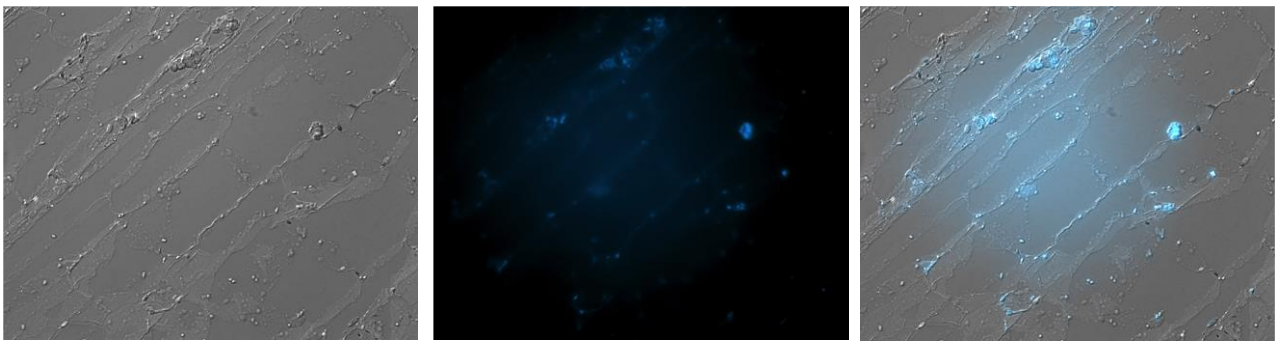
Figure 57. Histological sections (longitudinal) of *I. walleriana* leaf infected by *P. obducens*



DIC Alexa-350 Merged: DIC + Alexa-350

Above: Images of an *Impatiens walleriana* leaf section (longitudinal) infected by *Plasmopara obducens* (shown in blue, Alexa-350 probe) taken using a Zeiss Axio Imager 2 fluorescent microscope. Sections are approximately 10 µm thick. Although many details of *P. obducens* colonization of host tissue can be seen when the whole leaf is used for hybridization, these structures become less apparent when the histological sections are assessed. Pathogen mycelia can still be observed within the leaf tissue, but due to the thickness of the cut, the extent of the tissue colonization cannot be seen. Thicker sections may be necessary for greater distinctiveness in structural visualization. No oospores or zoospores were observed from this sample.

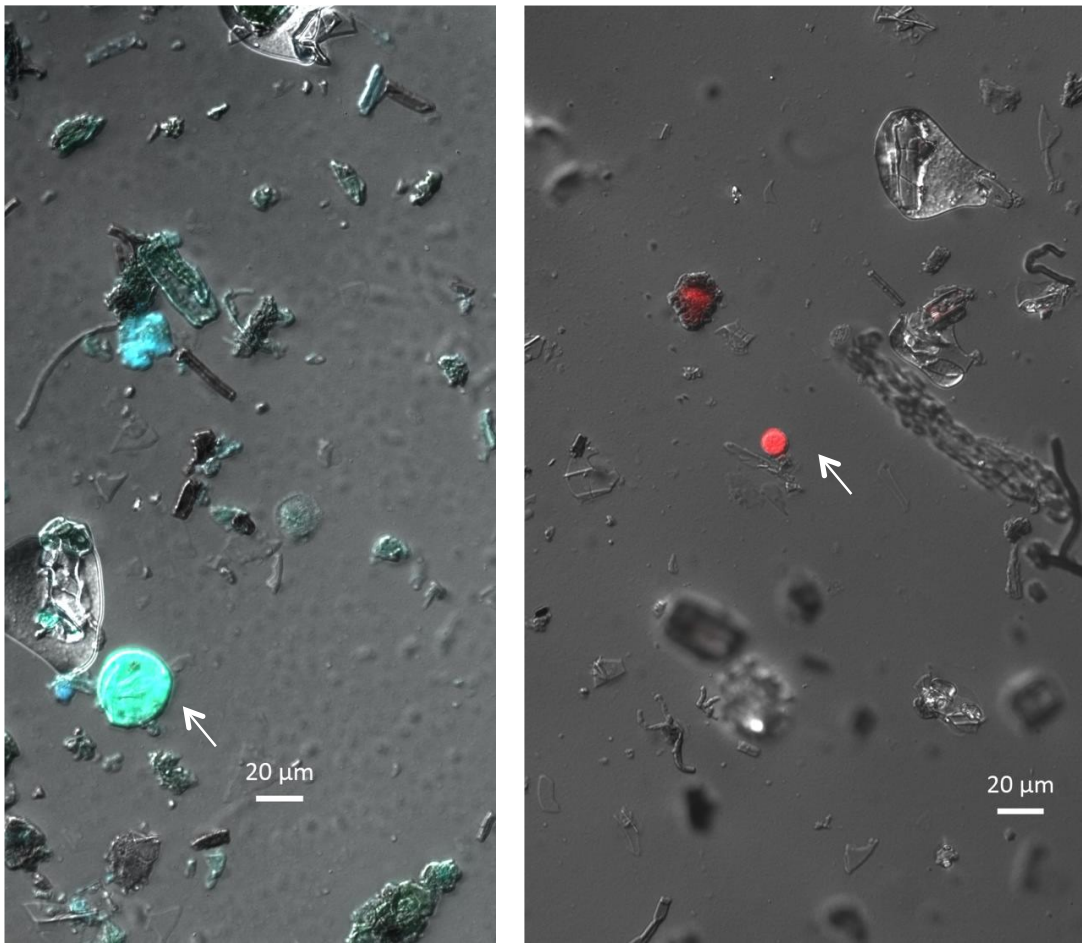
Figure 58. Histological sections (longitudinal) of *I. walleriana* leaf infected by *P. obducens*



DIC Alexa-350 Merged: DIC + Alexa-350

Above: Images of an *Impatiens walleriana* leaf section (longitudinal) infected by *Plasmopara obducens* (shown in blue, Alexa-350 probe) taken using a Zeiss Axio Imager 2 fluorescent microscope. Sections are approximately 10 µm thick. As it was observed in the previous sample (top of page), details of the structure of the stem and *P. obducens* were not clear, but the presence of the pathogen can be diagnosed using the FISH assay. No oospores or zoospores observed inside these tissues.

Figure 59. Soil samples, taken from 6" pots of *I. walleriana* plants with severe *P. obducens* infection



Above: Images of soil taken from 6" pots of an *Impatiens walleriana* plant heavily infected by *Plasmopara obducens* using a Zeiss Axio Imager 2 fluorescent microscope. Assessments in soil are ongoing to optimize. These images were prepared by dissolving a small amount of soil in distilled water, then fixing the liquid to glass microscope slides, followed by hybridization with the probe. Based on size and shape, the round structures identified by arrows in the images above may be oospores or sporangia (left and right images, respectively), but additional optimization and evaluation is required.

Monitor P. obducens populations; persistence of oospores in field soil using genomic diagnostic tools (Gen-ARSB-4, Gen-Rut-4)

Establishment of impatiens downy mildew sentinel plots. Fourteen sentinel plots were established in 2017 to monitor for IDM, sample the causal pathogen *P. obducens*, and quantify the pathogen in soil prior to and following plot establishment. By establishing a portion of the plots in gardens and arboreta within the Sentinel Plant Network, these plots will also help promote the role of gardens and arboreta as a resource for plant science research.

In preparation for plot establishment, they submitted a proposal for IDM sentinel plot establishment to the Sentinel Plant Network (http://www.sentinelplantnetwork.org/). Protocols were newly developed for establishing, monitoring, and sampling IDM sentinel plots, and web-content was generated to provide updates from the IDM sentinel plots (see Figure 60 and https://www.ornamentalpathology.com/idm-

plots). The fourteen plots were established across the Mid-Atlantic, Midwest, and a single plot on the west coast of the US (Table 36). Throughout the summer of 2017, disease samples were collected and stored as dried specimens at the USDA-ARS Mycology & Nematology Genetic Diversity and Biology Lab. DNA was extracted from samples, and genotyping was performed using mtDNA rps10 and SSR markers. Final data analysis is underway.

Figure 60. Example of the online graphic updates for the IDM sentinel plots.

The inset image shows material that pops up when a location symbol is clicked.

Table 36. Locations and establishment dates for seventeen IDM sentinel plots in the US

Location	State	City	Established
Boerner Botanical Gardens	Wisconsin	Hales Corners	6/20/17
Central Maryland Research & Education Center	Maryland	Upper Marlboro	6/22/17
Dumbarton Oaks Gardens	N/A	Washington, DC	6/27/17
James Madison's Montpelier	Virginia	Orange	6/29/17
Lower Eastern Shore Research & Education Center	Maryland	Salisbury	6/26/17
Norfolk Botanical Garden	Virginia	Norfolk	6/23/17
North Willamette Research & Extension Center	Oregon	Aurora	6/21/17
Phipps Conservatory and Botanical Gardens	Pennsylvania	Pittsburgh	6/27/17
Residential	Maryland	Silver Spring	6/10/17
Residential	Pennsylvania	Philadelphia	6/24/17
Residential	Delaware	Newark	7/1/17
Taylor Conservatory Foundation	Michigan	Taylor	6/30/17
University of Delaware	Delaware	Newark	6/27/17
Wellfield Botanic Garden	Indiana	Elkhart	6/21/17

Objective 3: Genomic Resources & Diagnostic: Population Characterization

Characterize IDM populations (Gen-ARSB-5, Gen-Rut-5)

Herbarium and modern sample assessment

A preliminary assessment of the population structure of the *P. obducens* isolates evaluated so far was done using the program Structure with no predefined populations, in order to determine if previous grouping based on rDNA/ITS were still recovered using different and more polymorphic markers. Based on the results obtained from the program Structure (Figure, below) only one of the previously described populations obtained using rDNA/ITS data (group G3). The remaining isolates cluster in at least 5 subpopulations that do not correspond to the remaining groups observed with rDNA/ITS data. The isolates in the different subpopulations do not group based on host or geographic origin.

Figure 61. Results of Bayesian clustering of specimens of *Plasmopara obducens* with no prior assumption of population structure. The most probable number of subpopulations recovered with current data is 6 (K=6).

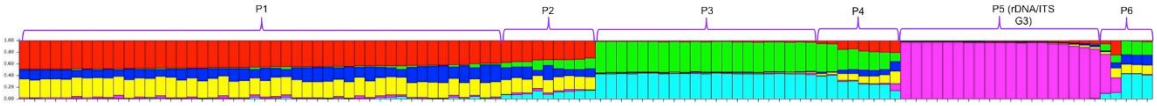
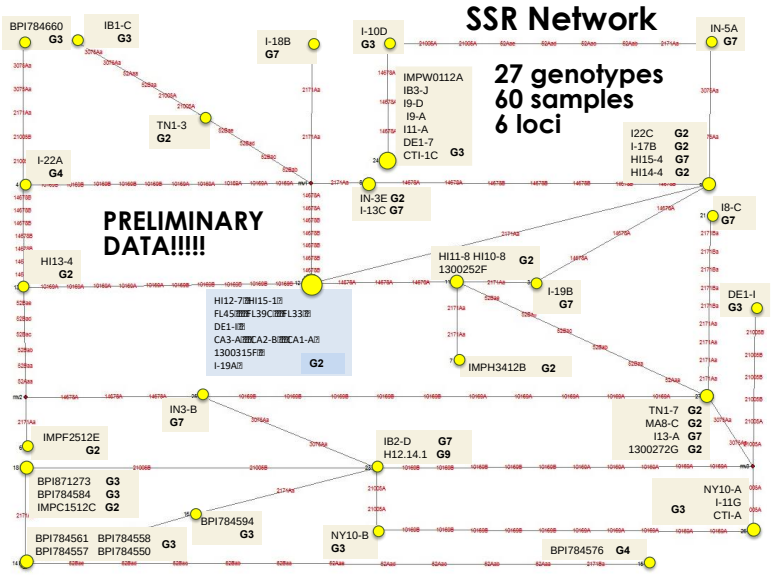


Figure 62. Network diagram constructed from just six of the SSR markers and used to screen 60 *P. obducens* samples. Twenty-seven unique genotypes were identified from this small sample.



Characterize pre- and post- IDM populations

The Crouch lab amassed a collection of IDM samples from as many locations as possible to ensure they identify the full complement of *P. obducens* genetic variation and to monitor any changes in pathogen populations. The sources of collected IDM arose partially from a "Wanted" posters sent in 2013 to diagnostic clinics, extension pathologists, etc. to request IDM foliar samples. This poster also included four other pathogens. The Crouch lab extracted DNA from ~1000 new IDM samples from 173 unique sample sites and 52 US herbarium specimens. The new US IDM collections were from 31 states (Figure 63, Figure 64). IDM collections from outside the US were from 15 countries; 18 from *I. walleriana*, 17 from other *Impatiens* species (Figure 65). This collection is stored in the US Fungal Collections

herbarium in Beltsville, Maryland, where the materials were catalogued and are maintained permanently for the benefit of the scientific community.

Figure 63. Geographic origin of *Plasmopara obducens* collections from the continental USA. and Hawai'i. Left: 894 IDM samples, derived from 173 unique sample sites (right).

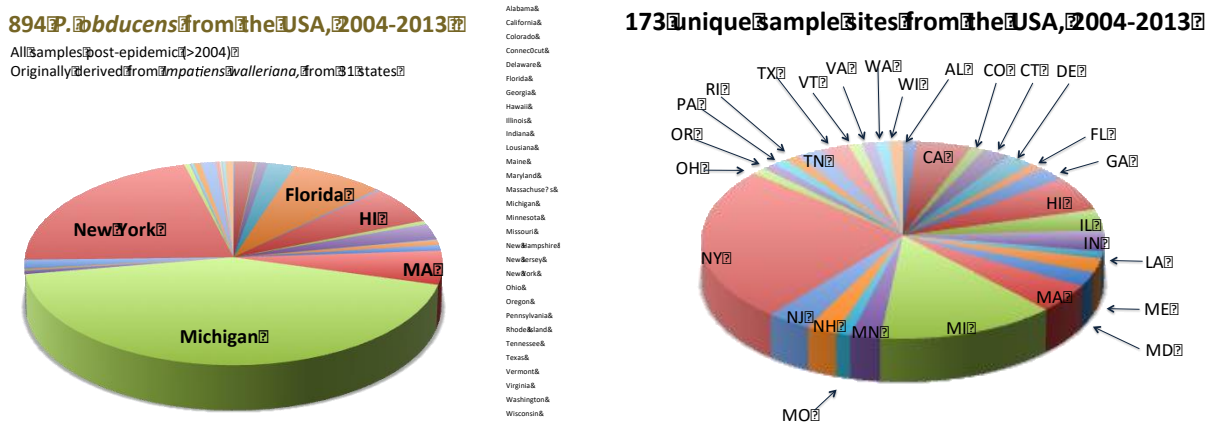


Figure 64. Geographic distribution of pre- and post-epidemic IDM samples collected in the USA.

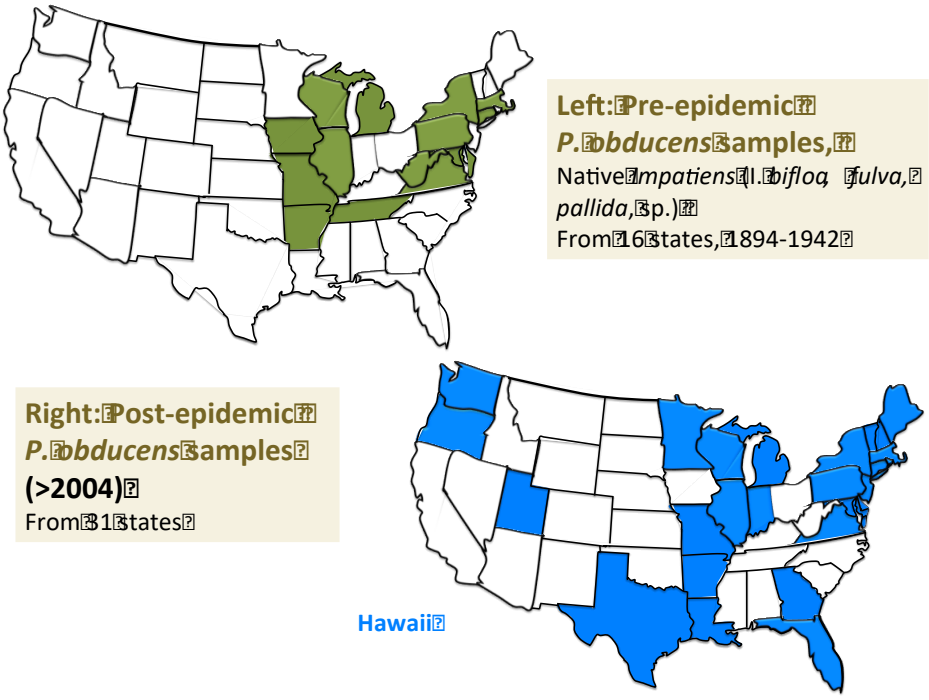
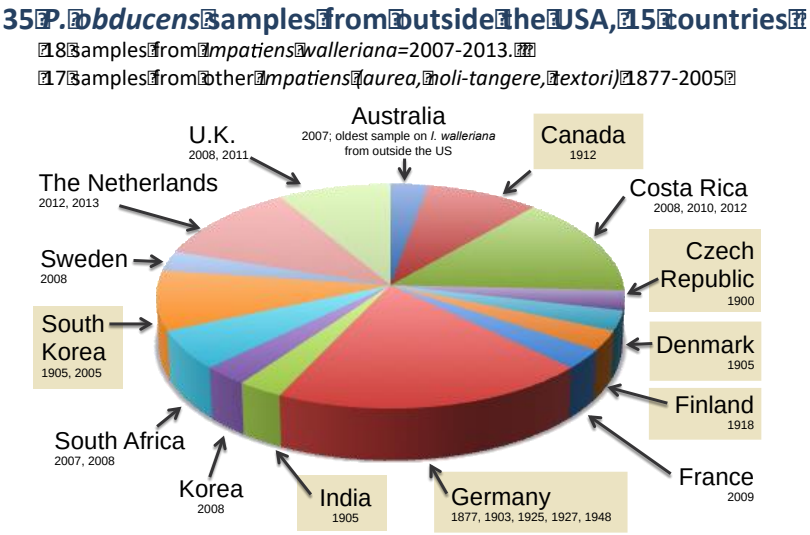


Figure 65. Geographic origin of *Plasmopara obducens* collections outside of the USA. through the end of 2013.



Ribosomal/ITS DNA Marker Genotypes

Over 800 samples of IDM have been analyzed using a set of four co-dominant molecular markers in the rDNA. In addition, 87 pre-epidemic herbarium specimens of *P. obducens* inhabiting native impatiens species and 20 samples of related *Plasmopara* were screened. The markers utilize real-time PCR endpoint detection of a set of SNPs, and were designed using DNA sequences from 134 individuals and clones. Eighteen haplotypes were identified. Five haplotypes were limited to modern samples, nine haplotypes were limited to historic samples, and four haplotypes were identified from both modern and historic samples. The numerically dominant haplotypes were identified from both modern and historic samples. *P. obducens* haplotypes overlapped with those of related *Plasmopara* species, indicating that these markers need to be supplemented with additional datasets to provide discriminatory signal. Therefore, development of simple sequence markers (SSRs) is underway using genome resources (see below).

Figure 66. Median joining network diagram constructed from ribosomal DNA dataset, showing nine major rDNA genotypes.

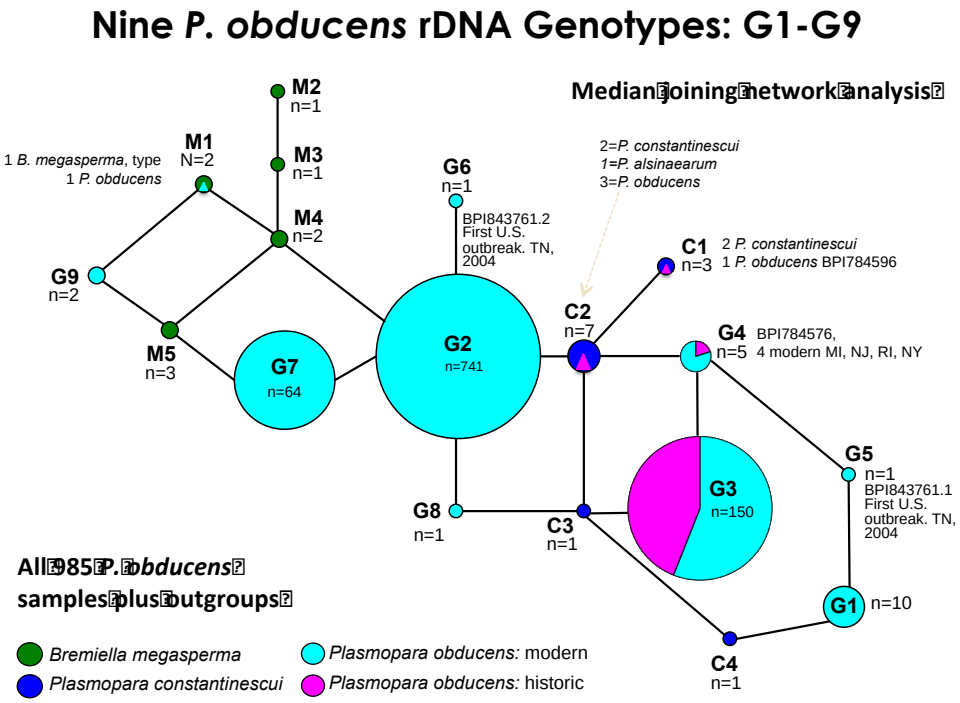
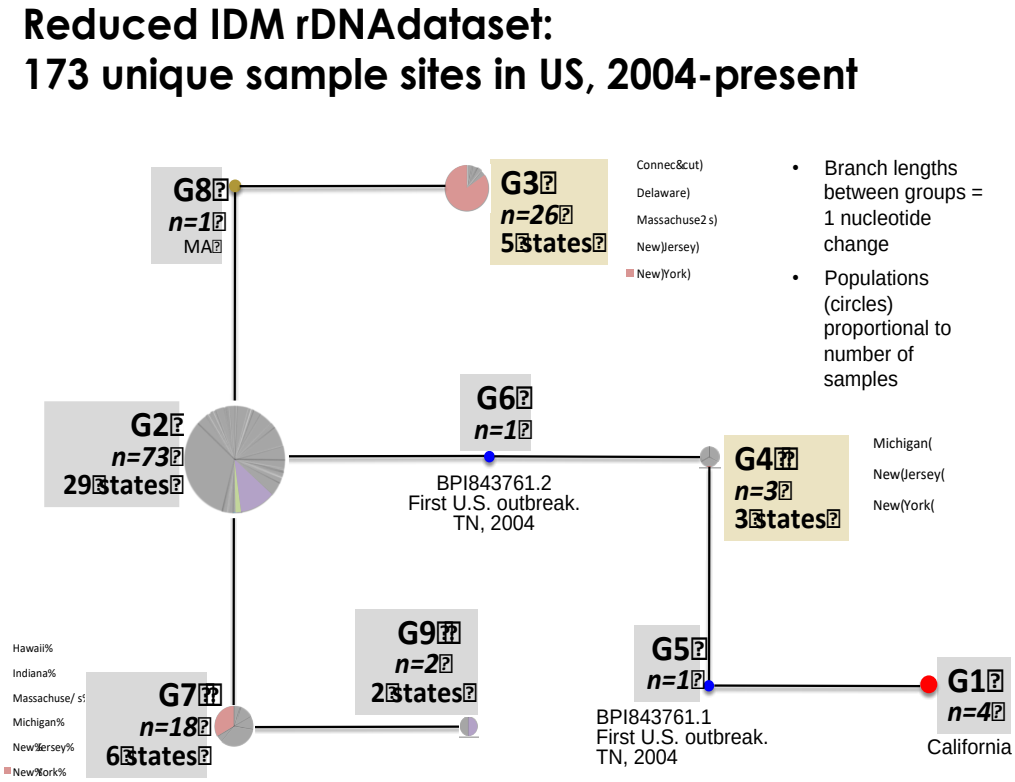


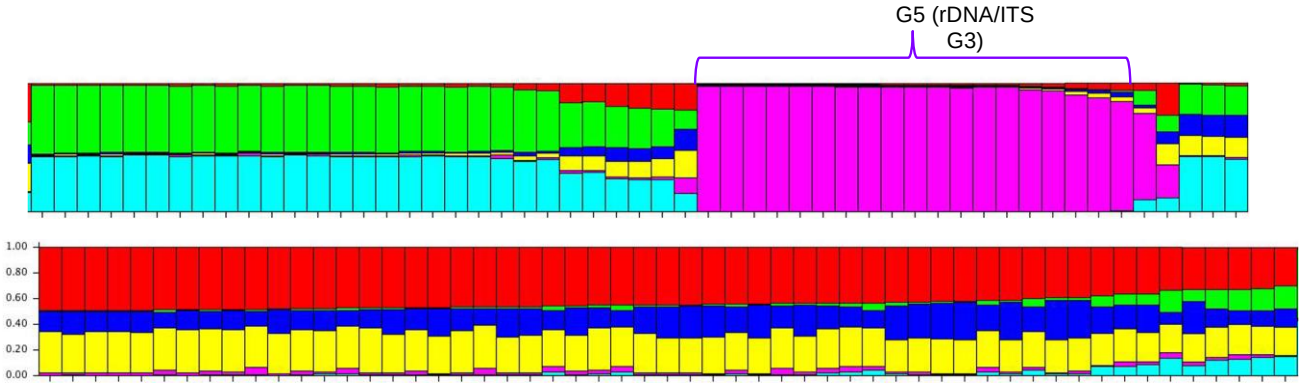
Figure 67. Median joining network diagram constructed from the ribosomal DNA datasets, with only the 173 unique sample sites and IDM samples in the analysis.



Five variable SNPs identfied in ribosomal/ITS regions showed little variation across 1400 *P. obducens* samples. IDM groups overlapped with other *Plasmopara* species, indicating that markers with higher variability are needed. Because *P. obducens* cannot be cultured and DNA is extracted directly from environmental samples, accurate genotyping of samples may be hindered by the presence of heterogenous DNA. Therefore, highly variable codominant markers that specifically target organismal DNA, such as microsatellites, would be ideal for this pathosystem.

Using rDNA ITS as a tool to distinguish isolates yielded the following results. Real-time PCR assay identified nine *P. obducens* rDNA genotypes. It was shown that rDNA/ITS genotype 3 (G3) was the only genotype present in North America prior to IDM epidemics on native *Impatiens* species (1880s). The rDNA genotype 2 (G2) was the most widespread and abundant of the rDNA/ITS genotypes in the US. The Crouch lab using this tool identified the emergence of new rDNA/ITS genotypes (G4-G9). The rDNA/ITS genotypes group *P. obducens* with other species of *Plasmopara*. Although *P. obducens* rDNA/ITS genetic groups were distinct and diagnosable, samples shared 98.1-100% similarity, with little statistical power associated with this single locus analysis (only ~1.20E-05 of the genome).

Figure 68. STRUCTURE diagram constructed from an 18 SSR dataset.



Each vertical rectangle represents a single sample, and colors refect genotypes. So far, only rDNA/ITS genotype G3 was recovered as a distinct population using these SSR markers from a subset of IDM samples. The species is far more diverse than what is observed using ITS/rDNA genotypes alone. Much admixture is observed between populations. Clustering by genetic similarities was not associated with geographic origin or host.

SSR Markers

The Crouch lab used the *P. obducens* genome assemblies to identify, develop and test 37 novel and species-specific SSR markers against a test panel of 96 *P. obducens* samples collected during 2012-2014. Thirty-four of the SSR markers were polymorphic, with 2-6 alleles observed. The markers were from gene regions, and based on homology to characterized oomycete proteins, were annotated as of unknown function (28 markers) or using putative identifications (6 markers, included one predicted CRINKLER family protein). All markers had sequence similarity to sequence data from other oomycete genomes. No markers produced a PCR product from host DNA. Observed and expected heterozygosity ranged from 0.013−0.892 and 0.023−0.746, respectively. Just 17 markers were sufficient to identify all multilocus genotypes. These are the first SSR markers available for this pathogen, and one of the first molecular resources. These markers will be useful in assessing variation in pathogen populations and determining the factors contributing to the emergence of destructive impatiens downy mildew disease. A paper describing these new SSR markers is in press with the peer-reviewed journal *Applications in Plant Science*, to be released in the December issue.

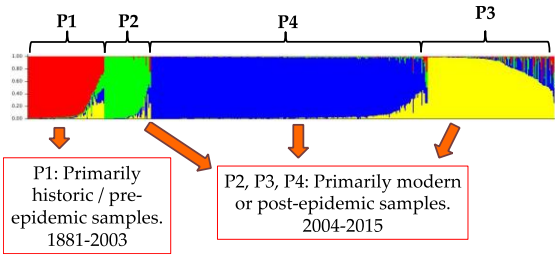
A sample consisting of 598 *P. obducens* was screened using the SSR markers to evaluate population groups. Of these 504 samples were of "modern" origin, collected between 2004 during the first IDM outbreak in TN and the current 2015 growing season. Ninety-four samples were pre-epidemic; herbarium specimens collected between 1881-2000 and were associated with native North American *Impatiens* species. Of these, 35 samples were from outside the US. SSRs fragment sizing was performed on an ABI3730xl DNA Analyzer, with 10% of all reactions repeated, scoring performed using GeneMarker software, and cluster analysis performed using STRUCTURE. Results are illustrated in Figure 69.

From the SSR genotypic analysis of 598 IDM samples, the Crouch lab found the following. There were four *P. obducens* groups (P1-P4). Genes flowed between populations. The SSR populations were consistent with rDNA-ITS groups. P1 largely consists of pre-epidemic samples, and all but three of the samples originated from native *Impatiens* hosts. P2, P3 and P4 largely consist of post-epidemic samples (Figure 69). No genotype was exclusive to cultivated or native impatiens species. Prior to the US IDM epidemic, all 4 major populations present, but P1 was the dominant population pre-epidemic. The sample collected from the 1st US outbreak in TN was admixed, but it carried the dominant pre-epidemic P1 genotype. For pre-epidemic populations, P2, P3, P4 genotypes were identified, however they were always found admixed with P1 genotype. For post-epidemic populations, clonal P2, P3, P4 genotypes were increasingly found as the sole genotype. During the epidemic, no pure P1 genotypes were identified, and the P1 genotype was a minority part of admixed samples. Non-US IDM samples had the same genotypes as US IDM samples.

From these data, it was concluded that there was a shift in *P. obducens* US population structure after 2004 and that the association with the cultivated species of impatiens has led to a shift in dominant *P. obducens* genotypes in the US. The dominant population on native impatiens species, P1, was supplanted by P2, P3, and P4 genotypes during the epidemics. P1 remains a component of epidemic populations, but only as a minority genotype. Although genotypes of populations P2, P3, and P4 were present as minority components prior to the epidemics, during the epidemics these genotypes emerged to become the dominant cause of IDM on cultivated *I. walleriana*.

Because post-epidemic genotypes of *P. obducens* were present in the US on native species of *Impatiens* since the 1880s, there was no evidence to support the introduction of novel populations from outside the US. However, the 35 non-US IDM samples showed the same genotypes as IDM samples from from within the US, so they cannot entirely rule out the possibility of non-indigenous *P. obducens* playing a role in the disease outbreaks.

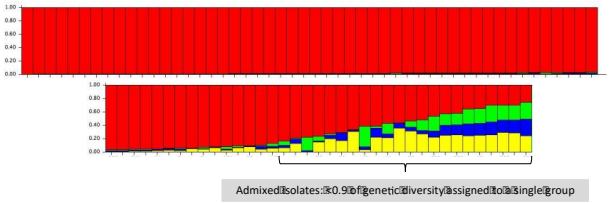
Figure 69. Visual summary of *P. obducens* population groups



Figure, left. Overall STRUCTURE diagram showing the four *P. obducens* populations identified from the 34 SSR markers. Below, individual panels illustrating SSR populations P1-P4 and their make-up.

P. obducens P1

- ❑ **Pre-epidemic samples 1880-2003⚜**
- ❑ **Six post-epidemic samples⚜**
- ❑ Hosts: primarily wild Impatiens: *I. balsamina, I. fulva, I. noli-tangere, I. pallida, I. textori*
- ❑ Distributed across the U.S.A.
- ❑ Some samples from Canada, Central Europe and Korea.

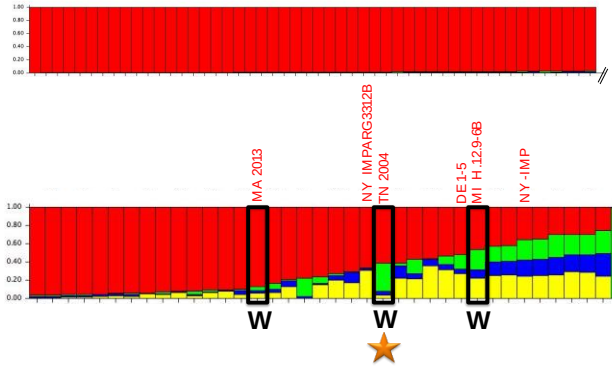


Admixed isolates: ≤ 0.9 of genetic diversity assigned to a single group

P. obducens P1

17 STATES: AL, CT, DC, DE, IA, IL, IN, KS, MA, MD, MO, NJ, NY, PA, TN, WI, WV

Six post-epidemic samples

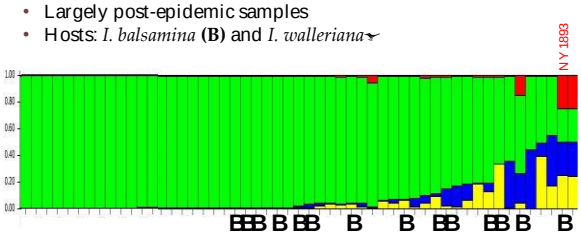


P. obducens P2

6 STATES: CT, DE, MD, ME, NJ, NY

One pre-epidemic sample

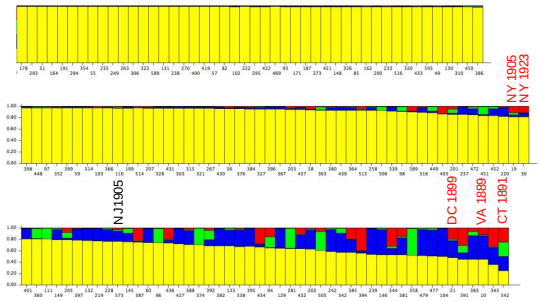
- Identifie only in the eastern U.S.
- Largely post-epidemic samples
- Hosts: *I. balsamina* **(B)** and *I. walleriana*



P. obducens P3

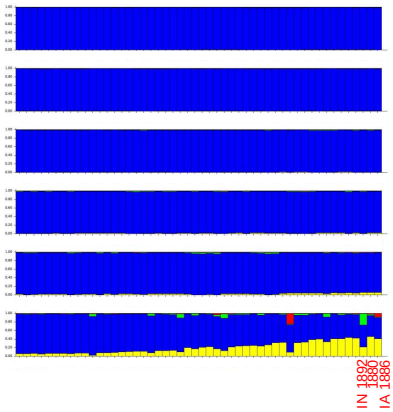
12 STATES: CA, CT, DC, DE, FL, HI, IN, MA, MI, NY, TN, VA

- Six pre-epidemic herbarium samples
- Hosts: *I. walleriana;* 37 samples from *I. arguta, I. balsamina, I. flanaganiae*



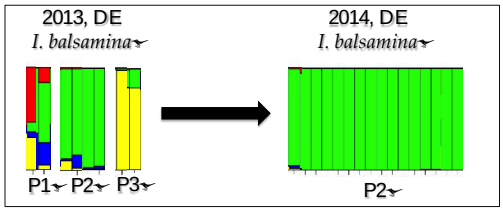
P. obducens P4

11 STATES: CA, HI, IN, MA, MD, ME, MI, NJ, NY, PA, TN

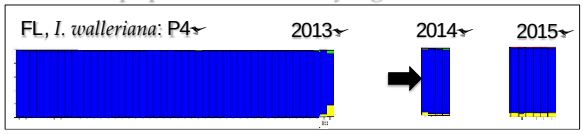


- Most abundant genotype in cultivated impatiens in U.S. nurseries
- **4 pre-epidemic samples ⚜**
- Hosts: *I. walleriana;* 14 samples from *I. auricoma, I. balsamina, I. flanaganiae*

Some populations are changing .....

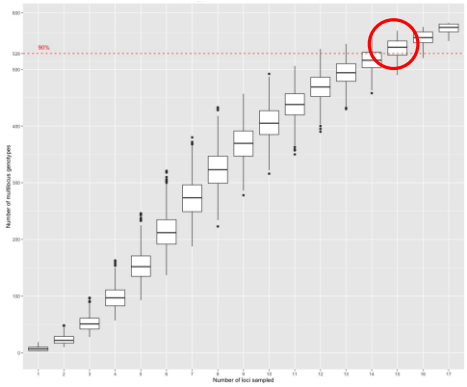


But some populations are staying the same!



The final population genetic analysis included 875 IDM samples collected between 2004-present day, plus 91 hebarium samples. Samples were genoptyped using 18 SSR markers. Genotype accumulation curve analysis shows that 15 markers are adequate to recover 90% of populaiton genetic diversity from the 966 samples (Figure 70).

Figure 70. Genotype accumulation curve illustrating number of SSR markers required to capture population genetic diversity of the IDM sample.



The IDM sample set was reduced to eliminate clones, and individual samples missing more than 50% of SSR data (due to non-amplification) were removed, leaving a total of 587 individuals. The clone-corrected data had 587 multi-locus genotypes (MLGT), meaning that the genetic diversity of the population is very high.

Table 37. Summary statistics for clone corrected dataset (n=587).

Locus	# individuals	Na	Ne	I	Ho	He	uHe	F
2910	507	3.000	2.223	0.868	0.917	0.550	0.551	-0.667
2933	556	2.000	1.984	0.689	0.906	0.496	0.496	-0.828
3024	423	5.000	2.300	1.011	0.757	0.565	0.566	-0.338
3075	490	5.000	2.433	1.070	0.776	0.589	0.590	-0.317
4176	435	3.000	1.692	0.646	0.529	0.409	0.409	-0.293
4357	531	6.000	2.425	1.042	0.863	0.588	0.588	-0.468
5494	523	6.000	1.775	0.938	0.315	0.436	0.437	0.277
6030	451	2.000	1.942	0.678	0.823	0.485	0.486	-0.696
7328	518	5.000	2.118	0.830	0.931	0.528	0.528	-0.763
8649	557	2.000	1.935	0.676	0.810	0.483	0.484	-0.676
11069	544	3.000	1.930	0.689	0.794	0.482	0.482	-0.648
12309	440	3.000	2.093	0.850	0.652	0.522	0.523	-0.249
21005	446	2.000	1.992	0.691	0.747	0.498	0.498	-0.500
29057	533	5.000	1.814	0.728	0.606	0.449	0.449	-0.350
33638	452	8.000	2.144	1.188	0.378	0.534	0.534	0.291
36128	396	5.000	1.265	0.469	0.093	0.209	0.209	0.553
47245	396	4.000	2.507	1.130	0.583	0.601	0.602	0.030
52381	496	2.000	1.992	0.691	0.935	0.498	0.498	-0.879

Na = No. of Different Alleles
Ne = No. of Effective Alleles = 1 / (Sum pi^2)
I = Shannon's Information Index = -1* Sum (pi * Ln (pi))
Ho = Observed Heterozygosity = No. of Hets / N
He = Expected Heterozygosity = 1 - Sum pi^2
uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He
F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)
Where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.

Figure 71. Using the computer program STRUCTURE, the 587 IDM samples from the clone-corrected dataset were subdivided into seven distinct population clusters.

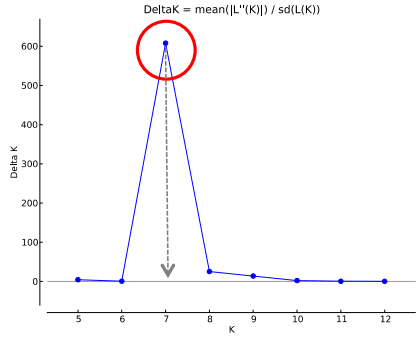


Figure 72. STRUCTURE diagram illustrating the seven populations (A-G).

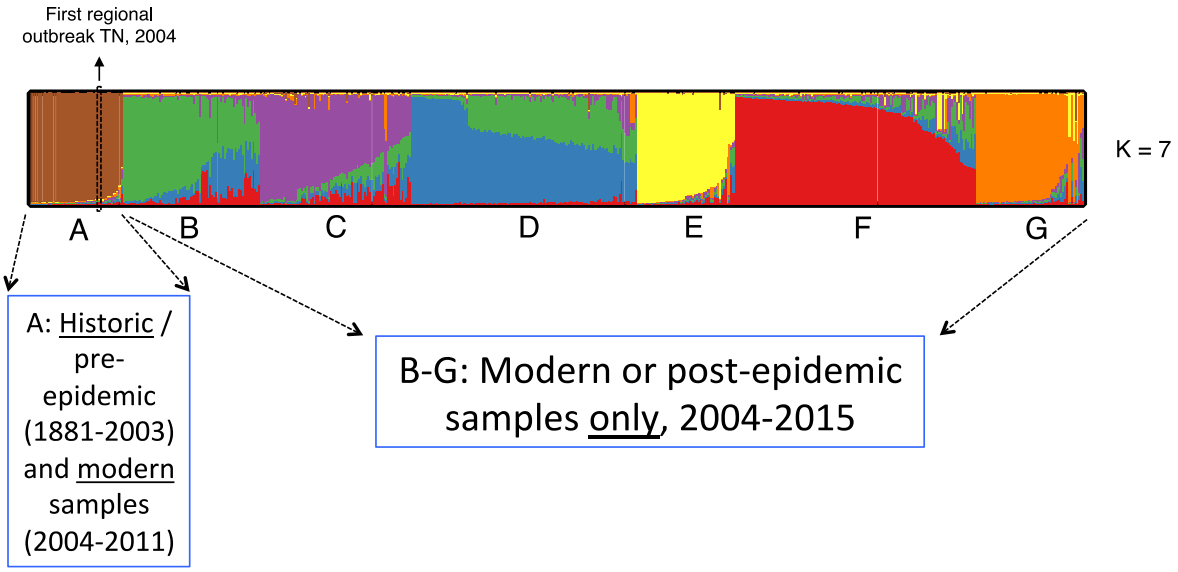
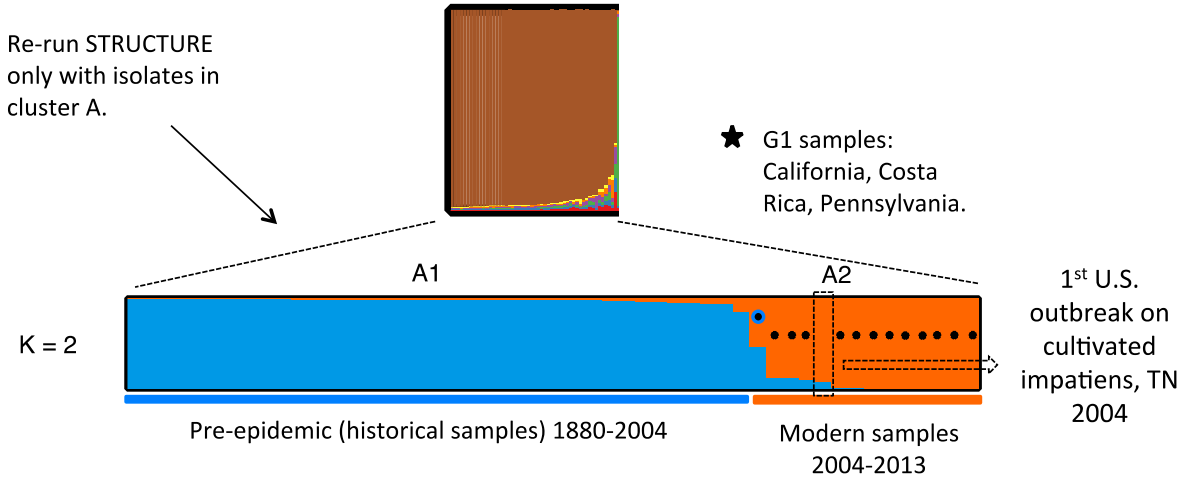
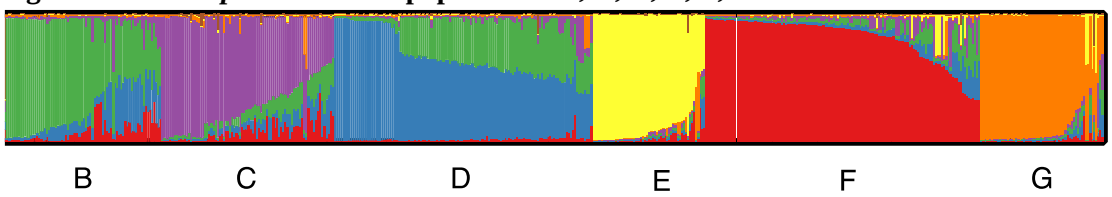


Figure 73. Population A is made up of ~11% of the total sample.



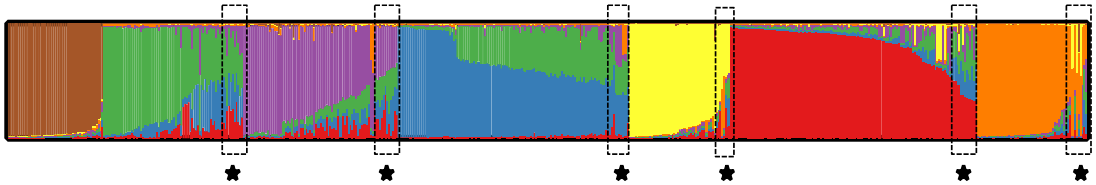
It contains all pre-epidemic IDM samples, IDM samples with rDNA genotype of G1, and includes the first IDM outbreak reported from *Impatiens walleriana* in the US in 2004. Hosts: wild Impatiens: *I. balsamina, I. fulva, I. noli-tangere, I. pallida, I. textori,* and cultivated *I. walleriana*. Distributed across the US Some from Costa Rica, Canada, Europe & Korea. STRUCTURE analysis of population A individuals shows that G1 samples cluster seperately from pre-epidemic samples.

Figure 74. *Plasmopara obducens* populations B, C, D, E, F, G.



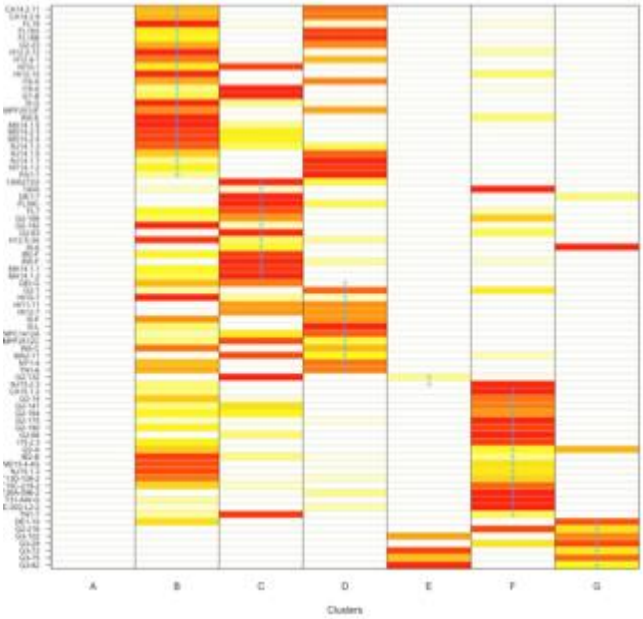
Distributed across the US (Hawaii-New Jersey, Michigan-Florida). Also from Holland, Costa Rica and Taiwan. These populations are responsible for the IDM outbreaks on *Impatiens walleriana.*

Figure 75. There is evidence of admixed genotypes between populations, where individuals have no more than 90% probability of membership in a single population cluster.



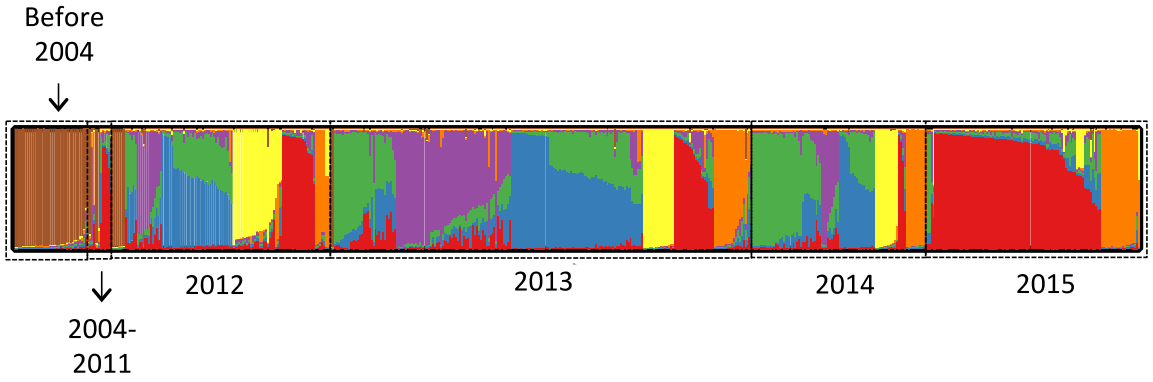
In the figure, admixed individuals are those displayed as bars have a mix of two or more colors (marked with a star).

Figure 76. Graphical translation of the posterior probability values of admixed individuals (n = 78).



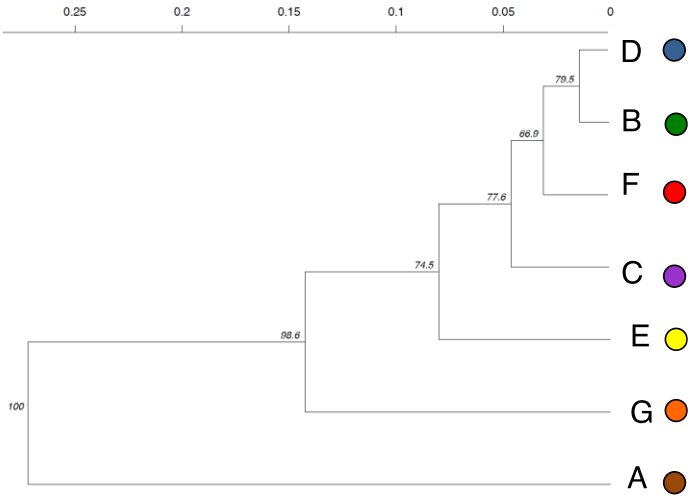
Heat colors represent membership probabilities (red=1, white=0); blue crosses represent the prior cluster provided. For some individuals, the DAPC classification is consistent with the original clusters (blue crosses are on red rectangles), except for those showing discrepancies (for example HI10-7), classified in group B while DAPC would assign it to group D. Such figure is particularly useful when prior biological groups are used, as one may infer admixed or misclassified individuals.

Figure 77. Distribution of IDM populations over time.



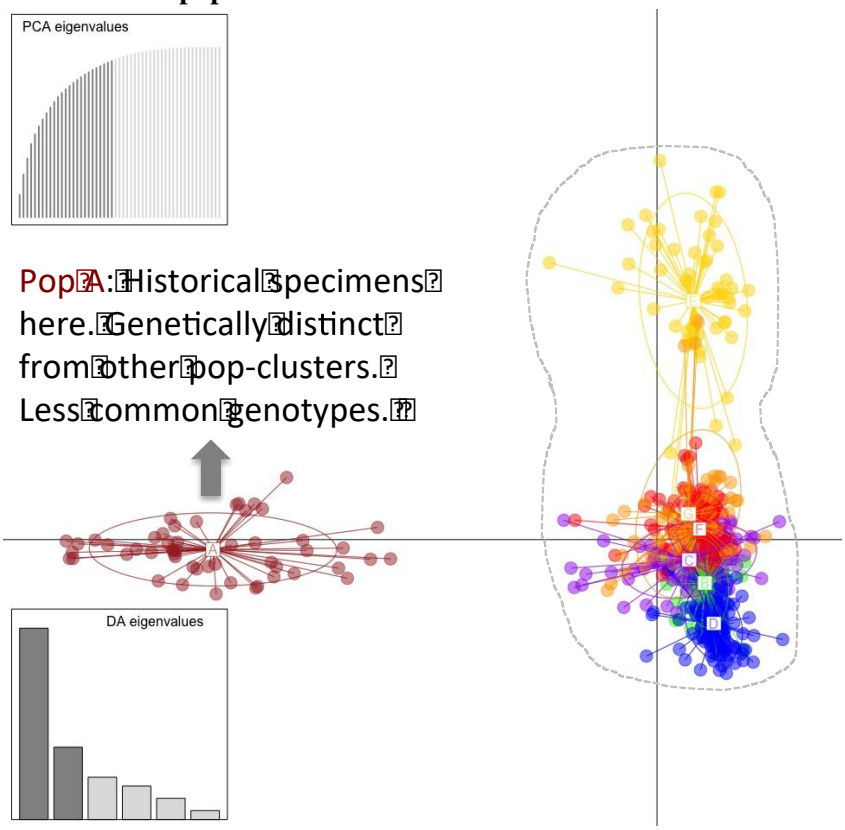
Before 2011 the dataset showed low genotypic diversity (but this also could be an artifact of the small sample size from this year). The years 2012 – 2015 show high diversity in the pathogen genetic structure. Except with few isolates in 2012 and 2013, population A is absent, and is not associated with the epidemics. By 2015 only two IDM populations (F-G) seem to dominate.

Figure 78. Bootstrapped dendrogram based on Nei's genetic distances among IDM populations.



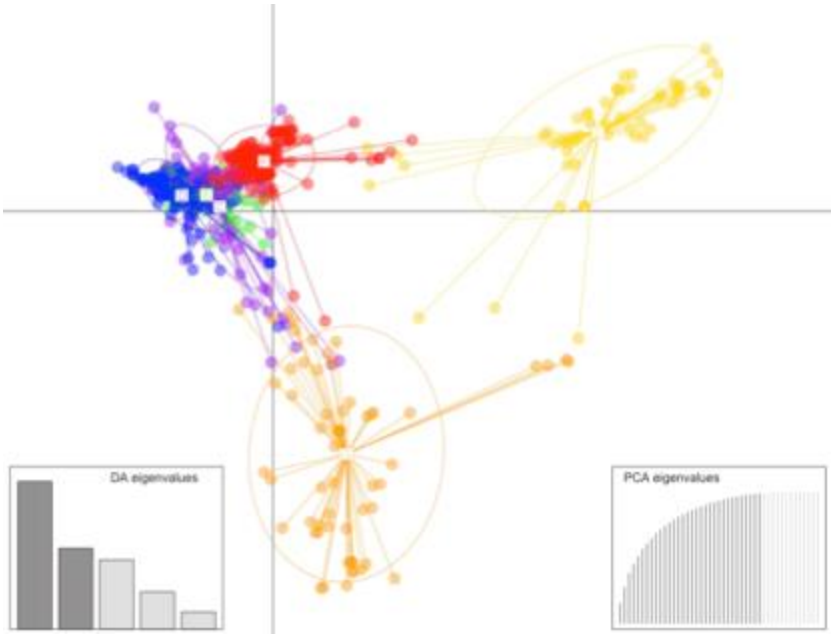
This dendrogram based tells us that the more divergent population clusters are A (pre-epidemic samples, G1 samples), followed by population G, and shows the relationships between the seven populations.

Figure 79. Discriminant analysis of principal components (DAPC) illustrating the relationship between IDM populations.



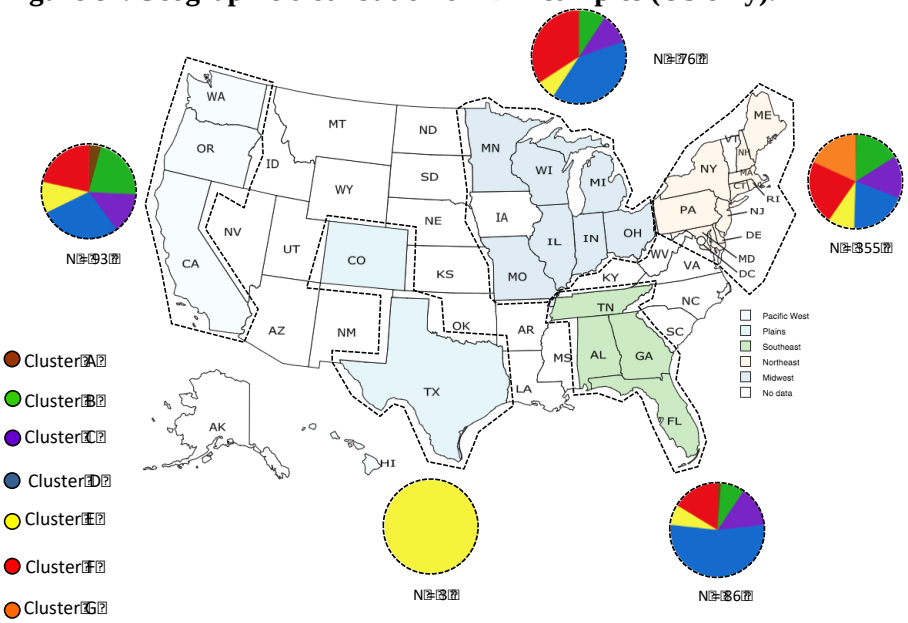
Population A is distinct and divergent from the remaning IDM samples.

Figure 80. Subset of the DAPC diagram above, showing just populations B-G, which are the incitants of IDM in the US between 2011-present day.



These populations are closely related, most likely share a common ancestry, and are recombining.

Figure 81. Geographic distribution of IDM samples (US only).



With the exception of population A, which is mostly found in California (and 2004 first report from Tennessee), all the remaining IDM populations are distributed along the US

Figure 82. Observed versus expected heterozygosity in the IDM populations.

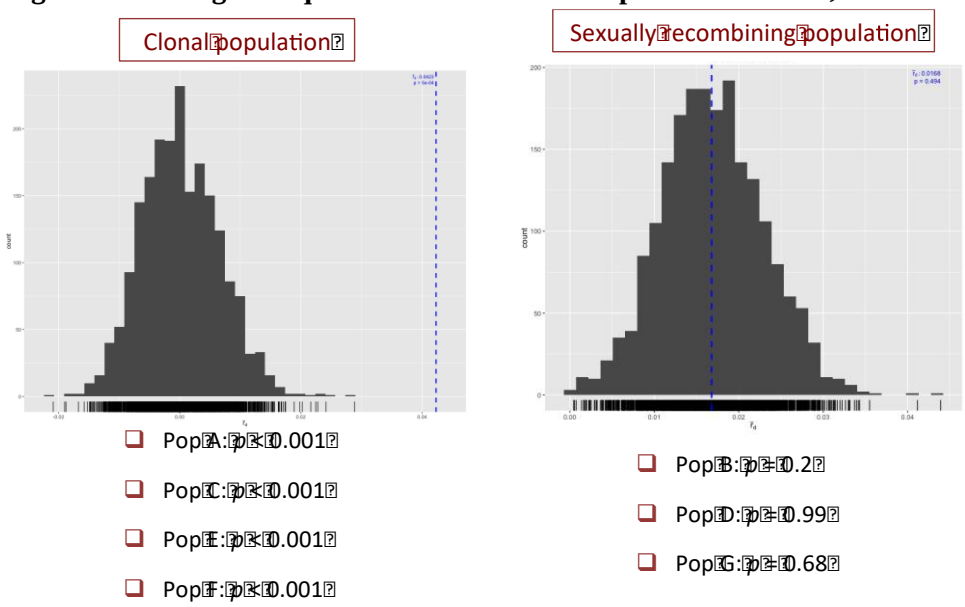
Ho=He; population A (historical specimens) at equilibrium, allele frequencies remaining constant

Population	Cluster		N	Na	Ne	Ho	uHe
A		Mean	31.056	2.611	1.621	0.333	0.329
		SE	3.969	0.325	0.173	0.074	0.056
B		Mean	69.111	2.167	1.901	0.807	0.461
		SE	0.928	0.121	0.065	0.062	0.030
C		Mean	63.222	2.500	1.595	0.445	0.336
		SE	2.553	0.185	0.088	0.079	0.043
D		Mean	110.889	2.222	1.878	0.795	0.455
		SE	2.436	0.101	0.062	0.060	0.027
E		Mean	46.222	2.722	2.057	0.664	0.468
		SE	2.143	0.266	0.151	0.087	0.045
F		Mean	113.167	2.333	1.890	0.770	0.444
		SE	3.475	0.181	0.091	0.076	0.037
G		Mean	49.333	2.500	1.775	0.620	0.421
		SE	2.771	0.185	0.071	0.082	0.031

Ho = Observed Heterozygosity = No. of Hets / N
He = Expected Heterozygosity = 1 - Sum pi^2

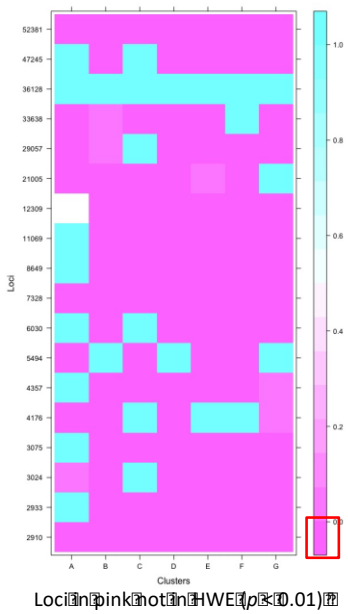
Ho>He: population clusters probably changing constantly over time.

Figure 83. Linkage disequilibrium estimates of reproductive mode, sexual or clonal.



Values of linkage disequilibrium will predict if populations are clonal (where significant disequilibrium is expected due to linkage among loci) or sexual (where linkage among loci is not expected). they obtained significant and non-significant values for the 7 population clusters found. Pops A, C, E , F showed significant values which indicates populations are clonal. However, Pops B, D and G showed no significant values, meaning they appear to be reproducing sexually.

Figure 84. Hardy-Weinberg equilibrium (HWE) across populations, broken down by SSR locus.



Loci in pink not in HWE ($p < 0.01$)

When a locus is not in HWE, it suggests one or more of the HTHEY assumptions is false. Departure from HTHEY has been used to infer the existence of either non-random mating, drift, mutation, migration, or natural selection. These HTHEY results go in agreement with the differences in expected and observed

heterozygosity, in that the IDM populations responsible for the disease epidemic seem to be evolving rapidly, versus population A, which is largely exhibiting HTHEY at most SSR loci.

Characterize CDM populations (Gen-MSU-4)

Michigan. Populations of CDM were collected in 2011 and 2012 from fields in Michigan and Canada. Samples from Michigan in 2011 and 2012 were collected from cucumbers at four locations at multiple times during the growing season. Canada samples from 2011 and 2012 were collected from multiple locations at a single time. In total, ~1000 isolates were collected in 2011 and an additional ~300 isolates were collected in 2012. For 2011 samples, DNA was successfully amplified for 439 isolates and genetic diversity was determined using seven microsatellite markers. they are currently running analyses on these isolates for population structure and genetic diversity. Population structure and genetic diversity will be compared among isolates collected within Michigan over the growing season, and between Canada and Michigan. Samples collected in 2012, were tested for PCR amplification using the *P. cubensis* specific primer for *Beta tubulin*.

Objective 3: Biological and Chemical Mitigation Strategies for IDM

Efficacy of biological and chemical mitigation strategies for IDM

Residual Control - Florida (Mit-UF-1)

2013. Dr. Aaron Palmateer examined length of residual efficacy as defined as no visible sporulation for active ingredients with known impact on oomycetes (Table 38). To compare residual fungicide efficacy for the control of impatiens downy mildew, plants received one of seventeen fungicide treatments including inoculated and non-inoculated controls. All fungicides were applied to visibly disease-free, 7-wk old, seed-raised impatiens as foliar sprays to runoff using a hand-pressurized sprayer. Foliar sprays delivered approximately 3.0 fl oz of spray solution per plant. Each fungicide treatment was applied once. The experimental design was a randomized complete block with ten replications per treatment. Each replicate consisted of a single plant grown in a 4-in. pot containing Pro Mix BX Professional Growing Mix. Plants were grown outside under 73% shade and watered daily by hand. The average daytime temperature was 78°F with 74% average relative humidity and average nighttime temperature was 71°F with 91% average relative humidity. Plants were spray inoculated with a suspension of *P. obducens* sporangia (3×106 sporangia/fl oz = 1x 105 sporangia/ml) 24 hr post treatment. Inoculum was prepared by rinsing sporulating impatiens leaves in water. Plants were monitored daily to record the first evidence of sporulation on leaf tissue. This research was started in early 2013 and is being replicated to confirm results with foliar applications and soil drenches.

Table 38. Residual efficacy of preventative fungicide applications with foliar applications for control of impatiens downy mildew, Palmateer 2013

Trade Name	Active Ingredient	Chemical Class	FRAC Code	Rate	Residual*
Untreated, non-inoculated					**8**
Untreated, inoculated					**6**
A14658C	Potassium Phosphite (54.5%)	Phosphonates	33	64 fl oz/100 gal	**38**
Actigard	Acibenzolar-S-methyl (50%)	Benzo-thiadiazole BTH	P 1	0.125 oz/100 gal	**10**
Adorn	Fluopicolide (39.5%)	Benzamides	43	1 fl oz/100 gal	**62**
Agri-Fos	Mono- and di-potassium salts of Phosphorous Acid (45.8%)	Phosphonates	33	1.125 fl oz/1 gal	**31**
Aliette	Fosetyl Al (80%)	Phosphonates	33	2 lbs/100 gal	**30**
Alude	Mono- and di-potassium salts of Phosphorous Acid (45.8%)	Phosphonates	33	1.125 fl oz/1 gal	**34**
Ele-Max Foliar Fosphite	Available Phosphoric Acid (28%) + Soluble Potash (26%)	Phosphonates	33	64 fl oz/100 gal	**34**
FosFiMax 0-40-20	Phosphorous Acid (60-70%) + Potassium Hydroxide (20-30%)	Phosphonates	33	32 fl oz/100 gal	**24**
Heritage	Azoxystrobin (50%)	QoI fungicide	11	4 oz/100 gal	**17**
Micora	Mandipropamid (23.3%)	Carboxylic Acid Amides	40	8 fl oz/100 gal	**24**
Nutriphite Magnum 2-40-16	Total Phosphoric Acid (40%)	Phosphonates	33	4 pts/100 gal	**34**
Orvego	Ametoctradin (26.9%) + Dimethomorph (20.2%)	QxI + CAA	45 + 40	14 fl oz/100 gal	**17**
Pageant	Pyraclostrobin (12.8%) + Boscalid (25.2%)	QoI + SDI	11 + 7	12 oz/100 gal	**10**
Phostrol	Mono- and dibasic sodium, potassium, and ammonium phosphites (53.6%)	Phosphonates	33	4 pts/100 gal	**34**
Prudent-44 15-0-0	Total Nitrogen (15%) [2.5% Ammoniacal Nitrogen + 12.5% Urea Nitrogen]	Phosphonates	33	4 pts/100 gal	**34**
Stature	Dimethomorph (43.5%)	Carboxylic Acid Amides	40	12.25 fl oz/100 gal	**24**
Subdue MAXX	Mefenoxam (22%)	Phenyl Amides	4	1 fl oz/100 gal	**27**

* Residual is the number of days post fungicide treatment with no evidence of sporulation

2016. At the University of Florida on 21 Nov 2016, plugs of *I. walleriana* 'Super Elfin White' and 'Super Elfin Red' were stepped up to 4" pots. In order to test residual efficacy of fungicides for impatiens downy mildew, impatiens were subjected to 14 fungicide treatments (10 plants/trt) at the highest label rate (for DM). Plants received foliar sprays on 21 Nov and 29 Nov.

Sporulation was noted on untreated controls on 5 Dec (2 weeks after start of trial). As of January 2017, plants in every treatment have completely died except for those in Treatment 7 (Segovis 2.4 fl oz). Plants in Treatments 12 and 13 (phosphonates) have succumbed to IDM but were still standing ("green stick syndrome").

Table 39 shows disease severity ratings over 4 weeks after the onset of disease. As stated previously, within the next month all plants had died except for those treated with Segovis. Phosphite-containing products provided the longest residual efficacy next to Segovis, which provided complete IDM control for the duration of the trial.

Table 39. Disease severity of treatments to control downy mildew of impatiens, Palmateer, 2016

Treatment	Rate/100 gal	Residual [z]	Disease Severity [y]
Untreated control	---	---	98.9 a [x]
Adorn	4 fl oz	5	96.8 a
Subdue Maxx	1 fl oz	5	75.6 b
Adorn + Subdue Maxx	4 fl oz + 1 fl oz	5	72.8 bc
Segway O	3.5 fl oz	6	48.9 cd
Stature SC	12.25 fl oz	10	42.4 d
Segovis	2.4 fl oz	---	0 g
Pageant Intrinsic	18 oz	7	26 e
Orkestra	10 fl oz	7	16.9 e
Orvego	14 fl oz	7	55 cd
Micora	8 fl oz	16	1.9 fg
Inosco	64 fl oz	21	1.3 g
Alude	64 fl oz	21	1.6 fg
Protect	2 lbs	7	24.1 e
Protect + Capsil	2 lbs + 6 fl oz	12	7.3 f

[z] Days after last application (29 Nov) until pathogen sporulation was evident on treated plants.
[y] Average weekly disease severity expressed as percent leaf canopy affected.
[x] Column means indicated with the same letters are not significantly different ($P \leq 0.05$) based on Student Newman Keuls test.

Active Ingredient Screens - Florida (Mit-UF-2)

2014. Starting in the winter of 2013/2014, Dr. Palmateer examined fungicide efficacy for managing IDM in the landscape. To determine fungicide efficacy for controlling downy mildew in the landscape disease free 4" potted impatiens, *I. walleriana* 'Super Elfin 'Red', were transplanted into raised beds located under 73% shade. Transplants were submitted to one of eight fungicide treatments, including inoculated and non-inoculated controls (Table 40). Experimental units were a plant treated with a single fungicide treatment, with 16 repetitions in a randomized complete block design (RCBD). Plants were watered daily by overhead irrigation. Fungicides were applied as a foliar spray, at a spray volume rate of 5 mL per plant, three days after transplanting on 1/17 and 28 days later on 2/13 for a total of two applications. Fungicide treatments were applied in the morning and plants were not watered for 24 hours following application. Fungicides were mixed according to rates listed in Table 1. Plants were spray inoculated with a suspension of *P. obducens* sporangia (3×106 sporangia/fl oz = 1x 105 sporangia/ml) 24 hr post treatment. Inoculum was prepared by rinsing sporulating impatiens leaves in water.

Table 40. Disease levels for 8 treatments to control impatiens downy mildew.

Treatment	Rate/ 100 gal	Disease Severity	AUDPC
Untreated non-inoculated control	----	75 a	360 a
Untreated inoculated control	----	77 a	368 a
Adorn + 3336F	1 oz + 14 oz	38 cd	151 b
Alude + Protect DF	2 qts + 1.5 lbs	44 bc	175 b
Confine Extra T & O	2 qts	34 d	126 b
Phostrol	64 fl oz	33 d	114 b
Aliette	2 lbs	50 b	206 b
Protect DF + Cuproxat	1.5 lb + 20 fl oz	50 b	218 b

Disease measures were severity (% canopy affected) and as average area under the disease progress curve (AUDPC).
Column means indicated with the same letters are not significantly different ($P \leq 0.05$) based on Student Newman Keuls test.

During fall 2014, an experiment was initiated to study fluopicolide efficacy. Results from the previous period indicate that products containing phosphorous, mefenoxam and fluopicolide are good candidates for long residual control of impatiens downy mildew. Due to much concern with mefenoxam resistant pathogen populations, mefenoxam was not evaluated this period. Due to the high level of downy mildew pressure in South Florida it has been challenging to conduct preventative disease management trials. Impatiens plants must be shipped in from downy mildew free locations and then treated upon arrival.

To determine efficacy of fluopicolide for controlling downy mildew in the landscape disease free 4" potted impatiens, *I. walleriana* 'Super Elfin 'white', were grown under 73% shade. Impatiens plants were submitted to one of seven treatments, including inoculated and non-inoculated controls (Figure 85, Table 41). Experimental units were a plant treated with a single fungicide treatment, with 10 repetitions in a randomized complete block design (RCBD). Plants were watered daily by overhead irrigation. Treatments were applied as a foliar spray, at a spray volume of 10 mL per plant. Potted impatiens plants received two fungicide applications seven days apart (11/12 & 11/19) and were transplanted to landscape beds 3 days after the second application (11/22). Two additional sprays were applied in the landscape on 28-day intervals (12/17 & 1/14).

All treatments were applied in the morning and plants were not watered for 24 hours following application. Treatments were mixed according to rates listed in Table 1. Plants were spray inoculated with a suspension of *P. obducens* sporangia (3×10^6 sporangia/fl oz = 1x 10^5 sporangia/ml) 48 hours post treatment on 11/14.

This trial was evaluated over a 3-month period (November 2014 – February 2015) and disease pressure was severe throughout. Disease severity ratings for all fungicide treated plants were statistically lower than the untreated controls. All treatments containing Adorn produced plants with statistically lower disease severity ratings and AUDPC values than plants treated with Daconil Ultrex or Pageant Intrinsic. Disease severity ratings for plants treated with the 2-ounce rate of Adorn tank mixed with Pageant Intrinsic or Daconil Ultrex were statistically lower than plants treated with Adorn alone. Plants treated with the 1-ounce rate of Adorn tank mixed with Pageant Intrinsic had statistically lower disease ratings than plants treated with the 1-ounce rate of Adorn tank mixed with Daconil Ultrex. No phytotoxicity was observed for any of the fungicide treatments tested in this trial.

Table 41. Disease levels for 7 treatments to control impatiens downy mildew in the landscape, Palmateer, 2014.

Treatment and rate/100 gal	Disease Severity (%)[z]		AUDPC[x]	
Non-treated, non-inoculated	94	b[y]	5275	a
Non-treated, inoculated	94	b	5271	a
Adorn 4SC 1 fl oz + Pageant Intrinsic 12 oz	36	de	2141	de
Adorn 4SC 2 fl oz + Pageant Intrinsic 12 oz	33	ef	1988	de
Adorn 4SC 2 fl oz	37	d	2202	d
Pageant Intrinsic 12 oz	76	a	4277	b
Daconil Ultrex 2.0 lb	79	a	4440	b
Adorn 4SC 1 fl oz + Daconil Ultrex 2.0 lb	45	c	2634	c
Adorn 4SC 2 fl oz + Daconil Ultrex 2.0 lb	30	f	1792	e

Disease measures were severity (% canopy affected) and as average area under the disease progress curve (AUDPC)

[z] Percent canopy area affected.

[y] Column means followed by the same letter are not statistically different (**α**=0.05) based on Tukey's HSD.

[x]Average Area Under the Disease Progress Curve

Figure 85. IDM fluopicolide trial, Palmateer, 2014

To determine efficacy of nutritional amendments for controlling downy mildew in the landscape disease free 6" potted impatiens, *I. walleriana* 'Super Elfin 'Orange', were transplanted into raised beds located under 73% shade. Transplants were submitted to one of six treatments, including inoculated and non-inoculated controls (Table 42, Figure 86). Experimental units were a plant treated with a single nutritional treatment, with 16 repetitions in a randomized complete block design (RCBD). Plants were watered daily by overhead irrigation. Treatments were applied as a foliar spray, at a spray volume rate of 10 mL per plant, three days after transplanting on 1/17 and 28 days later on 2/13 for a total of two applications. Treatments were applied in the morning and plants were not watered for 24 hours following application. Treatments were mixed according to rates listed in Table 2. Plants were spray inoculated with a suspension of *P. obducens* sporangia (3×10^6 sporangia/fl oz = $1x\ 10^5$ sporangia/ml) 24 hr post treatment. Inoculum was prepared by rinsing sporulating impatiens leaves in water.

All of the plants treated with fertilizer had statistically lower disease severity and area under the disease progress curve (AUDPC) rating values when compared to both sets of non-treated control plants. Plants treated with EleMax remained disease free for 21 days post inoculation (with the downy mildew pathogen), but Nutriphite Magnum provided the highest level of disease control. No phytotoxicity was observed for any of the fertilizer treatments tested in this trial.

Figure 86. IDM Landscape Nutrition Trial, Palmateer, 2014

Table 42. Disease levels for 6 fertilizer treatments to control impatiens downy mildew, Palmateer 2014.

Treatment and rate/100 gal	Days[z]	Disease Severity (%)[y]		AUDPC[x]	
Non-treated, non-inoculated	14	71	b[w]	2246	a
Non-treated, inoculated	14	80	a	2518	a
Actaphos 0-28-25 2 qts	14	61	c	1908	b
EleMax 64 oz	21	49	d	1543	c
FosfiMax 0-40-20 32 fl oz	7	47	d	1501	c
FosfiMax Copper 1 qt	14	59	c	1857	b
Nutriphite Magnum 64 fl oz	7	41	e	1299	c
Prudent-44 (15-0-0) 64 oz	14	47	d	1490	c

Disease measures were severity (% canopy affected) and as average area under the disease progress curve (AUDPC).

[z] Number of days from start of trial until signs of disease were present.

[y] Percent canopy area affected.

[x] Area Under the Disease Progress Curve

[w] Column means followed by the same letter are not statistically different (**α**=0.5) based on Tukey's HSD.

2015. To compare fungicide efficacy for the control of impatiens downy mildew, plants received one of 12 fungicide treatments including inoculated and non-inoculated controls. All fungicides were applied to visibly disease-free, 6-wk old, seed-raised impatiens as foliar sprays to runoff using a hand-pressurized sprayer. Foliar sprays delivered approximately 3.0 fl oz of spray solution per plant. Each fungicide treatment was applied once. The experimental design was a randomized complete block with ten replications per treatment. Each replicate consisted of a single plant grown in a 4-in. pot containing Pro Mix BX Professional Growing Mix. Plants were grown outside under 73% shade and watered daily by hand. Plants were spray inoculated with a suspension of *P. obducens* sporangia (3×106 sporangia/fl oz = 1x 105 sporangia/ml) 48 hours post fungicide treatment. Plants were monitored daily to record the first evidence of sporulation on leaf tissue and rated for disease incidence and severity weekly.

All treatments except for Nutrol provided some level of downy mildew control. Plants treated with Fenstop had statistically lower disease severity and AUDPC values when compared to all the other treatments. Impatiens treated with Fenstop or Prudent-44 remained disease free for 3 weeks under heavy downy mildew pressure. No phytotoxicity was reported.

Table 43. Disease levels for 12 fungicide and fertilizer treatments to control impatiens downy mildew, Palmateer 2015.

Treatment and rate[z]	Days[y]	Disease Severity (%)[x]		AUDPC[w]	
Non-treated, non-inoculated	7	83.79	b[v]	3657.5	ab
Non-treated, inoculated	7	91.22	a	3781.5	a
Fenstop 14 fl oz	20	39.472	h	2369.2	g
Dithane DF 24 oz	14	68.694	d	3347.3	cd
Segway 3.5 fl oz	12	72.72	c	3438.5	c
Alude 112.5 fl oz	15	61.625	ef	3148.8	ef
Phostrol 4 pt	14	63.658	e	3216.3	de
Fosfimax 0-40-20 32 fl oz	10	75.222	c	3491.8	bc
Nutriphite Magnum 4 pt	12	59.83	f	3101.8	ef
Prudent-44 4 pt	20	55.89	g	3006.8	f
Nutrol 36.28 g/1 gal	7	91.431	a	3782.5	a
Ele-Max 18.92 mL/1 gal	10	63.75	e	3213.8	de

Disease measures were severity (% canopy affected) and as average area under the disease progress curve (AUDPC).

[z] Unless noted otherwise all rates are per 100 gal

[y] Number of days from inoculation (22 Nov) until signs of disease present.

[x] Percent canopy area affected.

[w] Area Under the Disease Progress Curve

[v] Column means followed by the same letter are not statistically different (**α**=0.5) based on Tukey's HSD.

Active Ingredient Screens - Michigan (Mit-MSU-1)

2013. During 2013, Dr. Mary Hausbeck's laboratory conducted seven fungicide efficacy trials, testing 18 novel active ingredients. Reduced-risk, biopesticides, and experimental products were screened against industry standards. Two trials were conducted to determine the effectiveness of fungicide applications applied to impatiens seedlings with sporulation observed prior to the initial fungicide treatment. The efficacy of drench applications versus spray applications and varying application intervals were examined. One experiment was conducted to determine if greenhouse treatments could translate into disease protection in the landscape. Four experiments were conducted to determine the effect of drench applications of fluopicolide (Adorn) on plant health. Results of four experiments are included in this report (Table 44, Table 45, Table 46, Table 47).

2014. Two impatiens downy mildew efficacy fungicides screens were completed in 2014. One greenhouse trial was conducted to determine the longevity of control of drench applications of fluopicolide (Adorn), mefenoxam (Subdue MAXX), and phosphorous acid (Alude). Plants were inoculated 14-days after the treatments were applied and rated 14-days after inoculation. Disease severity was severe with 100% of the untreated control plants having pathogen sporulation. All of the Alude treated plants had IDM sporulation, although at a lower severity compared to the untreated control. Industry standards Adorn and Subdue MAXX remained efficacious 14-days after application with the Adorn treated plants remaining disease free and the Subdue MAXX treated plants with 16.7% disease incidence. A second trial was conducted in the greenhouse to test the efficacy of BASF experimental fungicide BAS 70301F. Three rates of the experimental fungicide were tested and all resulted in disease free plants post inoculation.

Table 44. Evaluation of greenhouse fungicide applications for the control of downy mildew of impatiens 'Accent Premium Red' in the landscape, Harlan and Hausbeck, 2013 PDMR 8:OT017

Treatment, rate/100 gal, and application method	No. Applic.[z]	Leaves with sporulation (%)					Disease severity[y]	
		12 Aug	21 Aug		27 Aug		1 Oct	
Untreated control	--	1.0[x]	57.9	c	99.4	d	10.0	c
Orvego SC 14 fl oz *Spray*	2	1.1	24.0	b	60.2	c	9.8	c
Orvego SC 14 fl oz + Droplex 0.25 % v/v *Spray*	2	0.0	27.5	b	76.5	cd	9.5	c
Adorn SC 1 fl oz + Subdue MAXX EC 1 fl oz *Drench*	1	0.0	0.0	a	0.0	a	2.2	a
Adorn SC 2 fl oz + Protect DF 2 lb + Capsil 6 fl oz *Spray*	1	0.0	2.9	a	27.6	b	7.0	b
Alude 12.75 fl oz *Drench*	1	0.0	4.6	a	27.3	b	7.8	b
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz *Drench*	1	0.0	0.0	a	1.7	a	3.3	a

[z] The first fungicide application was made 1 Jul in the greenhouse. The second fungicide application was made 11 Jul just prior to transplant into the landscape.

[y] Disease severity rating on a scale of 1 to 10 where 1=healthy, 2=minor chlorosis, 3=moderate chlorosis, 4=severe chlorosis, 5=moderate stunting, 6=severe stunting, 7=minor defoliation, 8=moderate defoliation, 9=severe defoliation, 10=complete defoliation/plant death.

[x] Column means with a letter in common or with no letter are not significantly different (LSD; *P*=0.05).

Table 45. Evaluation of drench and/or spray applications of experimental fungicides for the control of downy mildew of bedding impatiens in the greenhouse, Harlan and Hausbeck, 2013 PDMR 8:OT018

Treatment and rate/100 gal	Application method	Leaves sporulating (%)		Sporulation severity*	
		25 Nov		25 Nov	
Untreated control	--	53.1	b**	8.5	b
Subdue MAXX 1 fl oz + Alude 12.75 fl oz	drench	0.0	a	1.0	a
Segway SC 3.5 fl oz	spray	0.0	a	1.0	a
Acibenzolar 50WDG 0.25 oz + Heritage 50WG 0.9 oz	spray	0.0	a	1.0	a
V-10208 SC 4 fl oz	spray	0.0	a	1.0	a
V-10208 SC 4 fl oz	drench	0.0	a	1.0	a
Syngenta Experimental 9.6 fl oz	spray	0.0	a	1.0	a
Syngenta Experimental 9.6 fl oz	drench	0.0	a	1.0	a

*Sporulation severity is 1 to 10; where 1=no sporulation, 2=1-5% leaf area sporulating with pathogen, 3=6-10% sporulating, 4=11-20% sporulating, 5=21-30% sporulating, 6=31-40% sporulating, 7=41-50% sporulating, 8=51-60% sporulating, 9=61-70%, 10=>71% leaf area sporulating.

**Column means with a letter in common are not significantly different (Fisher's LSD; *P*=0.05).

Table 46. Evaluation of fungicides for the control of downy mildew of impatiens 'Accent Premium White', Harlan and Hausbeck, 2013 PDMR 8:OT019

Treatment, rate/100 gal, *application method*	Application schedule[z]	Leaves with sporulation (%)		Leaf area with sporulation (%)[y]		Incidence (% plants with sporulation)	
Untreated control	--	10.3	b	73.3	c	100.0	c
Adorn SC 1 fl oz + Subdue MAXX EC 1 fl oz *Drench*	1 dpi	0.0	a	0.0	a	0.0	a
Adorn SC 1 fl oz + Protect DF 2 lb + Capsil 6 fl oz *Spray*	1 dpi, 14 dai	0.0	a	0.0	a	0.0	a
ZeroTol 2.0 128 fl oz + Capsil 8 fl oz	1 dpi; 0.5, 3, 7, 14 dai	11.2	b	33.3	b	50.0	b
OxiPhos 50 fl oz *Drench* OxiPhos 102 fl oz *Spray*	2 dpi 0.5 dpi, 7 dai	3.1	ab	36.7	b	66.7	bc
OxiPhos 50 fl oz *Drench* ZeroTol 2.0 42.6 fl oz + Capsil 6 fl oz *Spray*	2 dpi 0.5, 3, 7, 14 dai	5.8	ab	14.2	ab	50.0	b
Alude 12.75 fl oz *Drench*	1 dpi, 14 dai	5.4	ab	41.7	b	66.7	bc
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz *Drench*	1 dpi	0.0	a	0.0	a	0.0	a

[z] dpi=days prior to inoculation. dai=days after inoculation.

[y] Based on a visual estimate of leaf area with sporulation.

[x] Column means with a letter in common are not significantly different (Student-Newman-Keuls; *P*=0.05).

Table 47. Evaluation of two experimental fungicides and a biopesticide for the control of downy mildew of bedding impatiens 'Accent Premium Red' in a greenhouse, Harlan and Hausbeck, 2013 PDMR 8:OT020

Treatment and rate/100 gal	Application method	Leaves with sporulation (%)		Leaf area with sporulation (%)[*]		Plants with sporulation (%)	
Untreated control	--	37.0	b[**]	61.0	bc	80.0	bc
Acibenzolar WDG 0.25 oz + Heritage WDG 0.9 oz	Drench	12.2	a	60.0	bc	100.0	c
Acibenzolar WDG 0.125 oz + Heritage WDG 0.5 oz	Drench	18.7	ab	70.0	c	100.0	c
Acibenzolar WDG 0.25 oz + Heritage WDG 0.9 oz	Spray	0.0	a	0.0	a	0.0	a
Acibenzolar WDG 0.125 oz + Heritage WDG 0.5 oz	Spray	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz	Drench	4.1	a	16.0	ab	40.0	abc
F9110 18 fl oz + Capsil 1 fl oz	Spray	7.0	a	40.0	abc	60.0	abc
F9110 30 fl oz + Capsil 1 fl oz	Spray	6.4	a	62.0	bc	100.0	c
F9110 45 fl oz + Capsil 1 fl oz	Spray	1.5	a	8.0	a	20.0	ab
Regalia 4 qt	Spray	0.4	a	34.0	abc	40.0	abc

[*] Based on a visual estimate of the leaf area covered with sporulation.

[**] Column means with a letter in common are not significantly different (Student-Newman-Keuls; *P*=0.05).

The effectiveness of the lower rate of Adorn was evaluated in a greenhouse study conducted in greenhouses on the campus of Michigan State University. Bedding impatiens ('Accent Premium Red') were seeded into 288-cell flats and transplanted into 4-in. plastic pots containing a soilless media (Suremix MI Cover Products Inc, Galesburg MI) on 31 May 2016. Plants were fertilized weekly with

200ppm of Peter's water soluble fertilizer (The Scotts Company, Marysville, OH). Six replications per treatment with one plant per replication were arranged in a completely randomized design. Plants were approximately 6-in. tall at the initiation of the experiment. Fungicides were applied as a drench (3 fl oz/pot) or foliar spray with a compressed air sprayer until runoff on the morning of 13 Jun. A sporangium suspension was prepared by placing impatiens leaves sporulating with downy mildew into distilled water and agitating to release spores. The suspension was sprayed onto the plants the afternoon of 13 Jun using a janitorial spray bottle, after which the bags were immediately closed. The plants were removed from the bags on 17 Jun. On 23 Jun, plants were placed into baskets covered in clear plastic for increased humidity until the rating date on 28 Jun. The total number of leaves and the number of leaves with pathogen sporulation were counted (% leaves with sporulation) and the severity of sporulation was estimated (1 to 5; 1= no sporulation, 2=minor sporulation, 3=moderate sporulation, 4=severe sporulation, 5=defoliation) on 28 Jun.

Results from this experiment were promising with the 0.5 fl oz rate of Adorn preventing downy mildew infection (Table 48). If results from the phytotoxicity screen show the 0.5 fl oz rate to be safe, future recommendations to growers will include Adorn in the rotational IDM control program. The drench application of Alude was not effective with 59.2% of leaves on treated plants showing downy mildew sporulation. This lack of efficacy is likely due to the 5 fl oz rate used in the experiment compared the 12.75 fl oz rate used in previous experiments where the product significantly reduced sporulation of the pathogen.

Table 48. Efficacy of multiple rates of Adorn alone and in combination with other fungicides against downy mildew of impatiens, Hausbeck and Harlan, 2014

Treatment and rate/100 gal, treated at 14-day intervals	Application Method	Sporulation Severity* 28 Jun		Plants with Sporulation (%) 28 Jun	
Untreated noninoculated	--	1.0	a	0.0	a
Untreated inoculated	--	9.0	c	82.1	c
Adorn 0.5 fl oz	Drench	1.0	a	0.0	a
Adorn 1.0 fl oz	Drench	1.0	a	0.0	a
Adorn 0.5 fl oz + Alude 5.0 fl oz	Drench	1.0	a	0.0	a
Adorn 1.0 fl oz + Alude 5.0 fl oz	Drench	1.0	a	0.0	a
Alude 5.0 fl oz	Drench	7.0	b	59.2	b
Adorn 4.0 fl oz	Spray	1.0	a	0.0	a

*Rated on a scale of 1 to 5, where 1= no sporulation, 2=minor sporulation, 3=moderate sporulation, 4=severe sporulation, 5=defoliation.
**Column means with a letter in common are not significantly different (Fisher LSD; *P*=0.05).

2015. Two *P. obducens* isolates (IDM001 and IDM004) collected in 2014 from Michigan landscape beds have been maintained in growth chambers on the campus of Michigan State University. These isolates were used in the 2015 greenhouse fungicide efficacy trials.

Nonregistered fungicides, Zing! (chlorothalonil/zoxamide) and Previcur Flex (propamocarb), were screened for efficacy against impatiens downy mildew. KPHITE (salts of phosphorous acid) was also included as a spray application. The industry standard, Subdue MAXX (mefenoxam) was applied as a drench. All treatments were applied one day prior to inoculation. Zing! was effective as a foliar spray protectant and completely prevented sporulation of the pathogen (Table 49). KPHITE was moderately effective with results similar to other phosphorous acid products tested in previous experiments. The nonregistered fungicide Previcur Flex, was not effective and will not be included in future studies. Data analysis was completed using SAS PROC GLM and statistical differences were compared using the Fisher's Protected Least Significant Differences test (*P*=0.05)

Table 49. Results of greenhouse experiment testing nonregistered fungicides against impatiens downy mildew, Hausbeck and Harlan, 2015

Treatment and rate/100 gal	Application method	Disease incidence (% of plants sporulating)		Mean sporulating leaves per plant (%)		Disease severity[y]	
Untreated noninoculated	N/A	100.0	c	46.2	c	3.0	c
Subdue MAXX EC 1 fl oz	Drench	0.0	a	0.0	a	1.0	a
Zing! 36 fl oz	Spray	0.0	a	0.0	a	1.0	a
KPHITE 2 qt	Spray	33.3	b	1.4	a	1.3	a
Previcure Flex 19.2 fl oz	Spray	100.0	c	24.6	b	2.5	b

2016. The newly registered fungicide Mural (azoxystrobin + benzovindiflupyr, Syngenta Professional Products) was tested for efficacy against impatiens downy mildew in the greenhouse. Although this product list downy mildew under "diseases controlled" on the label, they had not tested this product in previous experiments. Impatiens ('Accent Premium White') were seeded into a 288-cell flat. The seedlings transplanted into 4-in. pots containing a soilless media (Suremix MI Grower Products Inc, Galesburg MI). Four, single plant replicates per treatment were placed into a completely randomized design. The fungicide applications were applied prior to inoculation as either a drench (volume of 3 fl oz/pot) or as a foliar spray until glistening on 9 May. A sporangium suspension was prepared by placing impatiens leaves sporulating with downy mildew into distilled water and agitating to release spores. The suspension was sprayed onto the plants the afternoon using a janitorial spray bottle, after which the bags were immediately closed. The total number of leaves and the number of leaves with pathogen sporulation were counted (% leaves with *P. obducens* sporulation) and the severity of sporulation was estimated (1 to 5; 1= no sporulation, 2=minor sporulation, 3=moderate sporulation, 4=severe sporulation, 5=100% leaf area with *P. obducens* sporulation) on 22 May.

Disease pressure was moderate in this trial with 38.6% of leaves on the untreated control plants with sporulating *P. obducens*. All treatments in this trial prevented infection and pathogen sporulation. The newly registered fungicide Mural was effective in controlling downy mildew and will be included in future IDM control programs. Phytotoxicity was not observed on any of the treated plants in this study.

Table 50. Evaluation of the newly registered fungicide Mural against impatiens downy mildew, Hausbeck and Harlam, 2016

Treatment and rate/100 gal; *application type*	Sporulation severity[*] 22 May		Leaves with sporulating *P. obducens* (%) 22 May	
Untreated control	4.5	b[**]	38.6	b
Segovis 3.2 fl oz; *drench*	1.0	a	0.0	a
Micora 8 fl oz; *spray*	1.0	a	0.0	a
Mural 4 oz; *spray*	1.0	a	0.0	a
Mural 7 oz; *spray*	1.0	a	0.0	a

[*]Sporulation severity rated on a scale of 1-5; 1= no sporulation, 2=minor sporulation, 3=moderate sporulation, 4=severe sporulation, 5=100% leaf area with P. obducens sporulation.
[**]Column means with a letter in common are not significantly different (Fisher LSD; *P*=0.05).

Active Ingredient Screens - New Jersey (Mit-Rut-1)

2013. An experiment was established at the Rutgers Experiment Station in Cream Ridge, NJ to examine potential residential applications. Two phosphite materials were compared at two application intervals

with drenches of Adorn (fluopicolide) and Subdue Maxx (mefenoxam). For strictly sporulation, the best treatments were Adorn and Subdue drenches. However, A14658C and Alude treated plants appeared to be only slightly impacted by the infections (Table 51, Figure 87).

Table 51. Efficacy of fungicides for IDM, Palmer, 2013

	Sporulation Rating (0 – 5)		
Treatment	Dark Pink (Dazzler Punch)	Pink (Accent Rose)	White (Accent White)
A14658C 4 pt Weekly Foliar	1.3 ab	0.6 ab	1.7 b
A14658C 8 pt Biweekly Foliar	1.8 b	0.8 ab	1.2 b
Adorn 4 fl oz Monthly Drench	0.3 a	0.3 ab	0.0 a
Alude 1.25 qt Weekly Foliar	2.1 b	1.4 b	2.0 b
Alude 2.5 qt Biweekly Foliar	3.0 b	1.4 b	3.6 c
Subdue Maxx 2 fl oz Monthly Drench	0.0 a	0.0 a	0.0 a
Untreated	5.0 c	5.0 c	5.0 d

Figure 87. A14658C 4 pint per 100 gal applied weekly

2014. Two fungicide screening experiments were conducted for *P. obducens*. In the first experiment, three numbered compounds were screened along with tank mix combinations of several product plus Alude. The product list included A14658C, Adorn (fluopicolide), Alude (phosphorous acid generator), SP2770 WP, SYN546539 (A2100A, oxathiapiprolin), Subdue Maxx, (mefenoxam) Alude + Adorn, Alude + SYN546539, and Alude + Subdue Maxx. All treatments were applied as drenches with the exception of SP2770 WP and on Alude treatment which were applied as foliar sprays. The first application was made on 8/4/2014 (see details for remaining applications in the materials and methods section). The first observed disease occurred on 8/20/2014. Plants were rated for sporulation by examining the under surface of leaves and estimating area covered with visible sporangiophores (0 = 0 observed sporulation, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60 %, 4 = 61 to 80%, 5 = greater than 80%. Plants were also rated for quality using the visual guide shown below:
A14658C, reduced disease development fairly well across all three cultivars, while SYN546539 completed prevented any development of disease (Table 52) and plants from both treatments had high quality (Table 53). Alude and all the Alude combination treatments reduced disease and resulted in very high quality ratings. Sudbude Maxx drenches initially performed well, but low disease levels started to

occur 8/26/2014 and continued to build throughout the experiment. Adorn, the best treatment in a 2013 experiment, and the SP2770 WP treatments were not statistically different from the water sprays.

A second experiment was initiated in September to confirm Alude performance (Table 54). Similar to the first experiment, Subdue Maxx initially provided reduction of disease, but Alude, both the newly obtained sample and the previous used sample, were not statistically different from the water treatment.

Figure 88. Quality rating scale.

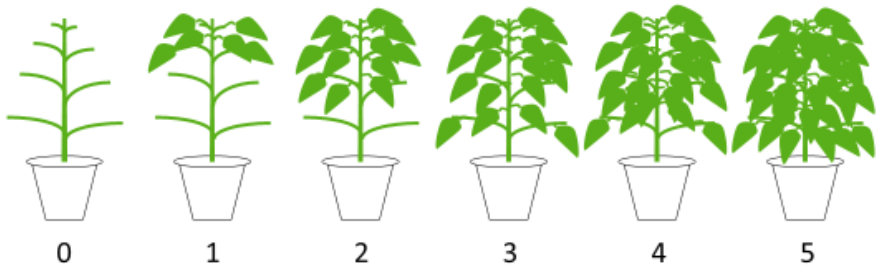


Table 52. Impatiens downy mildew development after applications of several new fungicides and tank mix combinations, Palmer, 2014.

Cultivar	Treatment	Sporulation (0 to 5 with 5 = 100% sporulation on under leaf surface)					
		8/20/2014	8/26/2014	9/3/2014	9/17/2014	9/24/2014	10/5/2014
Ruby	A14658C	0.0 a	0.2 a	0.0 a	0.2 a	0.7 a	2.2 bcd
	Adorn	1.5 abc	3.9 cd	4.5 d	5.0 f	5.0 c	5.0 ef
	Alude + Adorn	0.0 a	0.0 a	0.1 a	0.0 a	0.2 a	0.5 ab
	Alude + SYN546539	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Alude + Subdue	0.0 a	0.0 a	0.0 a	0.0 a	0.1 a	0.1 a
	Alude 28 d Drench	0.0 a	0.1 a	0.0 a	0.1 a	0.2 a	0.3 ab
	Alude Foliar	0.1 a	0.6 a	0.2 a	2.0 bc	3.5 c	3.8 def
	SP2770 14 d	2.3 ab	3.8 cd	4.8 d	5.0 f	5.0 c	na
	SP2770 7 d	1.0 ab	2.8 bd	3.8 cd	5.0 f	5.0 c	na
	SYN546539	0.0 ab	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Subdue Maxx	0.0 a	0.9 ab	1.4 ab	3.2 cde	5.0 c	4.5 f
	Water	3.4 bc	4.9 d	5.0 d	5.0 f	5.0 c	na
Impreza White	A14658C	0.0 a	0.0 a	0.0 a	0.4 a	1.2 ab	3.3 def
	Adorn	2.7 abc	4.9 d	5.0 d	5.0 f	5.0 c	na
	Alude + Adorn	0.0 a	0.0 a	0.0 a	0.2 a	0.3 a	0.6 ab
	Alude + SYN546539	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Alude + Subdue	0.0 a	0.0 a	0.0 a	0.0 a	0.1 a	0.1 a
	Alude 28 d Drench	0.0 a	0.1 a	0.1 a	0.0 a	0.1 a	0.6 ab
	Alude Foliar	0.1 a	0.4 a	1.3 ab	2.5 cd	3.8 c	4.5 f
	SP2770 14 d	1.4 abc	4.0 cd	4.8 d	5.0 f	5.0 c	5.0 bcdef
	SP2770 7 d	2.1 abc	4.7 cd	5.0 d	5.0 f	5.0 c	5.0 bcdef
	SYN546539	0.3 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Subdue Maxx	0.0 a	1.5 ab	2.4 bc	4.1 def	5.0 c	4.1 def
	Water	4.3 c	5.0 d	4.9 d	5.0 ef	5.0 c	na
White XP	A14658C	0.0 a	0.1 a	0.0 a	0.4 ab	0.9 ab	2.5 cde
	Adorn	2.5 abc	4.8 cd	4.8 d	5.0 cdef	na	na
	Alude + Adorn	0.0 a	0.0 a	0.0 a	0.3 a	0.5 a	0.8 abc
	Alude + SYN546539	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Alude + Subdue	0.0 a	0.0 a	0.0 a	0.1 a	0.2 a	0.4 ab
	Alude 28 d Drench	0.0 a	0.0 a	0.0 a	0.2 a	0.2 a	0.7 abc
	Alude Foliar	0.1 a	0.2 a	0.6 a	2.7 cd	4.4 c	4.8 f
	SP2770 14 d	2.5 abc	4.4 cd	5.0 d	5.0 ef	na	na
	SP2770 7 d	2.1 abc	4.7 cd	5.0 d	5.0 ef	5.0 bc	na
	SYN546539	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Subdue Maxx	0.3 a	1.4 ab	2.8 bc	4.8 f	5.0 c	5.0 bcdef
	Water	4.1 c	5.0 d	5.0 d	5.0 cdef	na	na

na = no plants available for rating sporulation because plants had completely succumbed to disease.

Table 53. Quality of plants after applications of several new fungicides and tank mix combinations with ambient *P. obduscens* inocula, Palmer, 2014

Cultivar	Treatment	Quality (0 to 5 with 5 = full lush plant with no visible decline)					
		8/20/2014	8/26/2014	9/3/2014	9/17/2014	9/24/2014	10/5/2014
Ruby	A14658C	5.0 b	5.0 d	5.0 f	5.0 h	4.9 f	4.2 abc
	Adorn	4.9 ab	4.3 bcd	3.4 bcd	1.6 bcd	1.0 bc	0.3 a
	Alude + Adorn	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude + SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude + Subdue	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude 28 d Drench	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude Foliar	5.0 b	5.0 d	5.0 f	5.0 h	4.0 e	2.0 a
	SP2770 14 d	4.9 ab	4.1 abcd	3.5 bcde	1.4 bcd	1.0 bc	0.9 a
	SP2770 7 d	4.9 b	4.5 cd	3.9 cdef	2.2 cde	1.3 bc	0.0 a
	SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Subdue Maxx	5.0 b	5.0 d	4.9 ef	3.8 fg	2.8 d	1.1 a
	Water	4.3 ab	3.3 ab	2.2 ab	0.8 ab	0.6 ab	0.0 a
Impreza White	A14658C	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	3.2 ab
	Adorn	4.9 ab	4.1 abcd	2.7 ab	1.2 abc	0.8 ab	0.0 a
	Alude + Adorn	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	4.9 bc
	Alude + SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude + Subdue	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	4.9 bc
	Alude 28 d Drench	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	4.9 bc
	Alude Foliar	5.0 b	5.0 d	5.0 f	4.6 gh	3.9 e	1.5 a
	SP2770 14 d	4.8 ab	4.3 bcd	3.7 cdef	1.1 ab	1.0 bc	0.1 a
	SP2770 7 d	5.0 b	4.6 cd	3.5 bcdef	1.2 abc	0.6 ab	0.3 a
	SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Subdue Maxx	5.0 b	5.0 d	4.9 ef	3.3 ef	2.6 d	1.1 a
	Water	4.3 a	3.0 a	1.6 a	0.8 ab	0.6 ab	na
White XP	A14658C	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	4.1 abc
	Adorn	4.8 ab	4.3 bcd	3.4 bcd	0.2 a	0.0 a	na
	Alude + Adorn	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude + SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude + Subdue	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Alude 28 d Drench	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	4.9 bc
	Alude Foliar	5.0 b	5.0 d	5.0 f	4.6 gh	4.1 e	1.2 a
	SP2770 14 d	4.9 ab	4.4 bcd	3.8 cdef	0.7 ab	0.0 a	0.0 a
	SP2770 7 d	5.0 b	4.4 bcd	3.7 cdef	0.6 ab	0.1 a	0.0 a
	SYN546539	5.0 b	5.0 d	5.0 f	5.0 h	5.0 f	5.0 c
	Subdue Maxx	5.0 b	5.0 d	4.8 def	2.5 de	1.6 c	0.1 a
	Water	4.7 ab	3.6 abc	2.1 ab	0.1 a	0.0 ab	na

na = no plants available for rating quality because plants had completely succumbed to disease an no plant parts were distinguishable.

Figure 89. Area under the Disease Progress Curve for Sporulation, Palmer, 2014.

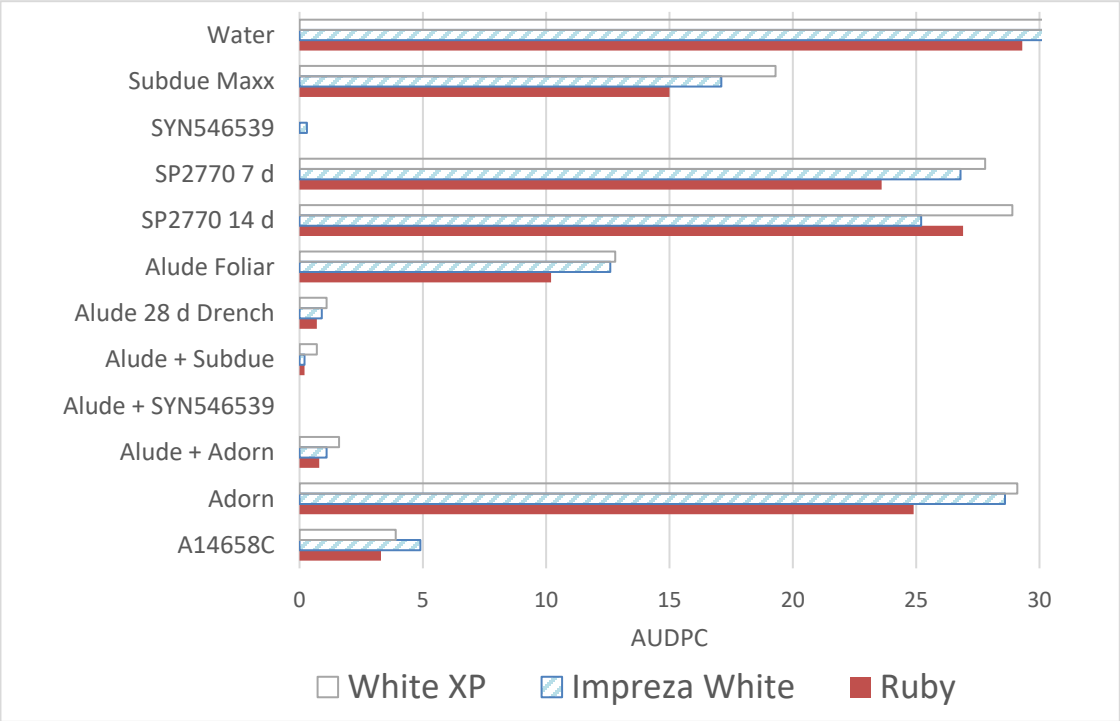


Figure 90. Area under the Curve for Quality, Palmer, 2014

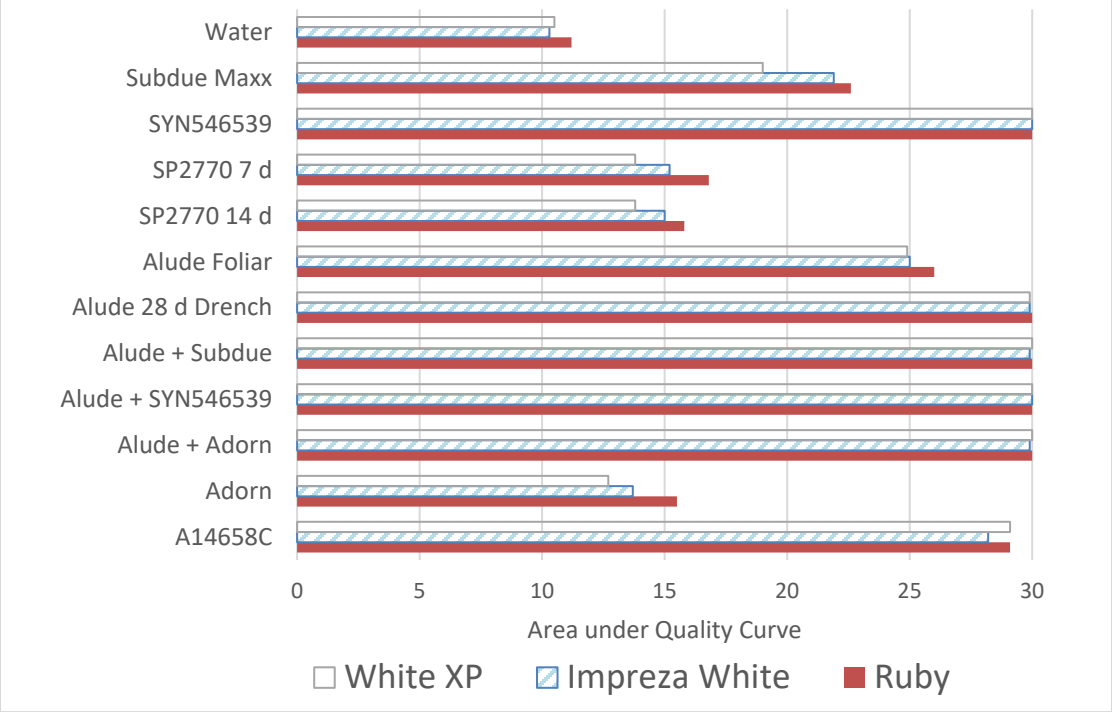


Table 54. Sporulation and quality ratings for fluopicolide resistance experiment, Palmer, 2014.

Cultivar	Treatment	Sporulation		Quality	
		9/17/2014	9/24/2014	9/17/2014	9/24/2014
Accent White	Adorn (New) 4 fl oz	4.9 a	5.0 a	4.0 a	2.8 a
	Adorn (Old) 4 fl oz	5.0 a	5.0 a	4.3 a	2.8 a
	Subdue Maxx	0.0 b	2.7 b	5.0 b	4.9 b
	Water	5.0 a	5.0 a	4.2 a	3.2 a
Dazzler Pink	Adorn (New) 4 fl oz	5.0 a	5.0 a	4.0 a	2.8 a
	Adorn (Old) 4 fl oz	4.9 a	5.0 a	4.2 a	2.8 a
	Subdue Maxx	0.8 b	3.2 b	5.0 b	5.0 b
	Water	4.9 a	5.0 a	4.3 a	3.3 a
SE Rose	Adorn (New) 4 fl oz	4.8 a	5.0 a	4.0 a	2.8 a
	Adorn (Old) 4 fl oz	4.9 a	5.0 a	4.0 a	2.8 a
	Subdue Maxx	0.5 b	2.3 b	4.8 b	4.6 b
	Water	4.7 a	4.9 a	4.1 a	2.8 a

2015. At Rutgers, Cream Ridge Experiment Station, a single experiment was conducted with impatiens planted in field containers and then grown under open shade house. Six fungicide treatments were applied as foliar sprays at weekly or biweekly intervals (A14658C, Adorn, SYN546539, BAS 703, Subdue Maxx, and Stature rotated with Pageant) and Alude was applied as a drench every 4 weeks (Table 55). Sporulation was collected on a scale of 0 to 5 with 0 equaling no visible sporulation and 5 equaling 90% or greater visible sporulation. Quality was collected on a scale of 0 to 5 with 0 being a plant with virtually no leaves and 5 being a healthy plant with full leaf canopy. The best treatment based on sporulation rating was SYN546539. Similar to previous results, Adorn and Subdue Maxx were not significantly different from water. A14658C, Alude, and BAS 703 reduced sporulation.

Table 55. Preliminary IDM efficacy results for 8 fungicide treatments, Palmer, 2015.

Cultivar	Treatment	Sporulation							
		7/28/15	8/6/15	8/12/15	8/18/15	8/25/15	9/1/15	9/8/15	9/15/15
Accent Star Red	A14658C	0.7 a	0.6 a	3.2 bcd	1.8 bc	1.6 cdef	1.6 abc	2.1 ab	2.8 c
	Adorn	1.3 a	1.8 abcde	4.8 a	4.8 a	4.7 a	5.0 fg	3.9 cd	4.8 d
	SYN546539	0.9 a	0.7 abc	1.7 ef	0.8 cd	0.7 ef	0.2 a	0.8 a	0.9 ab
	Water	1.8 a	2.0 abcde	4.9 a	4.9 a	4.4 a	5.0 efg	4.1 cd	4.6 d
	Alude 28 d Drench	1.1 a	1.0 abc	2.4 bcde	2.2 b	1.5 def	2.1 bcd	2.0 ab	2.4 bc
	BAS 703	0.7 a	0.6 a	2.7 bcde	2.5 b	1.8 cde	3.2 cdefg	3.2 bcd	3.8 cd
	Rotation: Stature / Pageant	1.1 a	1.3 abcd	4.1 abcd	3.8 abcd	3.0 bc	4.3 fg	4.7 d	4.9 d
	Subdue Maxx	0.9 a	1.3 abcd	4.0 abcd	4.3 a	4.6 a	4.7 g	3.9 cd	4.7 d
Xtreme Violet	A14658C	1.1 a	0.9 abc	2.7 bcde	1.9 bcd	1.6 cdef	1.1 ab	1.7 ab	2.4 bc
	Adorn	1.4 a	3.1 e	4.8 a	4.6 a	4.7 a	0.0	3.2 bcd	4.4 cd
	SYN546539	1.7 a	1.3 abcd	1.1 f	0.6 d	0.3 f	0.1 a	0.6 a	0.7 a
	Water	1.4 a	2.8 de	5.0 a	4.8 a	4.9 a	0.0	3.2 bcd	5.0 cd
	Alude 28 d Drench	1.2 a	1.7 abcde	2.8 bcde	2.6 bcd	2.4 cd	2.4 bcde	2.8 bc	3.6 cd
	BAS 703	1.6 a	2.2 bcde	3.1 bcd	2.9 bcd	1.8 cde	2.8 bcdef	3.2 bcd	4.0 cd
	Rotation: Stature / Pageant	1.2 a	2.4 bcde	4.2 abcd	4.1 abcd	2.8 cd	3.6 defg	4.4 d	5.0 d
	Subdue Maxx	1.2 a	2.3 bcde	4.1 abcd	4.6 a	4.3 ab	4.6 efg	4.2 cd	4.2 cd

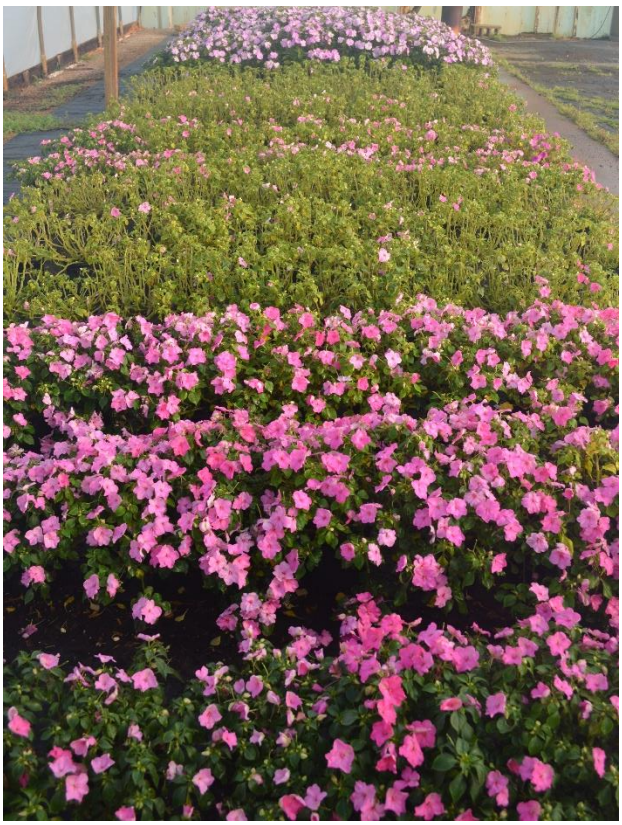
After completing a standard IR-4 foliar fungicide crop safety protocol where 1x, 2x, and 4x the standard efficacy rate is applied, the crew at the Rutgers Cream Ridge Experiment Station held the impatiens that had been treated with several products in the greenhouses. However, on July 19 when first evaluations were collected or the planned FY2016 IDM experiments, these leftover impatiens had started to exhibit signs of IDM, and they rated them on July 21. Nine weeks after treatment, approximately 1 week after symptoms were noticeable, some of the treatments were still effective; others not as much. While sporulation was not significantly different from the water control, the plants rated as higher quality. OxiPhos treated plants had very little sporulation, higher quality plants, and noticeably more blooms.

Table 56. IDM efficacy results for 3 fungicide treatments, Palmer, 2015.

Treatment (per 100 gal)	**Data Collected 7/21**			
	Sporulation (0 – 5)		**Quality (0 – 5)**	
Orkestra 8 fl oz	2.4	ab	3.0	d
Orkestra 16 fl oz	1.2	bcde	3.1	d
Orkestra 32 fl oz	1.3	abcde	4.0	c
OxiPhos 1 gal	0.5	de	4.3	bc
OxiPhos 2 gal	0.8	cde	4.6	ab
OxiPhos 4 gal	0.1	e	5.0	a
S2200 7.5 fl oz	2.3	ab	2.9	d
S2200 15 fl oz	1.8	abc	2.9	d
S2200 30 fl oz	1.4	abcd	2.8	d
Water Control	2.2	ab	2.1	e
No applications	2.6	a	2.0	e

3 foliar applications, 4/19, 5/3, 5/17

Figure 91. IDM impacted by several preventative treatments



The order of treatments from bottom to top are Oxiphos (low to high rates), S2200 (low to high rates), Orkestra (low to high rates with some flowers), followed by control and no application extras. The top row is a similarly-colored NG impatiens.

Active Ingredient Rotations - New Jersey (Mit-Rut-2)

2016. While planned as a preventative experiment, IDM appeared earlier in July during 2016. Previously, IDM symptoms and signs were evident starting in late July through mid August at this location. Quality ratings in both experiments were high at the first ratings on 7/19 (Table 57, Table 59), but sporulation ranged from 0.2 to 2.1 on a scale of 0 to 5 (Table 58, Table 60). There appeared to be some differences in susceptibility among cultivars, but all three cultivars did exhibit heavy sporulation by the end of the experiments. Alude, Alude rotated with Orkestra, Segovis, and Segovis rotated with Inosco provided high quality throughout Experiment A. Inosco also provided good quality through most of the experiment, but IDM started to cause noticeable plant decline on 9/6, three weeks after the last application. Adorn and Subdue were included in these experiments to monitor resistance. As anticipated, the *Peronospora obducens* population in these experiments was not greatly impacted by either product. Alude exhibited a different pattern this year than in previous research. Prior to this year, Alude treatments typically had high quality with heavy sporulation, but during 2016 very little sporulation occurred with Alude-treated plants. While this was observed in Experiment A, it was more apparent in Experiment B where the primary comparison was between Stature + Pageant with or without a rotation with Alude. The combination in rotation with Alude greatly reduced sporulation, and, by the end of the experiment, this treatment had significantly higher quality than Stature + Pageant alone.

Table 57. Experiment A. Quality Ratings on a scale of 0 to 5 (0 = No plant, 5 = Best quality with full canopy and high flower density)

Cultivar	Treatment	Quality																	
		7/19/2016		7/26/2016		8/1/2016		8/8/2016		8/16/2016		8/23/2016		8/30/2016		9/6/2016		9/13/2016		AUDPC (9/13)	
Super Elfin XP Coral	Adorn	5.0	a	5.0	a	3.5	cd	2.3	de	0.9	cd	0.4	de	0.2	de	0.0	e	0.0	f	17.3	de
	Alude	5.0	a	4.9	a	5.0	a	5.0	a	5.0	a	4.9	a	5.0	a	4.8	ab	4.7	ab	44.3	a
	Alude/ Orkestra	4.9	a	4.9	a	5.0	a	5.0	a	5.0	a	5.0	a	5.0	a	5.0	a	4.9	a	44.8	a
	Inosco	4.9	a	4.9	a	5.0	a	4.7	a	5.0	a	4.9	a	4.7	a	4.1	b	3.7	c	42.1	a
	Segovis	4.7	a	5.0	a	5.0	a	4.9	a	5.0	a	4.9	a	4.8	a	4.7	ab	4.4	abc	43.4	a
	Segovis/ Inosco	4.9	a	5.0	a	5.0	a	5.0	a	4.9	a	4.9	a	5.0	a	4.8	ab	4.8	a	44.4	a
	Subdue	4.8	a	4.9	a	4.4	ab	3.4	bc	1.6	c	1.7	c	1.7	c	1.5	d	1.0	e	24.9	c
	Water	4.9	a	4.8	a	3.1	d	1.5	e	0.6	d	0.1	e	0.0	e	0.0	e	0.0	f	14.9	e
Super Elfin XP Rose	Adorn	4.9	a	4.9	a	3.9	bc	2.5	cd	1.0	cd	1.3	c	0.8	d	0.7	e	0.2	ef	20.2	d
	Alude	5.0	a	5.0	a	5.0	a	5.0	a	4.8	a	4.7	a	4.8	a	4.8	ab	4.6	ab	43.7	a
	Alude/ Orkestra	4.8	a	4.8	a	5.0	a	4.8	a	5.0	a	5.0	a	4.9	a	5.0	a	4.9	a	44.3	a
	Inosco	4.8	a	4.4	a	4.9	a	4.9	a	5.0	a	4.9	a	5.0	a	4.4	ab	3.9	bc	42.3	a
	Segovis	4.8	a	4.7	a	4.7	a	4.9	a	5.0	a	5.0	a	4.8	a	4.4	ab	4.3	abc	42.8	a
	Segovis/ Inosco	4.9	a	4.4	a	4.8	a	4.8	a	4.7	a	4.8	a	5.0	a	4.8	ab	4.8	a	43.1	a
	Subdue	4.7	a	4.9	a	4.8	a	4.1	ab	2.6	b	2.7	b	2.8	b	2.3	c	2.1	d	30.9	b
	Water	4.8	a	4.5	a	3.3	cd	2.3	de	0.8	d	1.0	cd	0.5	de	0.1	e	0.0	f	17.3	de

Numbers followed by the same letter are not significantly different with Scheffe's post hoc at p = 0.05

Table 58. Experiment A. Sporulation Ratings on a scale of 0 to 5 (0 = No sporulation; 5 = 100%)

Cultivar	Treatment	Sporulation																			
		7/19/2016		7/26/2016		8/1/2016		8/8/2016		8/16/2016		8/23/2016		8/30/2016		9/6/2016		9/13/2016		AUDPC (9/13)		AUDPC (9/13) * Missing = 5	
Super Elfin XP Coral	Adorn	0.3	b	3.1	abcd	4.8	a	4.9	a	3.4	a	5.0	a	5.0	a	--		--		22.3	b	36.4	abc
	Alude	0.6	ab	0.4	e	0.7	ef	0.1	d	0.0	b	0.2	c	0.2	de	0.6	d	1.0	cd	3.7	ef	3.7	fg
	Alude/ Orkestra	0.5	ab	0.3	e	0.7	ef	0.0	d	0.0	b	0.1	c	0.0	e	0.1	d	1.2	c	2.8	ef	2.8	fg
	Inosco	0.5	ab	0.2	e	1.6	def	1.8	b	0.8	b	1.5	b	1.8	c	3.2	b	4.1	a	15.4	c	15.4	d
	Segovis	0.2	b	0.0	e	0.2	f	0.0	d	0.0	b	0.0	c	0.0	e	0.0	d	0.0	d	0.4	f	0.4	g
	Segovis/ Inosco	1.1	ab	0.0	e	0.2	f	0.0	d	0.0	b	0.1	c	0.0	e	0.1	d	0.0	d	1.4	ef	1.4	g
	Subdue	1.1	ab	1.6	bcde	3.2	bc	4.8	a	3.8	a	4.9	a	3.8	b	4.6	a	5.0	a	30.2	a	33.0	bc
	Water	0.8	ab	4.1	a	5.0	a	5.0	a	3.5	a	5.0	a	--		--		--		24.0	b	39.0	a
Super Elfin XP Rose	Adorn	0.6	ab	3.5	abc	4.6	ab	4.9	a	4.4	a	4.8	a	5.0	a	4.7	a	5.0	a	31.2	a	37.6	ab
	Alude	0.6	ab	1.7		1.7	de	0.8	cd	0.0	b	0.3	c	0.3	de	0.6	d	1.2	c	7.2	cd	7.2	ef
	Alude/ Orkestra	0.5	ab	1.0	de	1.1	ef	0.3	cd	0.0	b	0.1	c	0.0	de	0.1	d	0.7	cd	3.7	ef	3.7	fg
	Inosco	1.1	ab	1.4	cde	1.4	ef	1.2	bc	0.6	b	0.7	c	0.9	e	1.5	c	2.7	b	11.4	cd	11.4	de
	Segovis	2.1	a	1.8	bcde	1.2	ef	0.1	d	0.0	b	0.4	c	0.3	de	0.1	d	0.0	d	5.9	def	5.9	efg
	Segovis/ Inosco	1.7	ab	1.7	bcde	1.3	ef	0.0	d	0.1	b	0.0	c	0.1	de	0.1	d	0.0	d	5.0	ef	5.0	fg
	Subdue	0.6	ab	1.6	bcde	2.9	cd	4.9	a	4.2	a	4.4	a	3.9	b	4.4	a	4.7	a	31.6	a	31.6	c
	Water	1.4	ab	3.7	ab	4.8	a	5.0	a	4.4	a	5.0	a	5.0	a	5.0	a	--		27.8	ab	39.5	a

Numbers followed by the same letter are not significantly different with Scheffe's post hoc at p = 0.05

Table 59. Experiment B. Quality Ratings on a scale of 0 to 5 (0 = No plant, 5 = Best quality with full canopy and high flower density)

		Quality																			
Cultivar	**Treatment**	**7/19/2016**		**7/26/2016**		**8/1/2016**		**8/8/2016**		**8/16/2016**		**8/23/2016**		**8/30/2016**		**9/6/2016**		**9/13/2016**		**AUDPC (9/13)**	
Super Elfin XP Bright Orange	Adorn	5		4.9	a	4.4	a	3.3	bc	1.8	c	1.2	b	1.6	b	1.5	c	1.3	c	25.1	c
	Subdue	5		4.7	a	4.5	a	4.2	a	2.8	ab	2.6	a	2.8	a	2.8	b	2.6	b	32.1	b
	Water	5		4.7	a	4.2	a	3.3	c	2.3	bc	1.2	b	1.3	b	1.1	c	1.0	c	24.0	c
	Stature+ Pageant	5		4.7	a	4.5	a	4.0	ab	3.0	a	3.0	a	3.3	a	3.0	b	2.1	b	32.6	b
	Stature+ Pageant/ Alude	5		4.5	a	4.8	a	4.5	a	3.3	a	3.1	a	3.4	a	3.9	a	3.8	a	26.2	a

Numbers followed by the same letter are not significantly different with Scheffe's post hoc at p = 0.05

Table 60. Experiment B. Sporulation Ratings on a scale of 0 to 5 (0 = No sporulation; 5 = 100%)

		Sporulation																			
Cultivar	**Treatment**	**7/19/2016**		**7/26/2016**		**8/1/2016**		**8/8/2016**		**8/16/2016**		**8/23/2016**		**8/30/2016**		**9/6/2016**		**9/13/2016**		**AUDPC (9/13)**		**AUDPC (9/13) * Missing = 5**	
Super Elfin XP Bright Orange	Adorn	0.3	a	1.8	a	3.5	ab	5.0	a	3.7	ab	4.0	a	4.0	a	4.7	a	4.7	a	30.1	a	31.9	a
	Subdue	1.0	a	2.3	a	2.4	bc	4.4	ab	3.3	b	3.2	b	2.5	b	3.2	b	4.1	a	26.6	ab	26.6	b
	Water	0.3	a	2.3	a	3.7	a	5.0	a	4.2	ab	4.5	a	4.7	a	4.9	a	5.0	a	31.3	a	34.6	a
	Stature+ Pageant	0.6	a	2.1	a	2.9	bc	4	b	2.3	c	1.2	c	1.8	c	3.9	b	4.6	a	23.4	b	23.4	b
	Stature+ Pageant/ Alude	0.6	a	1	a	1.5	c	2.8	c	1.3	d	0.3	d	0.1	d	0.2	c	0.5	b	8.4	c	8.4	c

Numbers followed by the same letter are not significantly different with Scheffe's post hoc at p = 0.05

Active Ingredient Rotations for IDM- New York (Mit-CU-1)
There were not sufficient people and space resources to run fungicide rotation trials during the 2017 or 2018 seasons, as other experiments ran for longer than expected. A 2017 trial comparing the effect of spray applications of Mural WG, Segovis SC, Micora SC and a SubdueMAXX drench on one genetically resistant vs. one highly susceptible impatiens showed significant control from all fungicides on both plants, but fewer infected leaves per plant when fungicides were used on resistant cultivars.

Additional Relevant Information: Rotations for CDM Management

Evaluation of cultivars and fungicides for control of downy mildew on cucumber in Clinton, North Carolina
The experiment was conducted at the Horticultural Crops Research Station in Clinton, NC (N35°01.423'; W078°16.489'). Plots were double row beds on 5-ft centers covered with white plastic mulch; 20-ft long with 5-ft fallow borders on each end with non-treated guard rows on the perimeter of the field. The previous year the field was planted with sweetpotato. Cucumber was direct seeded on 7 Aug (1-ft in-row spacing, 1 seed/hill) in raised beds (40 plants/plot). Irrigation and fertilization were applied via drip tape. Treatments were randomized into four complete blocks. Fungicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with a two-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 45 psi. The three spray applications were made with a two-nozzle boom (19-in. spacing). Applications were made on: 16, 23, and 30 Sep. Disease severity was assessed on 4 Oct as percent leaf area with necrosis per plot. Fruit were harvested on 5 Oct. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means.

Downy mildew was first detected on 16 Sep at approximately 5% disease severity in the field and progressed throughout the course of the trial. Orondis Opti, Ranman, and Previcur Flex tank mixed with Bravo Weather Stik on Peacemaker were outstanding in controlling *P. cubensis* and produced a high level of marketable fruit. Ranman, Previcur Flex, and Zampro with Bravo Weather Stik tank mixed on Peacemaker as well as Bravo Weather Stik on Peacemaker, Orondis Opti, Ranman, and Previcur Flex tank mixed with Bravo Weather Stik on Citadel and non-treated Peacemaker also provided good downy mildew control. All treatments were significantly different from the untreated susceptible cultivars Expedition and Vlaspik, which had 33-34% disease severity at the end of the trial on 4 Oct. No additional disease ratings were taken under higher disease pressure due to hurricane Matthew. No phytotoxicity was observed. In the table, treatments are sorted by disease severity on 4 Oct.

Table 61. Fungicide efficacy for *P. cubensis* on cucumber

Treatment and rate of product per acre	Application No.	Disease Severity* (%)	
		4-Oct	Mkt Yield (lb/plot)
Orondis Opti 1.67 SC 2 fl oz Bravo Weather Stik 720 SC 32 fl oz Ranman 400 SC 2.75 fl oz Previcur Flex 66.5 SL 19.2 fl oz Peacemaker	1,4 1-6 2,5 3,6	1.3 e**	40.27 a
Ranman 400 SC 2.75 fl oz Bravo Weather Stik 720 SC 32 fl oz Previcur Plex 66.5 SL 19.2 fl oz Zampro 525 SC 14 fl oz Peacemaker	1,4 1-6 2,5 3,6	3.0 de	37.67 ab
Bravo Weather Stik 720 SC 32 fl oz Peacemaker	1-6	3.7 de	33.27 abc
Orondis Opti 1.67 SC 2 fl oz Bravo Weather Stik 720 SC 32 fl oz Ranman 400 SC 2.75 fl oz Previcur Flex 66.5 SL 19.2 fl oz Citadel	1,4 1-6 2,5 3,6	4.7 de	42.53 a
Nontreated; Peacemaker	N/A	6.3 de	32.67 a-d
Ranman 400 SC 2.75 fl oz Bravo Weather Stik 720 SC 32 fl oz Previcur Flex 66.5 SL 19.2 fl oz Zampro 525 SC 14 fl oz Citadel	1,4 1-6 2,5 3,6	9.3 cd	30.00 a-d
Bravo Weather Stik 720 SC Citadel	1-6	10.0 cd	33.47 abc
Orondis Opti 1.67 SC 2 fl oz Bravo Weather Stik 720 SC 32 fl oz Ranman 400 SC 2.75 fl oz Previcur Flex 66.5 SL 19.2 fl oz Vlaspik	1, 4 1-6 2,5 3,6	15.7 bc	29.87 a-d
Nontreated; Citadel	N/A	17.0 bc	33.97 abc
Ranman 400 SC 2.75 fl oz Bravo Weather Stik 720 SC 32 fl oz Previcur Plex 66.5 SL 19.2 fl oz Zampro 525 SC 14 fl oz Vlaspik	1,4 1-6 2,5 3,6	17.0 bc	22.13 b-e
Ranman 400 SC 2.75 fl oz Bravo Weather Stik 720 SC 32 fl oz Previcur Plex 66.5 SL 19.2 fl oz Zampro 525 SC 14 fl oz Expedition	1,4 1-6 2,5 3,6	18.0 bc	22.53 b-e
Bravo Weather Stik 720 SC 32 fl oz Expedition	1-6	19.3 b	22.67 b-e
Bravo Weather Stik 720 SC 32 fl oz Vlaspik	1-6	20.3 b	19.60 cde
Orondis OPti 1.67 SC 2 fl oz Bravo Weather Stik 720 SC 32 fl oz Ranman 400 SC 2.75 fl oz Previcur Flex 66.5 SL 19.2 fl oz Expedition	1,4 1-6 2,5 3,6	21.0 b	26.07 a-e
Nontreated; Expedition	N/A	33.0 a	15.80 de
Nontreated; Vlaspik	N/A	34.0 a	11.20 e

* Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.

** Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Waller-Duncan k=100).

Evaluation of fungicides for control of downy mildew on cucumber, Clayton North Carolina.
The experiment was conducted at the Central Crops Research Station in Clayton, NC (N35°40.054'; W078°30.299'). Plots were single 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. The previous year the field was planted with tobacco. Cucumber was direct seeded on 9 Aug (2-ft in-row spacing, 2 seed/hill) in raised beds and thinned to one plant per hill after emergence (7 plants/plot). Irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape on 31 Aug, 7, 14, 21 and 28 Sep and 6 Oct. Treatments were randomized into four complete blocks. Fungicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with a single-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 45 psi. The first three spray applications were made with a single-nozzle boom and the last one with a two-nozzle boom (19-in. spacing). Applications were made on 13, 20 and 29 Sep and 5 Oct. Disease severity was assessed on 27 Sep and 5 Oct as percent leaf area with necrosis per plot. Fruit were harvested on 23 and 30 Sep and 7 Oct. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means.

Downy mildew was first detected on 13 Sep at approximately 2% disease severity in the field and progressed throughout the course of the trial (Table 62). Ranman alternated with Previcur Flex and Bravo Weather Stik controlled *P. cubensis* when compared to the non-treated. No other treatment provided a commercially acceptable level of downy mildew control. No phytotoxicity was observed. In the table, treatments are sorted by disease severity on 5 Oct.

Table 62. Fungicide efficacy for *P. cubensis* on cucumber

Treatment and rate of product per acre	Application no.[y]	Disease severity[z] (%)	
		27-Sep	5-Oct
Ranman 3.33SC 2.75 fl oz Previcur Flex 6F 19.2 fl oz Bravo Weather Stik 6SC 2 pt	1, 4 2 3	11.5 c[x]	39.3 b
OxiPhos 14L 2 qt	1-4	34.5 a	71.3 a
CX-4380 SP 2.1 lb/100 gal	1-4	28.5 ab	71.3 a
ProPhyt 4.2SC 4 pt Kocide 3000 46.1DF 1 lb Cabrio 20EG 10 oz	1-4 1, 3 2, 4	25.3 b	72.0 a
CX-4380 SP 8.4 lb/100 gal	1-4	27.8 ab	72.3 a
CX-4380 SP 4.2 lb/100 gal	1-4	33.0 a	76.5 a
Non-treated	N/A	36.0 a	78.5 a

[z] Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.

[y] Application dates: 1=13 Sep, 2=20 Sep, 3=29 Sep and 4=7 Oct.

[x] Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Waller-Duncan k=100).

Objective 3: Mitigation Strategies: Management Longevity

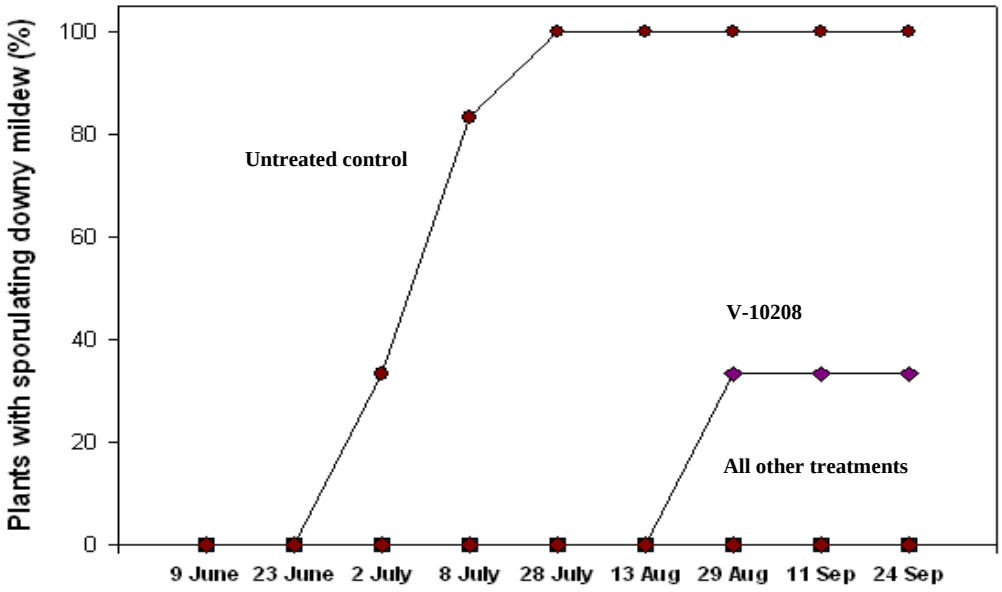
Longevity of tools applied during production after transplanting to the landscape (Mit-MSU-2, Mit-FL-3)

Michigan

2013. Experiments were conducted in Michigan to determine the effectiveness of greenhouse fungicide applications to control impatiens downy mildew in the landscape. Two sites were selected based on knowledge of IDM at the location in previous years. Each site had a trial with a white variety 'Accent Premium White' and a red variety 'Accent Premium Red'. Greenhouse applications were initiated on May 9th and reapplied at either 7- or 14-day intervals. The final application was applied June 2nd. The treated plants were transplanted June 4th for trial 1 (Figure 92), and June 9th for trial II (Figure 93). Plants were observed weekly for symptoms of downy

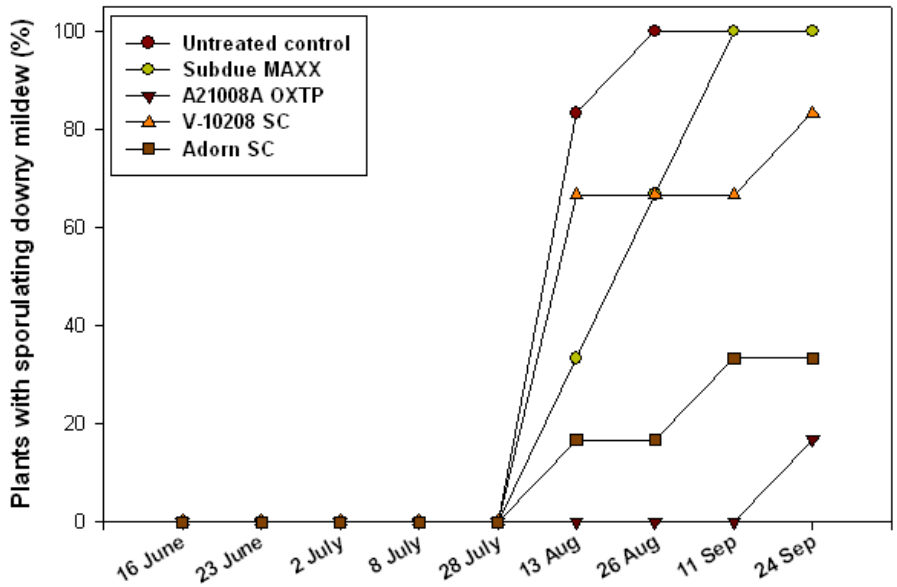
mildew infection. The figures below show the incidence (%) of 'Accent Premium White' impatiens from each treatment that were observed with downy mildew sporulation.

Figure 92. Greenhouse application residual efficacy after plants moved to landscape, Experiment I, Harlan and Hausbeck, 2013.



For Experiment I, the untreated control plants were 100% infected by the end of July. Treatment V-10208 was the only other treatment to show infection with ~35% of plants with sporulation at the 29 Aug rating date. All other treatments prevented pathogen sporulation by the end of September. All other treatments include Subdue MAXX, A21008A, Adorn, and various rotational programs of all products.

Figure 93. Greenhouse application residual efficacy after plants moved to landscape, Experiment II, Harlan and Hausbeck, 2013.



For Experiment II, the untreated control plants were 100% infected by the end of the study. Drench application in the greenhouse of the industry standard Adorn and experimental product A21008A significantly reduced incidence of IDM when compared to the untreated control.

2014. Four experiments were conducted in Michigan to determine the effectiveness of greenhouse fungicide applications to control infection in the landscape. Two sites were selected based on knowledge of IDM at the location in previous years. Each site had a trial with a white variety 'Accent Premium White' and a red variety 'Accent Premium Red'. Although data from the trials are still being analyzed, at one site, industry standards Adorn and Subdue MAXX were extremely efficacious and treated plants remained disease free for 12 weeks after infection was observed in the untreated control plants. At the second landscape location, industry standard Subdue MAXX had infection rates similar to the untreated control and the isolate is being tested for possible resistance to the fungicide. Experimental fungicide oxathiapiprolin was extremely effective and resulted in the lowest amount of infection at both sites. A variety trial was also transplanted at each site. Fourteen varieties (seven white and seven red varieties) were grown from seed in the greenhouse and each variety consisted of an untreated set of plants and a set that was treated with the grower standard IDM control program. Although data from this experiment is still being analyzed, no noticeable differences were observed between the varieties.

Table 63. Results from 2014 landscape mitigation trials – Site PFF 'Accent Premium Red'.

Site PFF, 'Accent Premium Red'	Leaves sporulating/defoliated (%)*									
Treatment and rate/100 gal, *application method* (*interval*)	7/2		7/8		7/17		8/13		8/29	
Untreated control	18.4	b**	30.1	b	27.6	b	81.7	b	83.3	b
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) QGU42 SC 9.6 fl oz, *drench* (*week 3*) Alude 12.75 fl oz + V-10208 SC 4 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
QGU42 SC 9.6 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) QGU42 SC 9.6 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz, *drench* (*28-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
QGU42 SC 9.6 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
V-10208 SC 4 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	9.2	a
Adorn SC 1 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	8.3	a

*Based on a visual estimation of percentage of leaves sporulating/defoliated.
**Column means with a letter in common are not significantly different (LSD t Test; *P*=0.05).

Table 64. Results from 2014 landscape mitigation trials – Site PFF 'Accent Premium White'.

Site PFF, 'Accent Premium White'	Leaves sporulating/defoliated (%)									
Treatment and rate/100 gal, *application method* (*interval*)	7/2		7/8		7/17		8/13		8/29	
Untreated control	2.58	b	14.8	b	29.3	b	100.0	b	100.0	b
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) QGU42 SC 9.6 fl oz, *drench* (*week 3*) Alude 12.75 fl oz + V-10208 SC 4 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
QGU42 SC 9.6 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) QGU42 SC 9.6 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz, *drench* (*28-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
QGU42 SC 9.6 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a
V-10208 SC 4 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	21.7	a
Adorn SC 1 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a	0.0	a	0.0	a

*Based on a visual estimation of percentage of leaves sporulating/defoliated.
**Column means with a letter in common are not significantly different (LSD t Test; *P*=0.05).

Table 65. Results from 2014 landscape mitigation trials – Site M 'Accent Premium Red'.

Site M, 'Accent Premium Red'	Leaves sporulating/defoliated (%)					
Treatment and rate/100 gal, *application method* (*interval*)	8/13		8/26		9/24	
Untreated control	88.3	b	100.0	c	100.0	d
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	8.3	a	9.2	a	34.2	bc
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) QGU42 SC 9.6 fl oz, *drench* (*week 3*) Alude 12.75 fl oz + V-10208 SC 4 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a
QGU42 SC 9.6 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) QGU42 SC 9.6 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz, *drench* (*28-day intervals*)	15.8	a	18.3	ab	47.5	c
QGU42 SC 9.6 fl oz, *drench* (*14-day intervals*)	0.0	a	0.0	a	0.0	a
V-10208 SC 4 fl oz, *drench* (*14-day intervals*)	17.5	a	35.8	b	53.3	c
Adorn SC 1 fl oz, *drench* (*14-day intervals*)	3.2	a	6.7	a	8.3	ab

*Based on a visual estimation of percentage of leaves sporulating/defoliated.
**Column means with a letter in common are not significantly different (LSD t Test; *P*=0.05).

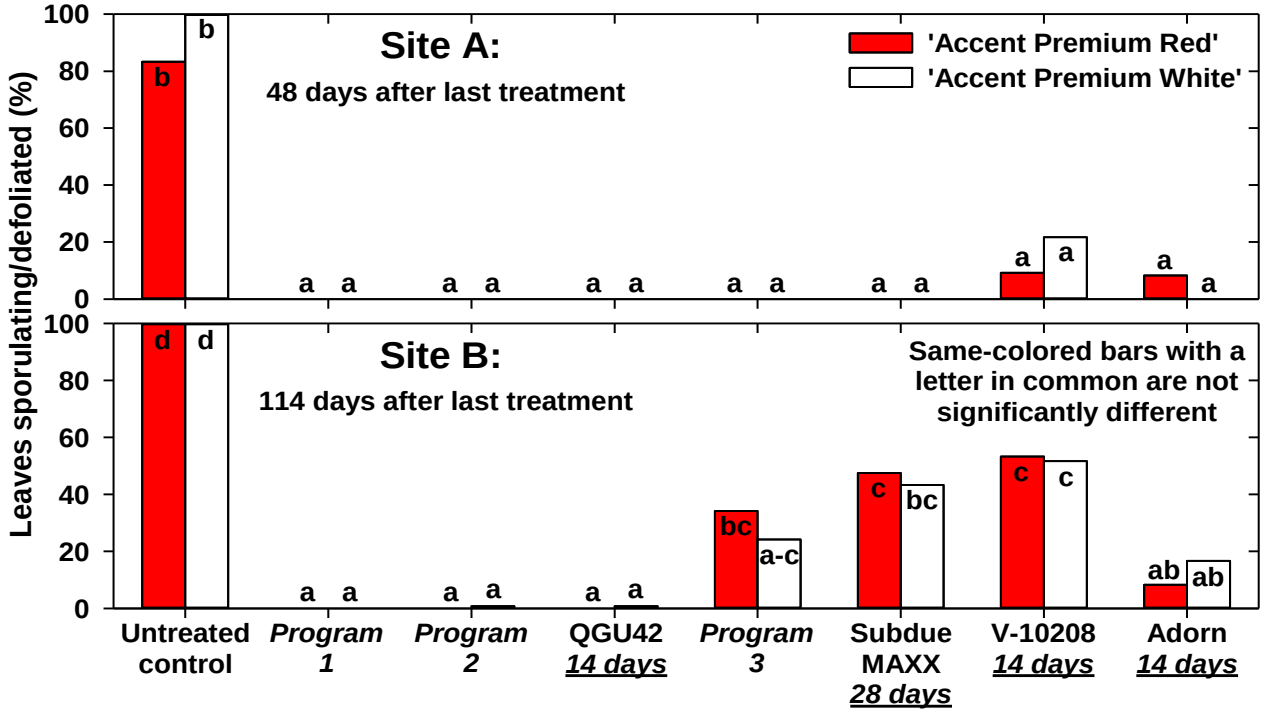
Table 66. Results from 2014 landscape mitigation trials – Site M 'Accent Premium White.

Site M, 'Accent Premium White'	Leaves sporulating/defoliated (%)					
Treatment and rate/100 gal, *application method* (*interval*)	8/13		8/26		9/24	
Untreated control	98.3	c	100.0	c	100.0	d
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	6.7	ab	5.5	a	24.2	a-c
Subdue MAXX EC 1 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) QGU42 SC 9.6 fl oz, *drench* (*week 3*) Alude 12.75 fl oz + V-10208 SC 4 fl oz, *drench* (*week 4*) Subdue MAXX EC 1 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	0.0	a	0.8	a	0.8	a
QGU42 SC 9.6 fl oz + Alude 12.75 fl oz, *drench* (*week 1*) Adorn SC 1 fl oz + Heritage WDG 0.9 oz, *drench* (*week 2*) Segway SC 3.5 fl oz, *spray* (*week 3*) Alude 12.75 fl oz, *drench* (*week 4*) QGU42 SC 9.6 fl oz + Adorn SC 1 fl oz, *drench* (*week 5*)	3.7	a	0.0	a	0.0	a
Subdue MAXX EC 1 fl oz, *drench* (*28-day intervals*)	16.7	ab	25.8	b	43.3	bc
QGU42 SC 9.6 fl oz, *drench* (*14-day intervals*)	0.0	a	0.5	a	0.8	a
V-10208 SC 4 fl oz, *drench* (*14-day intervals*)	25.0	b	35.8	b	51.7	c
Adorn SC 1 fl oz, *drench* (*14-day intervals*)	0.0	a	1.7	a	16.7	ab

[*]Based on a visual estimation of percentage of leaves sporulating/defoliated.

[**]Column means with a letter in common are not significantly different (LSD t Test; *P*=0.05).

Figure 94. Comparison of fungicide efficacy for IDM on two cultivars at two sites, Harlan and Hausbeck, 2014



Longevity of DM fungicides applied in the greenhouse to two impatiens cultivars planted in the landscape at Sites A and B. ***Program 1***: (QGU42+Alude)*/wk 1*, (Adorn+ Heritage)*/wk 2*, (Segway)*/wk 3*, (Alude)*/wk 4*, (QGU42+Adorn)*/wk 5*. ***Program 2***: (Subdue+Alude)*/wk 1*, (Adorn+Heritage)*/wk 2*, (QGU42)*/wk 3*, (Alude+V-10208)*/wk 4*, (Subdue+Adorn)*/wk 5*. ***Program 3***: (Subdue+Alude)*/wk 1*, (Adorn+Heritage)*/wk 2*, (Segway)*/wk 3*, (Alude)*/wk 4*, (Subdue+Adorn)*/wk 5*. All fungicides were drenched except Segway, which was applied as a foliar spray. Fungicide rates were: Adorn 1 fl oz, Alude 12.75 fl oz, Heritage 0.9 oz, QGU42 9.6 fl oz, Segway 3.5 fl oz, Subdue MAXX 1 fl oz, V-10208 4 fl oz.

2015. Four landscape studies were initiated in the spring of 2015. Treatments for the landscape trials were applied to impatiens in 4-in. pots in a greenhouse starting on May 6th (Table 67). The treated impatiens were transplanted into the landscape sites on May 29th. Plants were not treated post transplanting. At two sites, downy mildew was not observed on any of the untreated or treated plants by the final rating. At the site located in Hudsonville, MI, downy mildew was not observed until mid-October (data not shown). However, disease pressure was severe at the Byron Center, MI location with >80% of the untreated control plants leaves with sporulating downy mildew by mid August.

Statistical analysis of the data was completed using SAS PROC GLM and statistical differences were compared using the Fisher's Protected Least Significant Differences test (*P*=0.05). All treatments that included oxathiapiprolin (Segovis) had statistically fewer leaves with downy mildew when compared to the all other treatments. Although no significant differences were observed between the treatments with Segovis, it should be noted that the treatment of Segovis alone resulted in the fewest percentage of leaves with sporulating downy mildew. Drench applications of the labeled product Adorn and the experimental product V-10208 offered some long-term control of downy mildew with treated plants having <50% of leaves with sporulating downy mildew into early September. Protectant fungicides Zing! and V-10365 did not offer any extended control when compared to the untreated control plants. Surprisingly, Subdue MAXX treated plants had a similar percentage of leaves with sporulating downy mildew when compared to the untreated control. Phytotoxicity in the form of minor plant stunting and leaf distortion were observed on all Adorn-treated plants; however, by the end of June these differences were no longer evident.

Figure 95. Impatiens downy mildew landscape fungicide screen at Byron Center, MI, Harlan and Hausbeck, 2016.

Untreated plants and plants treated with ineffective fungicides are defoliated (center) while plants treated with effective fungicides (left, right) remain healthy.

Figure 96. Greenhouse application residual efficacy after plants moved to landscape, Experiment 1, Byron Center, MI, Harlan and Hausbeck, 2015.

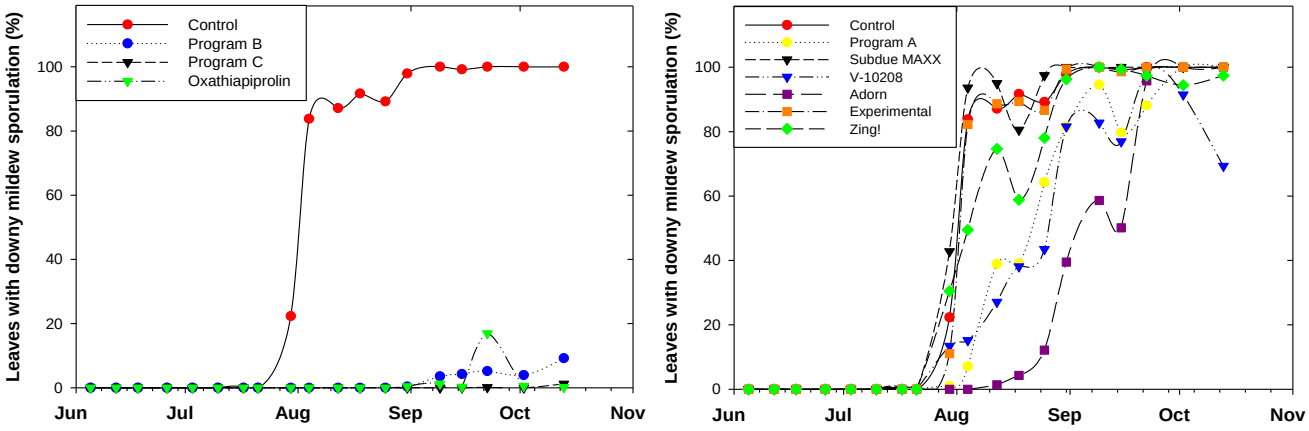


Table 67. Monthly results for greenhouse application residual efficacy after plants moved to landscape, Experiment 1, Byron Center, MI, Harlan and Hausbeck, 2015.

Treatment, rate/100 gal, drench unless otherwise noted	Interval, # of applications	Leaves with sporulating downy mildew (%)							
		30 Jul		25 Aug		15 Sep		13 Oct	
Untreated control	--	22.3	a-c	89.2	cd	99.2	d	100.0	c
A. Subdue MAXX 1 fl oz + Alude 12.75 fl oz B. Adorn SC 1 fl oz + Heritage WG 0.9 oz C. Segway 3.5 fl oz (spray) D. Alude 12.75 fl oz E. Subdue MAXX 1 fl oz + Adorn 1 fl oz	7-day, 5	1.0	a	64.3	bc	79.7	c	100.0	c
A. Subdue MAXX 1 fl oz + Alude 12.75 fl oz B. Adorn SC 1 fl oz + Heritage WG 0.9 oz C. Segovis 9.6 fl oz D. Alude 12.75 fl oz + V-10208 SC 8 fl oz E. Subdue MAXX 1 fl oz + Adorn 1 fl oz	7-day, 5	0.0	a	0.0	a	4.3	a	9.2	a
A. Segovis 9.6 fl oz + Alude 12.75 fl oz B. Adorn SC 1 fl oz + Heritage WG 0.9 oz C. Segway 3.5 fl oz (spray) D. Alude 12.75 fl oz E. Segovis 9.6 fl oz + Adorn 1 fl oz	7-day, 5	0.0	a	0.0	a	0.0	a	1.2	a
Subdue MAXX 1 fl oz	28-day, 2	42.8	c	97.5	d	100.0	d	100.0	c
Segovis 9.6 fl oz	14-day, 3	0.0	a	0.0	a	0.2	a	0.2	a
V-10208 SC 8 fl oz	14-day, 3	13.4	ab	43.5	b	76.9	c	69.3	b
Adorn SC 1 fl oz	14-day, 3	0.0	a	12.1	a	50.1	b	100.0	c
V-10365 SC 9.7 fl oz (spray)	14-day, 3	11.1	ab	86.6	cd	98.6	d	100.0	c
Zing! SC 36 fl oz (spray)	14-day, 3	30.5	bc	78.0	cd	99.4	d	97.4	c

In a second experiment to test preventative applications, disease pressure was severe throughout the duration of the trial. All treatments evaluated provided control of downy mildew on impatiens. Disease severity values for all treated plants were statistically lower than the untreated plants including the inoculated and non-inoculated controls (Table 68). The tank mix of Pageant Intrinsic with Protect DF provided statistically greater control than the rotation of Protect DF followed by Pageant Intrinsic 3 days later. Stature SC rotated with Pageant Intrinsic provided excellent disease control and disease severity values were statistically lower than back-to-back applications of Stature SC. The 10 and 13-ounce rates of BAS703F provided a statistically greater level of disease control than the 7-ounce rate of BAS703F, the 12-ounce Pageant Intrinsic rotation, the Protect DF and Pageant rotation and the Stature SC rotation. The 18-ounce rate of Pageant Intrinsic provided excellent control of impatiens downy mildew and when rotated with Stature SC the plants remained disease free throughout the duration of the trial. No phytotoxicity was observed for any of the treatments evaluated.

Table 68. Preventative applications for controlling impatiens downy mildew, Experiment 2, Harlan and Hausbeck, 2015

Treatments 1st application 2nd application 3rd application	**Severity** [z]	**Days until sporulation**
Untreated, non-inoculated control	69 a [y]	10
Untreated, inoculated control	69 a	10
Pageant Intrinsic 12 oz (4/2/15) Pageant Intrinsic 12 oz (4/9/15) ---	28 bc	18
Pageant Intrinsic 18 oz + Protect DF 1.5 lbs (4/2/15) Pageant Intrinsic 18 oz + Protect DF 1.5 lbs (4/9/15) ---	17 cd	18
Protect DF 1.5 lbs (4/2/15) Pageant Intrinsic 18 oz (4/5/15) ---	37 b	18
Stature SC 12.25 oz (4/2/15) Stature SC 12.25 oz (4/9/15) ---	29 bc	18
Stature SC 12.25 oz (4/2/15) Pageant Intrinsic 18 oz (4/9/15) ---	0 e	No sporulation
Catamaran 64 fl oz (4/2/15) Catamaran 64 fl oz (4/9/15) ---	0 e	33
BAS703F @ 7.0 fl oz (4/2/15) BAS703F @ 7.0 fl oz (4/9/15) BAS703F @ 7.0 fl oz (4/16/15)	7 d	18
BAS703F @ 10 fl oz (4/2/15) BAS703F @ 10 fl oz (4/9/15) BAS703F @ 10 fl oz (4/16/15)	0 e	33
BAS703F @ 13 fl oz (4/2/15) BAS703F @ 13 fl oz (4/9/15) BAS703F @ 13 fl oz (4/16/15)	0 e	33
Pageant Intrinsic @ 18.0 oz (4/2/15) Pageant Intrinsic @ 18.0 oz (4/9/15) Pageant Intrinsic @ 18.0 oz (4/16/15)	1 e	33

[z] Percent canopy area affected as an average across all rating dates.
[y] Column means followed by the same letter are not statistically different (P=0.05) based on Tukey's HSD.

2016. Two trials were initiated in 2016 to determine the effectiveness of greenhouse applied fungicides protect against downy mildew in the landscape. The fungicide applications were applied to the seedlings in 4-in. pots starting in early May and reapplied at the intervals described in the below table (Table 69). Multiple rates and application methods of the newly registered product Segovis were included. Rotational programs were also included. On 3 Jun, the impatiens were transplanted into landscape beds at two sites with history of IDM infection (Byron Center and Jenison, MI). The trials are observed weekly for signs of downy mildew on the plants. Downy mildew was observed in late September at both locations. At the Jenison site (Table 69), 74.0% of the foliage on the untreated control plants were observed with sporulating downy mildew. All of the treatment programs that included multiple active ingredients were highly efficacious and resulted in plants with no downy mildew sporulation for the entire 2016 season. Drench applications of Segovis completely prevented IDM sporulation while plants sprayed with Segovis did have some minimal sporulation by end of the year. Adorn

continued to be an effective product against IDM, however, some phytotoxicity was observed on the treated plants earlier in the season.

Table 69. Evaluation of greenhouse applied fungicides to control *P. obducens* , Harlan and Hausbeck, 2016.

Treatment and rate/100 gal (unless otherwise indicated), *drench treatment unless otherwise noted.*	**Leaves with sporulating downy mildew (%)**			
	27 Sept		**3 Oct**	
Untreated control	40.0	b*	74.0	b
Subdue MAXX 1.0 fl oz *week 1* Segovis 3.2 fl oz *week 2* Segway 3.5 fl oz *spray; week 3* Orvego 14.0 fl oz *spray; week 4* Subdue MAXX 1.0 fl oz + Segovis 3.2 fl oz *week 5*	0.0	a	0.0	a
Subdue MAXX 1.0 fl oz *week 1* Segovis 3.2 fl oz *week 2* Subdue MAXX 1.0 fl oz + Segovis 3.2 fl oz *week 5*	0.0	a	0.0	a
Segovis 3.2 fl oz + Alude 12.75 fl oz *week 1* Segway 3.5 fl oz *spray; week 3* Segovis 3.2 fl oz + Alude 12.75 fl oz *week 5*	0.0	a	0.0	a
Subdue MAXX 1.0 fl oz *weeks 1 and 5*	20.0	ab	28.0	a
Segovis 0.65 fl oz 14-day *weeks 1, 3, and 5*	0.0	a	0.0	a
Segovis 3.2 fl oz 14-day *weeks 1, 3, and 5*	0.0	a	0.0	a
Segovis 0.6 fl oz 14-day *spray; weeks 1, 3, and 5*	11.0	a	20.0	a
Segovis 2.4 fl oz 14-day *spray; weeks 1, 3, and 5*	10.8	a	30.0	a
Adorn 1.0 fl oz 14-day *weeks 1, 3, and 5*	0.0	a	0.0	a

*Column means with a letter in common are not statistically different (LSD; *P*=0.05).

2017. Two trials were initiated in 2017 to determine the effectiveness of greenhouse or landscape applied fungicides protect against downy mildew. The fungicide applications were applied either to the seedlings in 4-in. pots starting in late April or as a foliar spray to the impatiens in the landscape immediately after transplanting. The impatiens were transplanted into the landscape on 1st June into beds at two sites with history of IDM infection (Byron Center and Jenison, MI). The trials have been observed weekly for signs or symptoms of downy mildew. downy mildew was observed on the untreated plants at the Bryon Center site on September 13th and at the Jenison location on October 6th.

Figure 97. Untreated impatiens defoliated from downy mildew infection adjacent to disease free treated impatiens, September 2017, Byron Center, MI.

Florida

2015. Palmateer started a fungicide rotation experiment where impatiens (*Impatiens walleriana* 'Impreza Violet') plants were submitted to one of seven fungicide rotations, including untreated controls (Table 70). Disease free impatiens were grown in 4" pots with Promix BX in the shadehouse under 73% shade conditions. Plants were fertilized with Osmocote 14-14-14, and watered twice daily with overhead irrigation. Experimental units were a plant treated with a single fungicide treatment with 10 repetitions in a randomized complete block design (RCBD).

Fungicide treatments were applied as foliar spray providing 25ml per plant in the morning and treated plants were not watered for 24 hours following application. Fungicides were applied on a 7-day schedule with a total of four applications. Plants were transplanted into landscape beds on 12/10/15. Disease pressure was severe; thus natural inoculum was from diseased plants placed around the landscape beds.

The rotations with Segovis exhibited no symptoms and no sporulation (Table 70, Figure 99).

2016. The 2015 fungicide rotation experiment was repeated in 2016, where impatiens (*Impatiens walleriana* 'Impreza Violet') plants were submitted to one of seven fungicide rotations, including untreated controls. Disease free impatiens were grown in 4" pots with Promix BX in the shadehouse under 73% shade conditions. Plants were fertilized with Osmocote 14-14-14, and watered twice daily with overhead irrigation. Experimental units were a plant treated with a single fungicide treatment with 10 repetitions in a randomized complete block design (RCBD).

Fungicide treatments were applied as foliar spray providing 25ml per plant in the morning and treated plants were not watered for 24 hours following application. Fungicides were applied on a 7-day schedule with a total of four applications. Plants were transplanted into landscape beds on 12/10/15. Disease pressure was severe; thus, natural inoculum was from diseased plants placed around the landscape beds.

The rotations with Segovis exhibited no symptoms and no sporulation in both years (Table 70).

Figure 98. Four week rotation trial transplanted into landscape beds on 12/10/15

Figure 99. Left- 7-day rotation including Segovis, Inosco, Stature and Adorn. Right- Untreated Control. Photo taken 4 weeks after final application.

Table 70. Efficacy results for 7 fungicide rotation programs, Palmateer, 2015/2016 and 2016/2017.

	2015/2016		2016/2017 [w]	
Treatments (rate/100 gal)	**Disease Severity [z]**	**AUDPC [y]**	**Disease Severity**	**AUDPC**
Untreated control	99.7 a [x]	3678 a	84 a	1170 a
Week 1: Segway 3.5 fl oz Week 2: Stature 12.25 fl oz Week 3: Adorn 2 fl oz Week 4: Subdue Maxx 1 fl oz	72.2 b	1863 b	70.3 b	859 b
Week 1: Subdue Maxx 1 fl oz Week 2: Segovis 2.4 fl oz Week 3: Segway 3.5 fl oz Week 4: Inosco 64 fl oz	0 d	0 d	0 c	0 c
Week 1: Adorn 2 fl oz Week 2: Stature 12.25 fl oz Week 3: Inosco 64 fl oz Week 4: Segway 3.5 fl oz	24 c	519 c	67.5 b	877 b
Week 1: Segovis 2.4 fl oz Week 2: Inosco 64 fl oz Week 3: Stature 12.25 fl oz Week 4: Adorn 2 fl oz	0 d	0 d	0 c	0 c
Week 1: Stature 12.25 fl oz Week 2: Segway 3.5 fl oz Week 3: Adorn 2 fl oz Week 4: Subdue Maxx 1 fl oz	74 b	1984 b	69 b	886 b
Week 1: Inosco 64 fl oz Week 2: Adorn 2 fl oz Week 3: Subdue Maxx 1 fl oz Week 4: Segovis 2.4 fl oz	0 d	0 d	0 c	0 c
Week 1: Segway 3.5 fl oz Week 2: Stature 12.25 fl oz Week 3: Segovis 2.4 fl oz Week 4: Inosco 64 fl oz	0 d	0 d	0 c	0 c

[z] Average weekly disease severity expressed as percent canopy affected.
[y] Area Under the Disease Progress Curve.
[x] Column means indicated with the same letters are not significantly different (P=0.05) based on the Student Newman Keuls test.
[w] Year 1=2015-16; Year 2-2016-17.

Objective 3: Mitigation Strategies: Fungal Impact

Fungicide impact on oospore formation in leaves (Mit-CU-2)

To determine whether fungicide treatment affects oospore development in impatiens infected with *P. obducens*, impatiens transplanted to 6-in. pots filled with ProMix BX on 15 August were kept in the greenhouse prior to treatment to protect them from downy mildew inoculum. Treatments were applied as drenches in the greenhouse on 20 Aug. Alude was drenched at 10.0 fl oz/100 gal (2 pints/sq ft or 190 ml/6 in. pot), SubdueMAXX at 1.0 fl oz/100 gal (1.5 pint/sq ft or 142.5 ml/6-in. pot) and Micora at 8.0 fl oz/100 gal (180 ml/6-in. pot); non-sprayed plants served as a control. The drench treatment was not repeated during the experiment. There were 6 replications of the 4 treatments for harvest on each of 5 dates (thus 30 plants were given each treatment). The impatiens were held in the greenhouse for a week after the drench treatment. On 27 Aug 15, all plants were moved outside to a shade house. Once moved outside, plants were given overhead irrigation and also a natural inoculum source (diseased impatiens were placed evenly within the plot). Inoculum pressure was continuous during the trial.

Plants were inspected weekly for signs of downy mildew and symptoms of disease. The number of leaves with sporulation was counted. Plants were also rated for symptoms, with 5=no symptoms, 4=sporulation on leaf undersurface, 3=yellowing, 2=yellowing and defoliation and 1=complete defoliation and dying plant. At each rating, one plant from each replication of each treatment pre-marked for a particular harvest date was brought into the lab and one branch (one showing sporulation if available) was hand-sectioned at top, middle and base and examined microscopically for internal downy mildew structures.

Inspection 9 Sept, 13 days after exposure to inoculum (dai), showed no disease signs or symptoms in any treatment. Plants were all rated 5 for overall appearance (See Table 1 for data on plant ratings).

On 13 Sept (17 dai), sporulation was visible on all control plants and in some plants of the Alude and Micora treatments. Haustoria were visible in the stem at the top of all the inoculated control plants and in two reps of the Micora treatment. (see Table 2 for observations on downy mildew structures in the impatiens stems. Plants were all rated 4 or 5 for overall appearance on this date.

On 19 Sept (23 dai), following a period of cool night temperatures and low humidity, sporulation was evident on fewer leaves, but on the same treatments. Haustoria were visible in the upper stem tissue of the control and in all but three of the Micora treatments.

On 26 Sept (30 dai), sporulation was much more widespread than on the previous week. The first oospores were seen in one rep of the nontreated controls. Haustoria were seen in 3/6 of the Alude treated plants. The SubdueMAXX treatment continued to be free from signs of the disease (external or internal) and plants were all rated 5. This disease is so unique in that sporulation occurs before negative effects on the plant are evident. Inoculated controls were rated 3 on this date, as plants were showing both yellowing and defoliation.

On 4 Oct (38 dai), plant symptoms had progressed to include defoliation in nontreated controls. Oospores were seen in 6/6 of the inoculated control plants, and in 5/6 of the Micora treated plants. No symptoms or DM structures were seen in SubdueMAXX treatments, or in 3/6 of the Alude treated plants. Oospores in the controls were seen now in sections made mid-plant in 3/6 of the inoculated controls and in 2/6 of the Micora treated plants.

On 9 Oct (43 dai), all controls and all but one of the Micora treated plants were rated 1, indicated a severely diseased plant. Branches were harvested for sectioning, but there were no leaves left for sporulation observations in the controls or Micora treated plants. Haustoria and oospores were seen at top, middle and bottom of selected stems in all of the controls, and all but one of the Micora treated plants. No symptoms or structures were seen in the SubdueMAXX treated plants. Alude treated plants varied from 2 plants with no symptoms or signs and 4 that had 1-3 leaves with sporulation. These three had only haustoria (no oospores) at the top of the plant; 1/6 of the Alude-treated plants had no leaf sporulation, but haustoria were seen at the top of the plant.

On 17 Oct (51 dai), the Subdue MAXX treated plants still showed no symptoms. Oospores were in the tissue of all inoculated control and Micora treated plants, but none were seen in Alude treated plants.
On 8 Nov (73 dai) a final rating was taken on plants already rated, just after a light frost, on a second stem from the same plants tested 17 Oct. There were STILL no symptoms and no haustoria or oospores in the SubdueMAXX treated plants, but there were no leaves left on any of the other plants except for 3/6 of the Alude treated plants – the leaves remaining did not show sporulation. Alude treated plants were also interesting inthat the haustoria and oospores were seen only at the top section of the plant: they were never found in middle or bottom sections. Haustoria and oospores were present in top, middle and bottom sections of all control plants.

Conclusions: The protective effect of SubdueMAXX drench at the high labeled rate was extremely impressive on this obviously susceptible strain of *P. obducens*. Not only did plants fail to show sporulation during the 68 days of the trial (for 75 days after treatment), they also failed to show any internal structures of the pathogen. This was a consistent effect that was in sharp contrast to the inoculated controls, which deteriorated rapidly. Sporulation of the pathogen was uniformly apparent in the nontreated controls on Day 17. A few plants treated with Alude or Micora also showed sporulation on Day 17. Similarly, haustoria were seen for the first time on Day 17 in the controls, and in two of the Micora-treated plants. Micora appeared to have little effect on *P. obducens* infection in this trial, but treatment did delay oospore formation slightly. (The low effectiveness was surprising, and the material should be re-tested using a different batch of the fungicide). Symptoms, sporulation, and formation of both haustoria and oospores were slowed by Alude treatment, however. Intriguingly, there was more plant-to-plant variation seen in the Alude treatment than in the nontreated controls, Micora or SubdueMAXX treatments. This might indicate that fungicide was inadvertently distributed to the root system unevenly when it was applied, even though the dose per pot was the same. If the effect is highly dose-dependent and the critical dose is very close to the labeled rate, root systems receiving a more even distribution of the fungicide might result in healthier plants. Also, plants of slightly different size at time of treatment might respond differently to the same dose of fungicide. Because of reports of SubdueMAXX (mefenoxam) resistance in Florida, further study of the effects of phosphorous acid materials such as Alude may be the most fruitful for our improved understanding of how to manage downy mildew on impatiens with chemical plant protectants.

Table 71. Plant Health Rating on Impatiens Exposed to Inoculum on 27 Aug 2014[1]

Treatment	9 Sept	13 Sept	19 Sept	26 Sept	4 Oct	9 Oct	17 Oct	8 Nov
Nontreated	5.0	4.0	4.0	2.8	1.8	1.0	1.0	1.0
Alude	5.0	4.8	5.0	4.1	4.2	3.8	3.6	2.2
SubdueMAXX	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Micora	5.0	4.1	4.1	3.0	2.0	1.5	1.0	1.0

[1] Values are means of ratings of 6 plants for each treatment
[2] Plants rated on a 5-point scale: 5=healthy green, 4=green with sporulation, 3= some chlorosis, 2= chlorosis and some defoliation, and 1=completely defoliated plant.

Table 72. Observation of Internal Structures of Downy Mildew

Treatment	9 Sept	13 Sept	19 Sept	26 Sept	4 Oct	9 Oct	17 Oct	8 Nov
Nontreated	none	H 6/6	H 4/5	H 6/6 **OO 1/6**	H 6/6 **OO 6/6**	H 6/6 **OO 6/6**	H 6/6 **OO 6/6**	H 5/5 **OO 5/5**
Alude	none	none	none	H 2/6	H 3/6	H 2/6	H 5/6	H 6/6 **OO 5/6**
SubdueMAXX	none	none	none	none	none	none	none	none
Micora	none	H 2/6	H 3/6	H 5/6	H 5/6 **OO 5/6**	H 6/6 **OO 5/6**	H 6/6 **OO 6/6**	H 6/6 **OO 6/6**

[1] H=Haustoria, OO=Oospores observed in any of 3 sections made at top, middle and bottom of one stem per plant. Plants were different at each date except for the 17 Oct and 8 Nov ratings, which were made on the same plant roughly 3 weeks apart, at the end of the growing season.

Objective 3: Mitigation Strategies: Plant Impact

Plant Impact (Mit-MSU-3)

Michigan

Multiple product safety screens were conducted in the research greenhouses of Michigan State University to determine the effect of fungicide drenches on bedding impatiens. To determine if there was a varietal response to the fungicides, popular impatiens varieties Super Elfin XP, Dazzler, Impreza, and Accent Premium were included in each screen. As more growers have moved to soil drench applications for IDM control, the effect of soil type and its relation to the plant development issues from fungicides was previously unknown. Three substrate types were included in this study; Sunshine #1 Natural/Organic (70-80% Canadian Sphagnum peat moss), Sunshine-MVP/MM200 (50-60% vermiculite), and Farfard Mix #51 (60-65% composted pine bark). Fungicides tested in the various trials included Adorn (fluopicolide), Alude (phosphorous acid), oxathiapiprolin, and Subdue MAXX (mefenoxam). The Alude treatments included a NuFarm (53.6% a.i.) and Cleary (45.8% a.i.) formulation. These formulations were included based on perceived issues from greenhouse growers. The oxathiapiprolin product was tested at what they will believe be the lowest and highest rates on the eventual label. Tank mixes of Subdue MAXX + Adorn and Subdue MAXX + Alude were included as growers often apply these products together for extended disease control. Spray applications were not tested for safety as preliminary data has shown them to be safe on bedding impatiens. After the impatiens were transplanted into the various soil types, and fungicide drenches applied, the plants were rated for any signs of phytotoxicity. Plants were rated for health (1-5, 1=healthy, 5=plant death), plant height (cm), and the number of flowers. At the end of the study, fresh weight was collected by weighing (g) all above-ground plant materials.

An initial, large-scale experiment was completed in mid-Summer 2015. This experiment was conducted to collect preliminary data on how the relationship between substrate, cultivar, and fungicide would sort out. The results from this initial trial showed a strong negative correlation between the fluopicolide fungicide, the Sunshine vermiculite based substrate (Figure 100, Figure 101), and the Impreza series impatiens. Similar phytotoxicity was observed by commercial growersin MI. Phytotoxicity was not observed on any of the Alude (both formulations) treated plants. The number of flowers and flowering delay was not correlated to any product tested in the trial. A second followup experiment was completed that included Accent Premium White and Dazzler White impatiens; results mirrored those of the initial trials. Applications of Adorn to impatiens in the vermiculite based substrate resulted in smaller, distorted plants (Figure 101, Figure 102). The tank mixture of Adorn + Subdue MAXX resulted in more phytotoxicity when compared to either product applied alone (Figure 102).

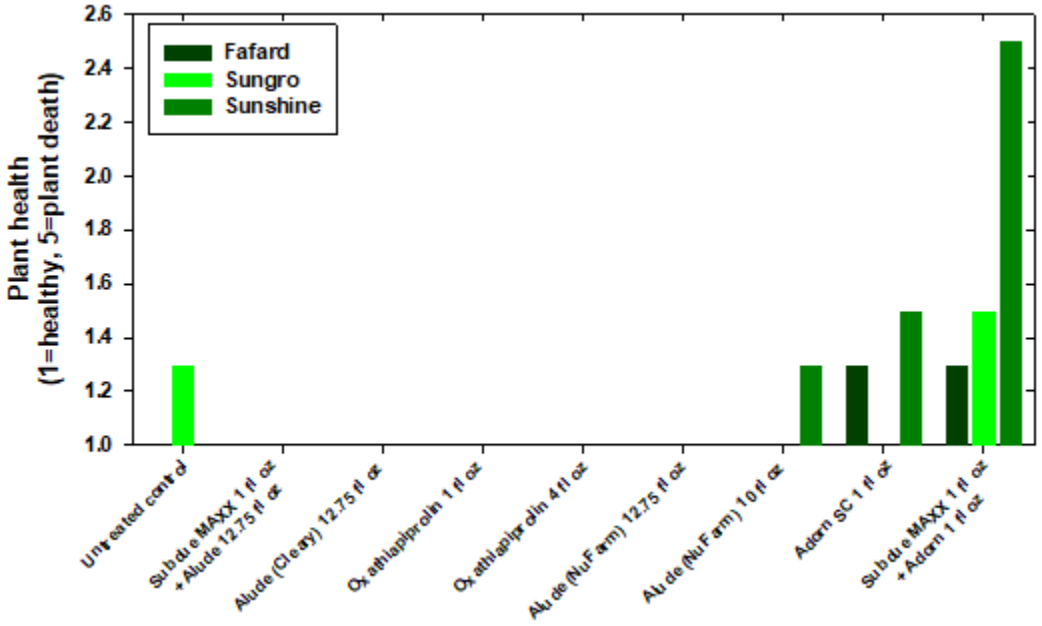
Figure 100. The relation between substrate type on phytotoxicity from mefenoxam + fluopicolide soil drenches, Harlan and Hausbeck, 2015.

Substrate type from left to right are; vermiculite based; peat moss based; and composted pine bark based.

Figure 101. Negative effect of the fungicide fluopicolide (Adorn) on the development of 'Impreza Red' impatiens, Harlan and Hausbeck, 2015.

Left, untreated 'Impreza Red' impatiens. Right, fluopicolide-treated, 'Impreza Red' impatiens.

Figure 102. Plant health of Accent Premium White impatiens in three soil types drenched with DM control fungicides, Harlan and Hausbeck, 2015.



To determine if Adorn would still have a place in IDM control programs, an experiment was initiated testing Adorn safety at a lower rate (0.5 fl oz) versus the currently used rate (1 fl oz). Applications of Adorn alone and in tank mixtures were included as previous studies concluded that phytotoxic effects were increased by when treatments were combined. This trial was initiated in Mid-July 2016 on four-week-old Accent Premium White seedlings in 4-in. pots. Applications of Adorn at 0.5 fl oz/100 gal resulted in some leaf distortion (Figure 103) and received a foliage health rating similar to plants treated with 1 fl oz of Adorn (Table 73). Interestingly, the 0.5 fl oz of Adorn when mixed with Alude (5 fl oz) resulted in statistically less phytotoxicity compared to the Adorn alone treatment. A drench application of Alude and spray applications of Adorn did not result in any foliar plant damage. In future recommendations to impatiens growers, they will advise only foliar applications of Adorn prior to flowering.

Table 73. Plant safety of Adorn at reduced rates on impatiens in the greenhouse, Harlan and Hausbeck, 2015.

Treatment and rate/100 gal (unless otherwise indicated), treated at 14-day intervals	Application Method	Foliar phyto severity		Avg. # flowers per plant		Flower phyto severity	
		12 Aug		12 Aug		12 Aug	
Untreated control		1.0	a	2.5		1.0	a
Adorn 0.5 fl oz	Drench	2.2	b	2.2		2.2	b
Adorn 1.0 fl oz	Drench	2.2	b	3.8		2.2	b
Adorn 0.5 fl oz + Alude 5.0 fl oz	Drench	1.3	a	7.0		1.5	ab
Adorn 1.0 fl oz + Alude 5.0 fl oz	Drench	2.8	b	1.5		2.0	b
Alude 5.0 fl oz	Drench	1.0	a	5.0		1.0	a
Adorn 4.0 fl oz	Spray	1.0	a	4.2	a	1.8	ab

*Rated on a scale of 1 to 5, where 1=no phytotoxicity to 5=plant death.
**Column means with a letter in common are not significantly different (Fisher LSD; *P*=0.05).

Figure 103. Adorn (0.5 fl oz/100 gal) drench applications resulted in some leaf distortion (right) compared to the untreated control (left)

Objective 3: Mitigation Strategies: Monitoring Resistance

Monitoring P. obducens resistance (Mit-UF-4, Mit-MSU-4)

Florida

2015. Impatiens downy mildew was not present during the Fall of 2014, so there were no efficacy trials until the disease appeared in the Spring of 2015. It's been since 2012 that we've evaluated mefenoxam in efficacy trials for impatiens downy mildew at the Tropical Research & Education Center, Homestead, FL. Each treatment was applied once on April 29 and impatiens were inoculated with *P. obducens* on May 2. Both spray and drench applications for all rates of Subdue Maxx provided control of impatiens downy mildew except disease rating values for the lowest rate of 0.5 and the 0.75 fl oz rate as a foliar spray were not statistically different from the controls. The lowest effective rate of Subdue Maxx was the 0.75 fl oz rate applied as a drench and the 1 or 2 fl oz rates applied as a drench or foliar spray provided an equal level of disease control.

Table 74. Subdue Maxx applications for controlling impatiens downy mildew

Treatment	Application	Severity [z]	Days until sporulation
Non-inoculated Control	---	45 a [y]	13
Inoculated Control	---	53 a	13
Subdue Maxx 0.5 fl oz/100 gal	Spray	37 a	13
Subdue Maxx 0.5 fl oz/100 gal	Drench	31 ab	13
Subdue Maxx 0.75 fl oz/ 100 gal	Spray	26 abc	13
Subdue Maxx 0.75 fl oz/ 100 gal	Drench	8 bcd	13
Subdue Maxx 1 fl oz/100 gal	Spray	12 d	20
Subdue Maxx 1 fl oz/100 gal	Drench	11 cd	20
Subdue Maxx 2 fl oz/100 gal	Spray	12 cd	20
Subdue Maxx 2 fl oz/100 gal	Drench	11 cd	20

[z] Percent canopy area affected as an average across all rating dates.
[y] Column means followed by the same letter are not statistically different (P=0.05) based on Tukey's HSD.

Michigan

2014. The discovery of an impatiens downy mildew isolate resistant to Subdue MAXX (mefenoxam) in Michigan caused great concern for growers as this product has been one of the most effective products available and the anchor of the control program recommended to growers. To determine if Subdue

MAXX should be included in the 2015 through 2018 IDM control program recommendations, the testing of whether isolates collected from 2014 through 2018 were sensitive became a high priority. Five IDM isolates were collected from Michigan landscapes in 2014. One of the locations included in the sampling was the site where the resistant isolate was collected in 2013. These isolates were separately propagated in the greenhouses on the campus of Michigan State University until enough inoculum could be produced for inoculation. To determine the sensitivity of each isolate to mefenoxam, a screen was conducted in the greenhouse using impatiens plants. For each isolate, six untreated impatiens, and six impatiens treated with Subdue MAXX (1 fl oz/100 gal; 24 hr prior to inoculation) were inoculated. After the infection and incubation period of the inoculation process was completed, the plants were place in containers at 100% humidity to induce sporulation. Of the five isolates tested, four were 100% susceptible to Subdue MAXX with no sporulation present at the end of the study (Table 75). Plants inoculated with the isolate collected from the site of the 2013 resistant isolate were the only ones with IDM sporulation.

Table 75. 2014 mefenoxam sensitivity testing, Harlan and Hausbeck.

Identification code	Collection date	County	Date inoculated	Date rated	Resistant to mefenoxam
IDM001	July 7, 2014	Ottawa	Aug 14, 2014	Aug 22, 2014	No
IDM002	Aug 26, 2014	Kent	Oct 15, 2014	Oct 31, 2014	No
IDM003	Sept 19, 2014	Ingham	Oct 15, 2014	Oct 31, 2014	No
IDM004	Sept 24, 2014	Ottawa	Oct 15, 2014	Oct 31, 2014	Yes
IDM005/IDM006	Sept 29, 2014	Kent	Oct 23, 2014	Nov 10, 2014	No

2015 and 2017. To determine if Subdue MAXX should be included in the 2016 IDM control program, the testing of all isolates collected in 2015 for resistance was a high priority. Four IDM isolates were collected from Michigan landscapes in 2015 and two in 2017 (Table 76). One of the locations included in the sampling was the site where the resistant isolate was collected in 2013 and 2014. These isolates were separately propagated in the greenhouses on the campus of Michigan State University until enough inoculum could be produced for inoculation. To determine the sensitivity of each isolate to mefenoxam, a screen was conducted in the greenhouse using impatiens plants. During 2015, four untreated impatiens, and four impatiens treated with Subdue MAXX (1 fl oz/100 gal; 24hr prior to inoculation) were inoculated with each isolate. During 2017, four untreated impatiens, and six impatiens treated with Subdue MAXX (1 fl oz/100 gal; 24hr prior to inoculation) were inoculated with each isolate. After the infection and incubation period of the inoculation process was completed, the plants were place in containers at 100% humidity to induce sporulation. All isolates tested in 2015 and 2017 were sensitive to mefenoxam. This was somewhat surprising as this testing included the isolate that was collected at a location with the isolate demonstrating resistance in 2013 and 2014

Table 76. 2015 and 2017 mefenoxam sensitivity testing, Harlan and Hausbeck.

Identification code	Collection Date	County	Resistant to mefenoxam
IDM007	Aug 26, 2015	Ingham	No
IDM008	Sept 22, 2015	Kent	No
IDM010	Oct 8, 2015	Kent	No
IDM011	Oct 13, 2015	Ottawa	No
IDM2017A	July 19, 2017	Kent	No
IDM2017B	Sept 13, 2017	Kent	No

Monitor P. cubensis resistance (Mit-MSU-5, Mit-NCSU-1)

Michigan

Single Product Fungicide Trial (Preventive Applications) in Michigan

Fungicides were tested to verify which products are working and to identify those that may no longer be effective. Although fungicides must be used in alternation in a commercial program to delay development of downy mildew resistance, testing products alone is needed to identify which products are effective enough to be included in a program. Seeds were planted on 19 July 2016. Fungicide treatments were applied ***preventively*** prior to disease development at 7-day intervals on 10, 17, 24, 31 August; and 7, 14 September. Priaxor SC was applied on 10, 17, 24, 31 August. Cucumber leaves were evaluated for downy mildew severity using the Horsfall-Barratt scale on 6, 9, 14, 19, 22 September (*only data from 6 and 22 September are shown*). Yields were not taken due to an uneven plant stand.

On 6 September, the untreated plants had a disease rating of 6.0 (6=25 to 50% disease) (Table 77). Five treatments protected plants effectively (rating <3.0: 3=3 to 6% disease) and included the following: Ranman SC, Zampro SC, V-10208 SC, Omega SC, Orondis Opti SC, and Orondis Ultra SC. The Orondis treatments resulted in healthy plants with no downy mildew evident at this early rating. By the end of the trial, the untreated plants were severely diseased (rating = 9.3; 9=87 to 94% disease). Three fungicide treatments were especially effective with ratings ≤3.5 (4=6 to 12% disease) and included Omega SC, Orondis Opti, and Orondis Ultra SC. In addition to the Orondis SC fungicides and Omega SC, other fungicides were proven to be helpful against downy mildew and included Ranman SC, Zampro SC, Gavel DF, V-10208 SC, and Bravo WeatherStik SC. Previcur Flex SL, Tanos DF, Revus SC, Priaxor SC, Cueva SC, Presidio SC, and Forum SC treatments resulted in high disease ratings (≥7.8). The last disease rating was one week after the final fungicide application with the exception of Priaxor SC that had been discontinued three weeks before the last rating. Although the fungicides including Tanos DF, Presidio SC and Previcur Flex SL have provided good downy mildew protection in the past, they did not protect the pickle crop from the disease in the 2016 campus trial. Results from 2016 are similar to results from the 2015 fungicide trial. However, Presidio SC is an effective fungicide for control of *Phytophthora* fruit rot, so its use in cucurbits is still recommended. Tanos DF can protect the crop from Alternaria leaf spot that can be a problem in hand-harvested pickle crops due to the extended time of harvesting.

Table 77. Foliar downy mildew severity of pickling cucumbers treated preventively with fungicides.

Treatment and rate/acre, applied at 7-day intervals	Disease severity*			
	9/6		9/22	
Untreated control	6.0	a**	9.3	a
Bravo WeatherStik SC 2 pt	3.3	de	6.0	de
Koverall DG 2 lb	5.3	a-c	7.5	c
Cueva SC 2 qt	5.5	ab	8.0	bc
Presidio SC 0.25 pt	4.5	bc	8.0	bc
Previcur Flex SL 1.2 pt	5.0	a-c	8.8	ab
Ranman SC 0.17 pt	2.3	e	4.3	fg
Zampro SC 0.88 pt	2.8	e	5.5	de
Gavel DF 2 lb	4.3	cd	5.0	ef
Tanos DF 0.5 lb	4.8	bc	7.8	bc
Curzate DG 5 oz	4.8	bc	8.3	a-c
Omega SC 1 pt	2.3	e	3.5	gh
Revus SC 8 fl oz	6.0	a	8.8	ab
Forum SC 6 fl oz	5.3	a-c	8.8	ab
Orondis Opti SC 34.2 fl oz	1.0	f	2.0	i
Orondis Ultra SC 9.64 fl oz	1.0	f	2.8	hi
V-10208 SC 8 fl oz	2.5	e	6.3	d
Priaxor SC 8 fl oz	5.3	a-c	8.0	bc

*Rated on the Horsfall-Barratt scale of 1 to 12, where 1=0% plant area diseased, 2=>0 to 3%, 3=>3 to 6%, 4=>6 to 12%, 5=>12 to 25%, 6=>25 to 50%, 7=>50 to 75%, 8=>75 to 87%, 9=>87 to 94%, 10=>94 to 97%, 11=>97 to <100%, 12=100% plant area diseased.

**Column means with a letter in common are not statistically different (LSD t Test; *P*=0.05).

Single Product Fungicide Trial (Rescue Applications) in Michigan

This trial was established using the same products included in the previous trial to determine which fungicides should be used when downy mildew develops in a field prior to any fungicide application. While 'rescue applications' are not optimal, there are instances when recommendations are needed for these situations. Seeds were planted on 25 July. Foliar fungicide sprays were applied to cucumber foliage ***after downy mildew symptom development***. Treatments were applied at 4-day intervals on 2, 7, 11, 15, 19, 23, 27 September. Cucumber leaves were evaluated for disease severity using the Horsfall-Barratt scale on 6, 19, 22, 26, 30 September and 4, 7, 11, 14 October (*data from selected dates are presented*). Yields were taken from the entire 20-foot row on 9, 16, 22 September (*sum of all harvests are shown*).

Disease was allowed to develop prior to the first fungicide application. On 6 September, all treatments including the untreated were uniformly diseased with a rating of 6.0-6.3 (6=25 to 50% disease). By 26 September, the untreated plants were rated at 9.8 (9=87 to 94% disease). Cueva SC, Previcur Flex SL, Presidio SC, and Forum SC were similar to the untreated plot. The Orondis treatments did not show any disease progression from the time that the sprays had begun and received a rating of 6.0 which was unchanged from the beginning of the spray program. While all other treatments were better than the untreated plots, most of the fungicides allowed significant disease development. On 7 October (10 days after last fungicide application), the Orondis treatments showed only limited disease progression with ratings of 6.3-6.5 and were the most effective fungicides. Plants treated with Ranman SC received a rating of 7.8 (7=50 to 75% disease). On 14 October (17 days after the last fungicide application), plots treated with either Orondis Opti SC or Orondis Ultra SC were similar to the V-10208 SC plot treatment and were the most effective treatments in limiting downy mildew disease. The untreated plot was almost entirely diseased (11.8; 12=100% disease) and treatments of Cueva SC, Presidio SC, Previcur Flex SC, Revus SC,

and Forum SC were similar to the untreated. All other treatments included in this study were better than the untreated control but still allowed unacceptable levels of disease to develop.

The untreated plot along with those treated with Cueva SC, Koverall DG, Bravo WS SC, Presidio SC, Previcur Flex SL, Gavel DF, Tanos DF, Curzate DF, Omega SC, Revus SC, and Forum SC treatments had the lowest total yields. The highest yields (>30 lb/plot) were harvested from plots treated with Ranman SC, Zampro SC, Orondis Opti SC, or Orondis Ultra SC. Although this trial provides helpful information to growers facing established downy mildew, fungicides are best applied preventively for maximum control and to delay the development of fungicide resistance to the downy mildew isolates.

Table 78. Foliar downy mildew severity and yield of pickling cucumbers treated with fungicides after disease was established.

Treatment and rate/acre, applied at 4-day intervals	Disease severity*						Total yield (lb)	
	9/26		10/7		10/14			
Untreated control	9.8	a**	10.8	a	11.8	a	17.7	f
Bravo WeatherStik SC 2 pt	7.8	e-h	8.8	d-g	9.0	c-e	23.2	b-f
Koverall DG 2 lb	8.5	c-e	8.8	d-g	9.3	cd	28.2	a-f
Cueva SC 1 qt	9.8	a	10.5	ab	11.5	a	19.0	ef
Presidio SC 0.25 pt	9.0	a-c	9.8	a-d	11.0	ab	25.1	b-f
Previcur Flex SL 1.2 pt	9.8	a	10.8	a	11.0	ab	22.2	c-f
Ranman SC 0.17 pt	7.3	gh	7.8	g	9.3	cd	38.4	a
Zampro SC 0.88 pt	7.5	f-h	8.8	d-g	9.5	cd	31.3	a-d
Gavel DF 2 lb	8.8	b-d	9.3	c-f	10.0	bc	22.9	b-f
Tanos DF 0.5 lb	8.3	c-f	8.5	e-g	10.0	bc	26.9	b-f
Curzate DG 5 oz	8.0	d-g	9.0	d-f	10.0	bc	25.5	b-f
Omega SC 1 pt	8.3	c-f	9.0	d-f	9.3	cd	21.9	c-f
Revus SC 8 fl oz	8.3	c-f	9.5	b-e	10.8	ab	24.4	b-f
Forum SC 6 fl oz	9.5	ab	10.3	a-c	11.3	a	20.4	def
Orondis Opti SC 34.2 fl oz	6.0	i	6.3	h	8.0	ef	33.8	ab
Orondis Ultra SC 9.64 fl oz	6.0	i	6.5	h	7.8	f	32.5	a-c
V-10208 SC 8 fl oz	7.0	h	8.3	fg	8.5	d-f	29.6	a-e

*Rated on the Horsfall-Barratt scale of 1 to 12, where 1=0% plant area diseased, 2=>0 to 3%, 3=>3 to 6%, 4=>6 to 12%, 5=>12 to 25%, 6=>25 to 50%, 7=>50 to 75%, 8=>75 to 87%, 9=>87 to 94%, 10=>94 to 97%, 11=>97 to <100%, 12=100% plant area diseased.

**Column means with a letter in common are not statistically different (LSD t Test; *P*=0.05).

North Carolina.

The experiment was conducted at the Cunningham Research Station in Kinston, NC (N35°18.172'; W077°34.341'). Plots were single 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. The previous year the field was planted with sweet potato. Cucumber was direct seeded on 10 Aug (2-ft in-row spacing, 2 seed/hill) in raised beds and thinned to one plant per hill after emergence (7 plants/plot). Three non-treated commercial cucumber varieties were included in the trial in addition to non-treated Vlaspik and Vlaspik treated with different fungicide applications. Irrigation and fertilization were applied via drip tape. Treatments were randomized into four complete blocks. Fungicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with hollow cone nozzles (TXVS-26) delivering 40 gal/A at 45 psi. The first three spray applications were made with a single-nozzle boom and the last one with a 2-nozzle boom (19-in. spacing). Applications were made on: 6, 14 and 23 Sep. and 3 Oct. Disease severity was assessed on 20 Sep and 3 Oct as percent leaf area with necrosis per plot. Fruit were harvested on 20 and 27 Sep and 4 Oct. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means.

Downy mildew was first detected on 6 Sep at approximately 3% disease severity in the field and progressed throughout the course of the trial (Table 79). Hurricane Matthew impacted Eastern North Carolina on 8 and 9 Oct and prematurely ended the trial due to wind/rain damage. Non-treated Peacemaker, Orondis Opti A and Non-treated Citadel controlled downy mildew well and produced the greatest weight of total marketable fruit. Ranman also managed *P. cubensis* when compared to the Non-treated Vlaspik. No other treatments provided commercially acceptable levels of disease control. No phytotoxicity was observed.

Table 79. Fungicide sensitivity of *P. cubensis* on cucumber.

Treatment and rate of product per acre	Application No.	Disease Severity* (%)		Mkt Yield (lb/plot)
		20-Sep	3-Oct	
Non-treated Peacemaker	N/A	9.8 h**	20.5 h	22.53 a
Orondis Opti A 0.83 SC 2 fl oz	1-4	14.5 g	26.5 h	13.24 b
Non-treated Citadel	N/A	16.3 fg	28.5 h	10.27 bc
Ranman 3.33 SC 2.75 fl oz Induce SL 0.25% V/V	1-4 1-4	16.5 fg	38.0 g	8.71 cd
Curzate 60 DF 5 oz	1-4	12.0 gh	41.0 fg	8.08 cde
Previcur Flex 6 F 19.2 fl oz	1-4	23.0 cde	43.0 efg	7.28 c-f
Gavel 75 WG 32 oz	1-4	23.0 cde	45.0 d-g	6.35 c-f
Presidio 4 SC 4 fl oz Induce SL 0.25% V/V	1-4 1-4	16.0 fg	46.5 c-f	8.19 cde
Manzate Pro Stick75 DG 48 oz	1-4	24.3 b-e	47.5 c-f	6.15 c-f
V-10208 4 SC 10 fl oz	1-4	20.3 ef	48.3 c-f	8.65 cd
Omega 500 F 24 fl oz	1-4	21.3 de	49.0 c-f	5.31 def
Zampro 4.33 SC 14 fl oz Induce SL 0.25% V/V	1-4 1-4	21.8 de	49.3 c-f	7.65 c-f
Revus 2.08 SC 8 fl oz	1-4	21.8 de	49.5 b-e	5.97 def
Tanos 50 WG 8 oz	1-4	22.3 de	50.8 b-e	7.31 c-f
Zing! 4.9 SC 36 fl oz	1-4	23.3 cde	51.8 bcd	6.59 c-f
Merivon 42.5 SC 6.7 fl oz	1-4	27.0 abc	52.0 bcd	6.02 def
Forum 43.5 SC 6 fl oz	1-4	23.5 cde	52.8 bcd	5.22 def
Bravo Weather Stik 6 SC 48 fl oz	1-4	23.3 cde	54.0 bc	8.73 cd
Ridomil Gold Bravo36.4 SC 40 fl oz	1-4	22.5 cde	54.8 bc	4.77 def
Cabrio 20 EG 12 oz	1-4	25.8 a-d	57.8 ab	5.79 def
Non-treated Expedition	N/A	28.5 ab	64.3 a	4.15 ef
Non-treated Vlaspik	N/A	29.5 a	64.3 a	3.71 f

* Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.

** Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Waller-Duncan k=100).

Outreach and Publications

Refining Best Management Practices

Based on the results presented here, management recommendations were provided to growers to improve production of cucurbits and impatiens. Not all technical bulletins and presentations may be listed below.

Scientific Abstracts/Posters

1. Cano, L. M., Withers, S., Gent, D., Noel, N., Quesada-Ocampo, L. M. 2015. Discovery of effectors in the hop downy mildew pathogen *Pseudoperonospora humuli*. Phytopathology 105: in press.

2. D'Arcangelo K., Miles T., and Quesada-Ocampo L. M. Occurrence of fungicide resistance in *Pseudoperonospora cubensis* populations causing cucurbit downy mildew in commercial and wild hosts. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
3. Eskandari, F and Shishkoff N. 2017. Systemic infection of Impatiens balsamina through inoculation of roots with vegetative sporangia of the Impatiens downy mildew (Plasmopara obducens) Phytopathology 107:S4.1.http://dx.doi.org/10.1094/PHYTO-107-7-S4.1
4. Granke, L., Morrice, J., VanOverbeke, M., Carnago, G., Pianosi, A., and Hausbeck, M.K. 2013. Influence of environmental factors on aerial concentrations of *Pseudoperonospora cubensis* sporangia and cucumber downy mildew disease severity. Phytopathology 103(Suppl. 2):S2.52.
5. Harlan, B. and M. Hausbeck. 2015. Epidemiology and Management of Impatiens Downy Mildew in the United States, (abstract approved) , GreenSys2015 International Symposium on New Technologies and Management for Greenhouses, Plant Protection Session, Evora, Portugal, July 2015.
6. Johnson, K. and Shishkoff, N. 2017. The Overwintering of Plasmopara obducens (Impatiens Downy Mildew). (Abstr.) Phytopathology 107:S4.1. http://dx.doi.org/10.1094/PHYTO-107-7-S4.1
7. Johnson, K. E., Shishkoff, N. 2017. Monitoring Impatiens Beds for Overwintering of *Plasmopara obducens* (Impatiens Downy Mildew) and Development of a Baiting Assay for Soil" Potomac Division 73rd Annual Meeting, March 22 - 24, 2017, Lakeview Golf Resort and Spa, Morgantown, WV
8. Keach, J., Daughtrey, M., Bridgen, M. and Salgado-Salazar, C. 2016. Susceptibility of Impatiens species to downy mildew caused by *Plasmopara obducens*. (Abstr.) Phytopathology 106:S2.3. http://dx.doi.org/10.1094/PHYTO-106- 4-S2.3
9. Keach, J., Daughtrey, M., Bridgen, M., Salgado-Salazar, C. 2016. Susceptibility of *Impatiens* species to downy mildew caused by *Plasmopara obducens* (Abstr.). Phytopathology 106:S2.3. http://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-106-4-S2.1
10. Naegele R. P., Kurjan J., Quesada-Ocampo L. M., and Hausbeck M. K. (2014) Population structure of *Pseudoperonospora cubensis* in Michigan and Canada. Cucurbitaceae, Bay Harbor, MI, October 2014.
11. Naegele R. P., Kurjan J., Quesada-Ocampo L. M., and Hausbeck M. K. (2014) Population structure of *Pseudoperonospora cubensis* in Michigan and Canada. Proceedings of Cucurbitaceae 2014. Pages 45-47.
12. Naegele R. P., Kurjan J., Quesada-Ocampo L. M., and Hausbeck M. K. (2014) Temporal changes in *Pseudoperonospora cubensis* field populations in Michigan cucumber. Phytopathology 104: S3.84.
13. Naegele, R. P., Quesada-Ocampo, L. M., Kurjan, J., Saude, C., and Hausbeck, M. K. 2015. Spatiotemporal population structure of *Pseudoperonospora cubensis* isolates in Michigan and Ontario, Canada. Phytopathology 105: in press.
14. Neufeld, K. N., Quesada-Ocampo, L. M., and Ojiambo, P. S. 2015. Development of 5' nuclease probes for detection and quantification of *Pseudoperonospora cubensis* sporangia. Phytopathology 105: S2.8.
15. Quesada-Ocampo, L. M., Withers, S., Gongora-Castillo, E., Gent, D. H., and Ojiambo, P. S. 2015. Using next-generation sequencing to develop species-specific molecular diagnostics for cucurbit downy mildew. Phytopathology 105: in press.
16. Shands A., Wallace E., Miles T., and Quesada-Ocampo L. M. Detection of fungicide resistant *Pseudoperonospora cubensis* isolates using novel molecular tools. Oomycete Molecular Genetics Network Meeting, Pacific Grove, CA, March 2017.
17. Shishkoff, N. 2016. Evidence points to homothally in the Impatiens downy mildew. (Abstr.) Phytopathology 106:S3.1.http://dx.doi.org/10.1094/PHYTO-106-S-S3.1
18. Shishkoff, N. 2018. Systemic spread of Plasmopara obducens in Impatiens plants with roots exposed to sporangia or oospores. https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-108-10-S1.1

19. Shishkoff, N. and Eskandari, F. 2016. Germination of oospores of Plasmopara obducens and characterization of megasporangia. (Abstr.) Phytopathology 106:S3.1.http://dx.doi.org/10.1094/PHYTO-106-S-S3.1
20. Shishkoff, Nina, 2016. Evaluating nine boxwood cultivars for susceptibility to *Calonectria pseudonaviculata* and *C. henricotiae.* APS Annual Meeting. Tampa, FL. August 6 – 10, 2016.
21. Shishkoff, Nina. 2016. Evidence points to homothally in the Impatiens downy mildew. APS Potomac Division Meeting. Richmond VA. March 23-25, 2016.
22. Shishkoff, Nina. 2016. Germination of oospores of *Plasmopara obducens* and characterization of its megasporangia. APS Potomac Division Meeting. Richmond VA. March 23-25, 2016.
23. Wallace E. C. and Quesada-Ocampo L. M. (2014) *In silico* identification and analysis of microsatellite location and frequency in downy mildew transcriptomes. Phytopathology 104: S3.123.
24. Wallace, E. C. and Quesada-Ocampo, L. M. 2015. Validation of transcript SSR markers in *Pseudoperonospora cubensis* from commercial and non-commercial cucurbits. Phytopathology 105: in press.
25. Withers S., Gongora-Castillo E., Bowman M. J., Childs K., Gent D. H., Ojiambo P. S., and Quesada-Ocampo L. M. (2014) Developing genomic resources for species-specific molecular diagnostics of cucurbit downy mildew. Phytopathology 104: S3.130.

Oral Scientific Research Presentations

1. Cano L. M., Withers S, Gent D., Bowman M., Childs K. and Quesada-Ocampo L. M. Genome sequence and effector repertoire of the hop downy mildew pathogen *Pseudoperonospora humuli*. APS Meeting, Tampa, FL, August 2016.
2. Cano, L. M., Withers, S., Gent, D. H., and Quesada-Ocampo, L. M. 2015. Embracing discovery of effectors in the hop downy mildew plant pathogen. Oomycete Molecular Genetics Network Meeting, Pacific Grove, CA, March.
3. Cano, L. M., Withers, S., Gent, D., Noel, N., and Quesada-Ocampo, L. M. 2015. Discovery of effectors in the hop downy mildew pathogen *Pseudoperonospora humuli*. APS Meeting, Pasadena, CA, August.
4. Crouch, J.A. 2016. Where are all these newly emergent downy mildew diseases coming from? University of Maryland, Department of Plant Science and Landscape Architecture, College Park, MD, 25-February-2016.
5. Crouch, J.A. 2018. Emerging downy mildews: The same old pathogens or new enemies? International Congress of Plant Pathology, 6th International Oomycetes Workshop, Boston, MA, July 28, 2018.
6. Crouch, J.A. 2018. Where are all these newly emergent downy mildew diseases coming from? PROCINORTE International, Plant Health Task Force: Beyond Boundaries to Secure Plant Health and Productivity, Beltsville, Maryland, October 11-13, 2017.
7. Crouch, JA. (2014) "Where in the world are all these downy mildews coming from? 19th Ornamental Workshop on Diseases and Pests, Henderson, NC, 30-Sept-2014.
8. Crouch, JA. (2014) Genomes for the rest of us! American Phytopathological Society Potomac Division Meeting, March 12-14, 2014, Annapolis, MD.
9. Daughtrey, M. 2018. Impatiens downy mildew host range. 2018 Boxwood Blight/Downy Mildew Meeting, BARC, Beltsville, MD Feb. 21, 2018
10. Daughtrey, M. 2018. Production experiments with impatiens downy mildew. 2018 Boxwood Blight/Downy Mildew Meeting, BARC, Beltsville, MD Feb. 21, 2018
11. Daughtrey, M. The Role of Oospores in Impatiens Downy Mildew Outbreaks. (with Nina Shishkoff). North Carolina Ornamentals Workshop. Hendersonville, NC. October 26, 2016.
12. Daughtrey, M. Why It Isn't Safe For Pears, Impatiens or Boxwood Any More. Seminar, Department of Plant Pathology, North Carolina State University, Raleigh, NC. September 28, 2016.
13. Hausbeck, M. 2015. How can they minimize our losses? Control strategies in downy mildew systems. APS Meeting, Pasadena, CA, August.

14. Hausbeck, M.K., L.L. Granke and R.P. Naegele, '*Pseudoperonospora cubensis* sporangia trapping as a tool for early detection and epidemiological studies of downy mildew in Michigan,' Cucurbitaceae 2014, Bay Harbor, MI, Oct 2014.
15. Keach, J. E., Daughtrey, M. L., Bridgen, M. P. and Salgado-Salazar, C. Susceptiblity of *Impatiens* species to downy mildew caused by *Plasmopara obducens*. Northeastern Plant, Pest and Soils Conference. Philadelphia, PA. January 4, 2016.
16. Mandal M. K., Ikerd J., Wallace E., Turecheck W., Grace R., Quesada-Ocampo L. M. and Kousik C.S. 2017. Population biology of the downy mildew pathogen on tolerant and susceptible cucumber in the Southeastern United States. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
17. Palmateer, A.J. 2013. Impatiens downy mildew: Management options for the greenhouse, nursery, and landscape industries. Annual meeting of the American Phytopathological Society, Austin, TX. Abstract and Oral Presentation.
18. Palmateer, A.J. 2013. Managing impatiens downy mildew in the greenhouse and landscape. May 7th, Florida Phytopathological Society, Fort Lauderdale, FL. Oral Presentation
19. Quesada-Ocampo L. M. 2016. Next generation sequencing to develop molecular diagnostics for *Pseudoperonospora cubensis*. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
20. Quesada-Ocampo L. M. 2016. Using genomics approaches for rapid development of species-specific diagnostics for cucurbit downy mildew. Canadian Phytopathological Society Annual Meeting, Biovigilance Symposium, Moncton, Canada, June 2016.
21. Quesada-Ocampo L. M. 2016From the field to the lab and back: translational strategies to improve disease control in vegetable crops. VIB-PSB-NC State Plant Sciences Workshop, Ghent, Belgium, May 2017.
22. Quesada-Ocampo L. M. Improving detection and control of cucurbit downy mildew through research and extension. Plant Pathology Society of North Carolina Meeting, Raleigh, NC, October 2014.
23. Quesada-Ocampo, L. M., Withers, S., Gongora-Castillo, E., Gent, D. H., and Ojiambo, P. S. 2015. Using next-generation sequencing to develop species-specific molecular diagnostics for cucurbit downy mildew. APS Meeting, Pasadena, CA, August.
24. Rahman A. and Quesada-Ocampo L. M. 2016. Early detection and quantification of *Pseudoperonospora cubensis* airborne sporangia using real-time PCR. APS Meeting, Tampa, FL, August 2016.
25. Rahman A. and Quesada-Ocampo L. M. Early detection and quantification of *Pseudoperonospora cubensis* airborne sporangia using real-time PCR. APS Meeting, Tampa, FL, August 2016.
26. Rahman A., Martin F., Shands A., Miles T., and Quesada-Ocampo L. M. 2017. Using comparative genomics to develop biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Oomycete Molecular Genetics Network Meeting, Pacific Grove, CA, March 2017.
27. Rahman A., Wallace E., Crouch J., Martin F., and Quesada-Ocampo L. M. 2017. Unravelling historical shifts in *Pseudoperonospora cubensis* populations in the US that resulted in the 2004 cucurbit downy mildew epidemic. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
28. Ramon M. L., Martin F. N., and Quesada-Ocampo L. M. 2017. TaqMan assay for differentiation of *Pseudoperonospora humuli* and *P. cubensis* host specialized clades. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
29. Rivera, Y, Malapi-Wight, M, Ismaiel, A, Crouch, JA. 2014. Droplet digital PCR detection of aneuploidy in *Plasmopara obducens* causing impatiens downy mildew disease. American Phytopathological Society Potomac Division Meeting, March 12-14, 2014.
30. Rivera, Y, Salgado-Salazar, C, Gulya, T and Crouch JA. 2016. Emergent rudbeckia downy mildew epidemics caused by *Plasmopara halstedii* are genetically distinct from the pathogen populations

infecting sunflower. American Phytopathological Society Potomac Meeting, March 23-25, 2016, Richmond, VA.

31. Rivera, Y. 2014. Downy mildews on ornamental crops. University of Maryland Invasive Species Conference, March 24, 2014.
32. Rivera, Y., Malapi-Wight, M., Ismaiel, A. and Crouch, JA. (2014) Impatiens under attack: Genetic variability of the oomycete pathogen *Plasmopara obducens* in the US 3rd Annual BA-UMD Fall Symposium, Trends in Agriculture, January 23, 2014, Beltsville, MD.
33. Rivera, Y., Salgado-Salazar, C., Ismaiel, A., Saied, N. and Crouch, JA. (2014) Droplet digital PCR detection of aneuploidy in *Plasmopara obducens* causing impatiens downy mildew disease. Annual Beltsville Area Poster Day, USDA-ARS, April 21, 2014, Beltsville, MD.
34. Rivera, Y., Salgado-Salazar, C., Ismaiel, A., Saied, N. and Crouch, JA. 2014. Droplet digital PCR detection of aneuploidy in *Plasmopara obducens* causing impatiens downy mildew disease. American Phytopathological Society Potomac Division Meeting, March 12-14, 2014, Annapolis, MD.
35. Salgado, CS, Malapi-Wight, M, Rivera, Y, Crouch, JA. 2014. Next-generation sequencing technology for the study of newly emergent fungal and oomycete pathogens of ornamental crops. VIII Latin American Mycological Conference, November 4-7, 2014, Medellin, Colombia.Salgado-Salazar, C., Rivera, Y., Ismaiel, A., Saied, N. and Crouch, JA. (2014) Genome sequencing of *Plasmopara obducens*, the causal agent of impatiens downy mildew, for microsatellite marker development. 25th Annual Beltsville Area Poster Day, USDA-ARS, April 21, 2014, Beltsville, MD.
36. Salgado, CS, Rivera, Y, and Crouch, JA. 2014. Genome sequencing of *Plasmopara obducens*, the causal agent of impatiens downy mildew, for microsatellite marker development. American Phytopathological Society Potomac Division Meeting, March 12-14, 2014.
37. Salgado-Salazar, C, Rivera, Y, and Crouch, JA. 2016. Changes in *Plasmopara obducens* population structure corresponded with the emergence and rapid spread of the impatiens downy mildew epidemics. American Phytopathological Society Potomac Meeting, March 23-25, 2016, Richmond, VA.
38. Salgado-Salazar, C., Rivera, Y., Ismaiel, A., Saied, N. and Crouch, JA. 2014. Genome sequencing of *Plasmopara obducens*, the causal agent of impatiens downy mildew, for microsatellite marker development. 25th Annual Beltsville Area Poster Day, USDA-ARS, April 21, 2014, Beltsville, MD.
39. Salgado-Salazar, C., Rivera, Y., Ismaiel, A., Saied, N. and Crouch, JA. 2014. Genome sequencing of *Plasmopara obducens*, the causal agent of impatiens downy mildew, for microsatellite marker development. American Phytopathological Society Potomac Division Meeting, March 12-14, 2014, Annapolis, MD.
40. Shands A., Wallace E., Miles T., and Quesada-Ocampo L. M. Detection of fungicide-resistant *Pseudoperonospora cubensis* isolates using molecular techniques. NCSU Undergraduate Research Symposium. Raleigh, NC, August 2016.
41. Shishkoff, N. 2018. “Understanding the life cycle of the Impatiens downy mildew and its implications”. Symposium talk at the Potomac Division meeting of the American Phytopathological Society in Ocean City, MD, March 21-23.
42. Shishkoff, N., and Smallwood, E. 1017. The Impatiens downy mildew project: Citizen Scientists figure it out! Spring Research Festival, Fort Detrick, MD. May 2-3, 2016.
43. Suarez, S. N., Lopez, P., Chase, A. R., and Palmateer, A. J. 2017. Preserving efficacy of new FRAC group from resistance development in impatiens downy mildew. American Phytopathological Society Annual Meeting, San Antonio, TX, August 2017.
44. Suarez, S. N., Lopez, P., Chase, A. R., and Palmateer, A. J. Preventive fungicide applications in production and their impact on residual efficacy against impatiens downy mildew in the landscape. American Phytopathological Society Annual Meeting. Tampa, FL, July 30-August 3, 2016.
45. Walker A., Rahman A. and Quesada-Ocampo L. M. Phylogenetic relationships of *Pseudoperonospora cubensis* isolates causing downy mildew of cucurbit crops in the United States. NCSU Undergraduate Research Symposium. Raleigh, NC, August 2016.

46. Wallace E. C. and Quesada-Ocampo L. M. 2016. *Pseudoperonospora cubensis* on commercial and non-commercial cucurbits in North Carolina: population structure determine by simple sequence repeats (SSRs). APS Meeting, Tampa, FL, August 2016.
47. Wallace E. C. and Quesada-Ocampo L. M. 2017. Examining the population structure of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis,* by host, location, and time. American Phytopathological Society Potomac Division Meeting, Morgantown, WV, March 2017.
48. Wallace E. C. and Quesada-Ocampo L. M. Genetic structure of *Pseudoperonospora cubensis* populations infecting commercial and non-commercial cucurbits in North Carolina. XIth Eucarpia Cucurbitaceae Meeting, Warsaw, Poland, July 2016.
49. Wallace, E. C., and Quesada-Ocampo, L. M. 2015. Validation of transcript SSR markers in *Pseudoperonospora cubensis* from commercial and non-commercial cucurbits. APS Meeting, Pasadena, CA, August.
50. Withers S., Gongora-Castillo E., Gent D., Ojiambo P., and Quesada-Ocampo L. M. (2015) Developing species-specific molecular diagnostics for cucurbit downy mildew. Oomycete Molecular Genetics Network Meeting, Pacific Grove, CA, March 2015.
51. Yamagata J., Rahman A. and Quesada-Ocampo L. M. Detection of airborne *Pseudoperonospora cubensis* sporangia using spore trapping and quantitative PCR. NCSU Undergraduate Research Symposium. Raleigh, NC, August 2016.

Scientific Publications

1. Cespedes-Sanchez, M.C., Naegele, R.P., Kousik, C.S., and Hausbeck, M.K. 2015. Field response of cucurbit hosts to *Pseudoperonospora cubensis* in Michigan. Plant Disease 99:676-682.
2. Chase, A. R., Daughtrey, M. and Cloyd, R. 2018. Compendium of Bedding Plant Diseases and Pests. American Phytopathological Society, St. Paul, MN 170 pp.
3. Cohen, Y., Van den Langenberg, K. M., Wehner, T., Ojiambo, P., Hausbeck, M., Quesada-Ocampo, L. M., Lebeda, A., Sierotzki, H., and Gisi U. 2015. Resurgence of *Pseudoperonospora cubensis* – the agent of cucurbit downy mildew. Phytopathology 105: 998-1012.
4. Crandall S. G., Rahman A., Quesada-Ocampo L. M., Martin F. N., Bilodeau G. J., and Miles T. D. (201X) Advances in diagnostics of downy mildews: lessons learned from other oomycetes and future challenges. Submitted.
5. Crouch, JA, Ko, MP and McKemy, J. (2014) First report of impatiens downy mildew caused by *Plasmopara obducens* in the Hawai'ian islands. *Plant Disease* 98(5):696.
6. Granke, L.L., Morrice, J.J., and Hausbeck, M.K. 2014. Relationships between airborne *Pseudoperonospora cubensis* sporangia, environmental conditions, and cucumber downy mildew severity. Plant Disease 98:674-681.
7. Harlan, B.R., Granke, L., and Hausbeck, M.K. 2016. Epidemiology and management of impatiens downy mildew in the United States. Acta Horticulturae (in press).
8. Hausbeck, M.K., Naegele, R.P., and Granke, L.L. 2014. *Pseudoperonospora cubensis* sporangia trapping as a tool for early detection and epidemiological studies of downy mildew in Michigan. Proceedings of Cucurbitaceae 2014, 12-16 Oct, Bay Harbor, MI, pp. 41-44.
9. Holmes, G., Ojiambo, P., Hausbeck, M., Quesada-Ocampo, L. M., and Keinath, A. P. 2015. Resurgence of cucurbit downy mildew in the United States: A watershed event for research and extension. Plant Disease 99: 428-441.
10. Naegele R. P., Quesada-Ocampo L. M., Kurjan J. D, Saude C., and Hausbeck M. K. (2016) Regional and temporal population structure of *Pseudoperonospora cubensis* in Michigan and Ontario. Phytopathology 106: 372-379.
11. Naegele, R.P., Hausbeck, M.K., Quesada-Ocampo, L.M., and Saude, C. 2014. Population structure of *Pseudoperonospora cubensis* in Michigan and Canada. Proceedings of Cucurbitaceae 2014, 12-16 Oct, Bay Harbor, MI, pp. 45-47.

12. Ojiambo, P. S., Gent, D. H., Quesada-Ocampo, L. M., Hausbeck, M. K., and Holmes, G. J. 2015. Epidemiology and population biology of *Pseudoperonospora cubensis*: A model system for management of downy mildews. Annual Review of Phytopathology 53:223-246.
13. Palmateer, A.J., T.E. Seijo, P. Lopez, and N.A. Peres. 2013. Severe outbreak of downy mildew caused by *Plasmopara obducens* on *Impatiens walleriana* in Florida. Plant Disease 97(5): 687. http://dx.doi.org/10.1094/PDIS-08-12-0705-PDN
14. Rivera, Y, Rane, K and Crouch, JA. 2014. First report of Rudbeckia downy mildew caused by *Plasmopara halstedii* in Maryland. *Plant Disease 98(7): 1005.*
15. Rivera, Y, Salgado-Salazar, C, Creswell, T, Ruhl, G, Crouch, JA. (2016) First report of downy mildew caused by *Peronospora* sp. on *Agastache* in the United States. *Plant Disease*: In press: doi.org/10.1094/PDIS-10-15-1119-PDN.
16. Rivera, Y, Salgado-Salazar, C, Gulya, T and Crouch JA. (2016) Newly emerged populations of *Plasmopara halstedii* infecting rudbeckia exhibit unique genotypic profiles and are distinct from sunflower-infecting strains. *Phytopathology*: In press: doi.org/10.1094/PHYTO-12-15-0335-R.
17. Rivera, Y, Salgado-Salazar, C, Windham, AS, Crouch, JA. (2016) Downy mildew on coleus (*Plectranthus scutellarioides*) caused by *Peronospora belbahrii sensu lato* in Tennessee. *Plant Disease*: 100(3)655.
18. S.G. Crandall, A. Rahman, L.M. Quesada-Ocampo, F.N. Martin, G.J. Bilodeau, T.D. Miles. 2017. Advances in diagnostics of downy mildews: lessons learned from other oomycetes and future challenges: in press.
19. Suarez, S. N., Lopez, P., Chase, A. R., and Palmateer, A. J. 2016. Evaluating Fungicides in Production for Long Term Management of Impatiens Downy Mildew. Proc. Fla. State Hort. Soc. (In Press).
20. Thomas A., Carbone I., Choe K., Quesada-Ocampo L. M., and Ojiambo P. 2017. Resurgence of cucurbit downy mildew in the United States: Insights from comparative genomic analysis of *Pseudoperonospora cubensis*. Ecology and Evolution: in press.
21. Wallace E and Quesada-Ocampo L. M. 2017. Analysis of microsatellites from the transcriptome of downy mildew pathogens and their application for characterization of *Pseudoperonospora* populations. PeerJ 5: e3266.
22. Wallace E. and Quesada-Ocampo L. M. (201X) Population structure and host adaptation of *Pseudoperonospora* populations infecting commercial and wild cucurbit hosts in North Carolina. Submitted.
23. Wallace E., Adams M., and Quesada-Ocampo L. M. (2015) First report of downy mildew on buffalo gourd (*Cucurbita foetidissima*) caused by *Pseudoperonospora cubensis* in North Carolina. Plant Disease: in press.
24. Wallace E., Adams M., Ivors K., Ojiambo P., and Quesada-Ocampo L. M. (2014) First report of *Pseudoperonospora cubensis* causing cucurbit downy mildew on *Momordica blasamina* and *M. charantia* in North Carolina. Plant Disease 98: 1279.
25. Wallace E., Choi Y. J., Thines M., and Quesada-Ocampo L. M. (2016) First report of *Plasmopara* aff. *australis* on *Luffa cylindrica* in the United States. Plant Disease 100: 537.
26. Wallace, E., Adams, M., and Quesada-Ocampo L. M. 2015. First report of downy mildew on buffalo gourd (*Cucurbita foetidissima*) caused by *Pseudoperonospora cubensis* in North Carolina. Plant Disease 99:1861.
27. Withers S., Gongora-Castillo E., Gent D., Thomas A., Ojiambo P., and Quesada-Ocampo L. M. 2016. Using next-generation sequencing to develop molecular diagnostics for *Pseudoperonospora cubensis*, the cucurbit downy mildew pathogen. Phytopathology 106: 1105-1116.

Plant Disease Management Reports

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

2. Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Kinston 2015. PDMR 10: V086.
3. Adams M. L. and Quesada-Ocampo L. M. 2017. Evaluation of cultivars and fungicides for control of downy mildew on cucumber, Clinton 2016. Plant Disease Management Reports: V098.
4. Adams M. L. and Quesada-Ocampo L. M. 2017. Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2016. Plant Disease Management Reports: V100.
5. Adams M. L. and Quesada-Ocampo L. M. 2017. Evaluation of fungicides for control of downy mildew on cucumber, Kinston II 2016. Plant Disease Management Reports: V096.
6. Adams M. L. and Quesada-Ocampo L. M. 2017. Evaluation of fungicides and cultivars for control of downy mildew on cucumber, Kinston 2016. Plant Disease Management Reports: V097.
7. Adams M. L., Noel N. A., and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2015. PDMR 10: V084.
8. Adams M. L., Parada C. H., and Quesada-Ocampo L. M. (2015) Evaluation of fungicides for control of downy mildew on cucumber, Kinston II 2014. PDMR: in press.
9. Adams, M. L., and Quesada-Ocampo, L. M. 2015. Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2014. PDMR 9: V087.
10. Adams, M. L., and Quesada-Ocampo, L. M. 2015. Evaluation of fungicides for control of downy mildew on cucumber, Cleveland 2014. PDMR 9: V086.
11. Adams, M. L., Parada, C. H., and Quesada-Ocampo, L. M. 2015) Evaluation of fungicides for control of downy mildew on cucumber, Kinston II 2014. PDMR 9: V085.
12. Adams, M. L., Parada, C. H., and Quesada-Ocampo, L. M. 2015. Evaluation of fungicides for control of downy mildew on cucumber, Kinston I 2014. PDMR 9: V081.
13. Harlan, B.R., and Hausbeck, M.K. 2013. Control of impatiens downy mildew with fungicide drenches and sprays, 2012. Plant Disease Management Reports 7:OT031. Online.
14. Harlan, B.R., and Hausbeck, M.K. 2013. Evaluation of fungicide drenches and sprays for control of downy mildew of impatiens, 2012. Plant Disease Management Reports 7:OT033. Online.
15. Harlan, B.R., and Hausbeck, M.K. 2013. Evaluation of registered fungicides for impatiens downy mildew control, 2012. Plant Disease Management Reports 7:OT032. Online.
16. Harlan, B.R., and Hausbeck, M.K. 2014. Evaluation of drench and/or spray applications of experimental fungicides for the control of downy mildew of bedding impatiens in the greenhouse, 2013. PDMR 8:OT018.
17. Harlan, B.R., and Hausbeck, M.K. 2014. Evaluation of fungicide applications for the control of downy mildew of impatiens, 2013. PDMR 8:OT019.
18. Harlan, B.R., and Hausbeck, M.K. 2014. Evaluation of greenhouse fungicide applications for the control of downy mildew of impatiens in the landscape, 2013. PDMR 8:OT017.
19. Harlan, B.R., and Hausbeck, M.K. 2014. Evaluation of two experimental fungicides and a biopesticide for the control of downy mildew of bedding impatiens in a greenhouse, 2013. PDMR 8:OT020.
20. Hausbeck, M.K. 2014. Evaluation of registered and unregistered fungicides for control of downy mildew of pickling cucumber, 2013. PDMR 8:V303.
21. Hausbeck, M.K., and Harlan, B.R. 2016. Greenhouse evaluation of experimental fungicides for the control of Botrytis blight on geranium, 2016. PDMR 10: (need OT number).
22. Hausbeck, M.K., and Linderman, S.D. 2014. Evaluation of fungicides for control of downy mildew of cucumber, 2013. PDMR 8:V304.
23. Rahman A., Miles T. D., Martin F. N., and Quesada-Ocampo L. M. 2017. Molecular approaches for development of biosurveillance tools for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Canadian Journal of Plant Pathology: in press.
24. Suarez, S. N. and Palmateer, A. J. (2017) Evaluation of fungicides for control of downy mildew of impatiens, 2015. Plant Disease Management Reports 11:OT024.

Oral Extension Presentations

1. Adams M. L. and Quesada-Ocampo L. M. Cucurbit downy mildew management in the greenhouse. 42nd Annual Conference of the North Carolina Greenhouse Vegetable Growers Association. Greensboro, NC, October 2014.
2. Adams M. L. and Quesada-Ocampo L. M. Cucurbit downy mildew resistance to fungicides in the field. 28th annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2013.
3. Adams M. L. and Quesada-Ocampo L. M. Disease management in vegetable production. Certified Crop Advisor Training. Jacksonville, NC, December 2014.
4. Adams M. L. and Quesada-Ocampo L. M. Evaluation of fungicides for control of cucurbit downy and powdery mildew in NC. Winter Vegetable Conference. Asheville, NC, February 2014.
5. Adams M. L. and Quesada-Ocampo L. M. Identification and management of cucurbit and tomato diseases. PLANT Farm Enterprise Incubator. Hillsborough, NC, February 2015.
6. Adams M. L. and Quesada-Ocampo L. M. Identification and management of cucurbit and tomato diseases. Northeast Agriculture Agents Association Meeting. Plymouth, NC, December 2014.
7. Adams M. L. and Quesada-Ocampo L. M. Identification and management of cucurbit and tomato diseases. PLANT Farm Enterprise Incubator. Hillsborough, NC, March 2014.
8. Adams M. L. and Quesada-Ocampo L. M. Vegetable disease control methods. Vegetable Growers Program. Reidsville, NC, February 2015.
9. Adams, M. L. and L.M. Quesada-Ocampo. Cucurbit downy mildew resistance to fungicides in the field. 28th annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2013.
10. Adams, M. L., and Quesada-Ocampo, L. M. 2015. Identification and management of cucurbit and tomato diseases. PLANT Farm Enterprise Incubator. Hillsborough, NC, February.
11. Adams, M. L., and Quesada-Ocampo, L. M. 2015. Vegetable disease control methods. Vegetable Growers Program. Reidsville, NC, February.
12. Crouch, JA and Salgado-Salazar, C. 2016. Understanding the emergence of impatiens downy mildew disease using molecular markers, genomics and herbarium collections. Ball Horticultural Company, West Chicago, IL, February 15, 2016.
13. Daughtrey, M. (2014) Division of Plant Industry, Horticultural Inspector Training, NYSDAM, Colonie, NY. Diseases they Need to be Ready For and How to Handle Them. April 3, 2014. (34 people) (3 hrs)
14. Daughtrey, M. (2014) Enhanced First Detector Training for New York, NPDN, Great River, NY. Scouting Walk. June 19, 2014. (30 people) (0.75 hr)
15. Daughtrey, M. (2014) GardenTalks Lecture Series. Cornell Cooperative Extension of Westchester County. Scarsdale Library, Scarsdale, NY. Flourishing Gardens vs. Plant Diseases and Pests. April 16, 2014. (110 people) (2 hrs).
16. Daughtrey, M. (2014) Morris County Parks Commission. Frelinghuysen Arboretum, Morris Township, NJ. Changing Climates, Changing Gardens: Protecting Plants in the 21st Century. April 17, 2014. (35 people)(2 hrs)
17. Daughtrey, M. (2014) Sustainable Landscape Horticulture Program Work Team, online webinar, Ithaca, NY. Downy Mildew and Other Current Disease Problems. (10 people) (0.5 hr)
18. Daughtrey, M. (2014) The Garden Conservancy. Rocky Hill Lecture Series. Chappaqua Library, Chappaqua, NY. Bees, Trees and Berries. April 16, 2014 (100 people) (1 hr).
19. Daughtrey, M. 2013. Changing Climates, Changing Gardens, one-hour presentation at the Chappaqua Library, Chappaqua, NY 10/30/13.
20. Daughtrey, M. 2013. Global Gardening: Protecting Plants in the 21st Century, one-hour presentation at the Organic and Sustainable Gardening in a Warmer World Conference, Ithaca, NY 10/5/13
21. Daughtrey, M. 2013. Landscape Diseases: The Least-Wanted List, one-hour presentation at the New York State Nursery and Landscape Association Leader's Forum in Ithaca, NY 10/23/13
22. Daughtrey, M. 2013. Outwitting the Diseases of Herbaceous Perennials and Annuals, one-hour presentation to Master Gardeners and the public at the Long Island Fall Gardening Symposium, Bridgehampton, NY 9/21/13.

23. Daughtrey, M. 2013. Presentation with Colleen Warfield, Ball Horticultural Company. Impatiens Downy Mildew: Are they Any Closer to Control? 75-minute presentation to growers at the OFA Short Course, Columbus, OH, 7/12/13.
24. Daughtrey, M. 2013. Rusty, Sandy and Downy Blights and Blasts to Challenge Your Gardens. 45 minute presentation at the Southampton Garden Club, Southampton, NY 6/15/13
25. Daughtrey, M. 2014 Hudson Valley Nursery and Greenhouse Growers School. Cornell Cooperative Extension. Middletown, NY. Coping with the Top Two Diseases for Annuals, Herbaceous Perennials, Shrubs and Trees, January 29, 2014. (60)
26. Daughtrey, M. 2017. Downy Mildew Update. Cultivate'17. Columbus, OH, 15 July 17.
27. Daughtrey, M. 2017. Pear Trellis Rust and Updates on Impatiens Downy Mildew, Boxwood Blight and Oak Wilt. LIAA General Membership Meeting, Polish Hall, Riverhead, NY, November 14, 2017.
28. Daughtrey, M. 2017. Research on Impatiens Downy Mildew. Cornell University Long Island Horticultural Research & Extension Center Plant Science Day, 11 July 17.
29. Daughtrey, M. 2017. Using Disease Management Tools: Getting the Most Out of Fungicides and Biologicals: Choices and Rotations. UConn Program: Keeping Those Dratted Diseases Out of Your Crops. New Haven, CT, 21 September 17.
30. Daughtrey, M. 2018. Beware the Blights! 2018 Hudson Valley Nursery & Greenhouse Growers; School, Cornell Cooperative Extension of Orange and Ulster Counties. Feb 27
31. Daughtrey, M. 2018. IDM and Its Cousins. Long Island Greenhouse Conference, Cornell Cooperative Extension of Suffolk County, Riverhead, NY April 30, 2018
32. Daughtrey, M. 2018. New bothers for NY ornamentals in landscape and nursery. 2018 Plant WNY Education Day, Buffalo, NY Feb. 1, 2018
33. Daughtrey, M. 2018. New Bothers. Long Island Nursery and Landscape Association Meeting. Polish Hall, Riverhead, NY Feb 16, 2018
34. Daughtrey, M. 2018. Spot spotting. Plant Health Virtual Conference. University of Massachusetts Webinar. Feb 14, 2018
35. Daughtrey, M. 2018. Understanding ornamental plant diseases. Cornell Cooperative Extension of Suffolk County Master Gardener Training, Riverhead, NY Feb 11 2018
36. Daughtrey, M. and Wallace, E. 2017. Managing Maniacal Mildews, Both Powder and Down. UConn Program: Keeping Those Dratted Diseases Out of Your Crops. New Haven, CT, 21 September 17.
37. Daughtrey, M. Annuals and Perennials: Disease Management for Low-Input Landscapes. New York State Turf and Landscape Association. Upsky, NY. December 1, 2016.
38. Daughtrey, M. Capital District Bedding Plant Conference and Trade Show. Cornell Cooperative Extension of Albany, Schenectady, Fulton/Montgomery, Greene, Columbia and Washington Counties. Latham, NY. No Impatiens Downy Mildew This Year? Now What Do they Do? January 28, 2014. (80)
39. Daughtrey, M. CENTS. Ohio Nursery and Landscape Assn and Ohio State University. Down With Impatiens Downy Mildew! January 13, 2014. (150)
40. Daughtrey, M. Cornell Gardeners Seminar Series. Long Island Horticultural Research & Extension Center, Riverhead, NY. Disastrous Diseases, February 25, 2014. (20)
41. Daughtrey, M. Diseases of Ornamental Plants, Certified Nursery and Landscape Professional Training, Holtsville, NY, March 3, 2015
42. Daughtrey, M. Diseases of Ornamental Plants. Master Gardener Training of Putnam, Westchester and Dutchess Counties, Carmel, NY. October 5, 2016.
43. Daughtrey, M. Diseases of Ornamental Plants. Master Gardener Training of Rockland County, Stony Point, NY. October 6, 2016.
44. Daughtrey, M. Diseases to deal with in cut flower crops. 2017 Long Island Agricultural Forum, Speonk, NY, January 12, 2017.

45. Daughtrey, M. Diseases they Saw in 2016. Long Island Floriculture Conference. Riverhead, NY, January 17, 2017.
46. Daughtrey, M. Disgusting Diseases: Don't Let Them Lower Your Flower Power, Farmingdale State University, Farmingdale, NY, March 25, 2015
47. Daughtrey, M. Dodging Downy Mildews. SAF 2015 Pest and Production Management Conference, Feb. 19-21, Orlando, FL. 137 pp.
48. Daughtrey, M. Downy Mildew Diseases on Flower Crops. Greenhouse Session, Mid-Atlantic Fruit and Vegetable Conference, Hershey, PA, February 1, 2017.
49. Daughtrey, M. Education Conference, Western New York State Nursery and Landscape Assn. Buffalo, NY, Flower Power Reduction? Impatiens Downy Mildew and Rose Rosette. February 7, 2014. (120)
50. Daughtrey, M. Facing Five Fierce Foes of Ornamental Plants, Cornell Alumni Association, Long Island Chapter. Hicks Nursery, Hicksville, NY. January 24, 2015.
51. Daughtrey, M. Keeping Diseases Out. 2017 Pesticide Applicator Update. Cornell University, Ithaca, NY, March 30, 2017.
52. Daughtrey, M. Keeping Those Dratted Diseases Out of Your Greenhouse Crops. Chesapeake Green, Maryland Nursery and Landscape Association, Linthicum, MD, February 24, 2017
53. Daughtrey, M. Mildew Madness. Commercial Flower Growers of Wisconsin 2016 Fall Greenhouse Conference. Denmark, Wisconsin. October 19, 2016.
54. Daughtrey, M. Pitting Your Wits Against Perennial Pathogens: Disease control in herbaceous perennials. Chesapeake Green, Maryland Nursery and Landscape Association, Linthicum, MD, February 23, 2017
55. Daughtrey, M. Plant Pathology for Ornamental Plants. Westhampton Beach AP Biology Class. Cornell University Long Island Horticultural Research and Extension Center. October 17, 2016.
56. Daughtrey, M. Plant Pathology in the Springtime. Commercial Flower Growers of Wisconsin 2016 Fall Greenhouse Conference. Denmark, Wisconsin. October 19, 2016.
57. Daughtrey, M. Research on Diseases of Floriculture Crops. Cornell University Long Island Horticultural Research & Extension Center, Riverhead, NY. September 14, 2016.
58. Daughtrey, M. SAF Pest and Production Management Conference. Society of American Florists. San Diego, CA. What Turned the Bedding Plant Industry Topsy-Turvy: Impatiens Downy Mildew. February 22, 2014. (100)
59. Daughtrey, M. Spring Diseases. Greenhouse Session, Mid-Atlantic Fruit and Vegetable Conference, Hershey, PA, February 1, 2017.
60. Daughtrey, M. The Role of Oospores in Impatiens Downy Mildew Outbreaks. With Nina Shishkoff. North Carolina Ornamentals Workshop. Hendersonville, NC. October 26, 2016.
61. Daughtrey, M. Those Dratted Downy Mildews! Iowa State University Short Course. Ames, Iowa, November 4, 2016.
62. Daughtrey, M. Wave Hill 2014 Horticultural Lecture Series. New York School of Interior Design, Manhattan, NY. Disastrous Diseases, February 19, 2014. (150)
63. Daughtrey, M. What's New and How Do they Stop It: Disease Management on Flowers and Herbs in the Greenhouse. 2016 Northeast Greenhouse Conference. November 10, 2016.
64. Daughtrey, Margery (2015) Diagnosis and IPM for Diseases of Ornamentals. Central Park Conservancy. NY, NY. Aug 2015.
65. Daughtrey, Margery (2015) Disease Walkabout. Cornell Floriculture Field Day, Ithaca, NY. Aug 2015.
66. Daughtrey, Margery (2015) Impatiens Downy Mildew: Is it Safe Yet? Far West Show. The Solution Center. Portland, OR. Aug 15.
67. Daughtrey, Margery (2015) Ornamental Disease Research. Long Island Horticultural Research & Extension Center Plant Science Day, Riverhead, NY, July 2015.
68. Daughtrey, Margery (2015) Stumpers and crop jumpers. Penn. State Flower Trials Field Day, Manheim PA, July 2015.

69. Daughtrey, Margery. 2017. Current Disease Challenges: Oak Wilt, SOD, Box Blight, Pear Rust and Impatiens Downy Mildew. Long Island Horticultural Society, Planting Fields Arboretum, Oyster Bay, NY, June 25, 2017.
70. Daughtrey, Margery. 2017. Diseases of Landscape Ornamentals. HOR 228, Current Hort Topics: Insects and Diseases of Lawns and Gardens, Farmingdale State University, Farmingdale, NY. April 3, 2017.
71. Daughtrey, Margery. 2017. Which Blight is Which? Nursery Greenhouse Seminar. CAPCA, Escondido, CA, June 7, 2017.
72. Daughtrey, Margery. Dealing with Dastardly Diseases. Pesticide Recertification Day, Cornell Cooperative Extension, Capital District. Latham, NY. March 22, 2016.
73. Daughtrey, Margery. Downy Mildews: Old Pathogens with Increasing Impact. Academy of Crop Production. University of Georgia, Athens, GA, June 14, 2016.
74. Daughtrey, Margery. Gruesome Garden Diseases and Mindful Management Strategies. 2015 Turf and Grounds Exposition, NYS Turf Association, Rochester, NY November 18, 2015.
75. Daughtrey, Margery. Most Important Disease Problems Confronting New York. New York State Department of Agriculture and Markets, Colonie, NY. February 24, 2016.
76. Daughtrey, Margery. News You Can Use. Long Island Greenhouse & Floriculture Conference, Riverhead, NY. January 19, 2016.
77. Daughtrey, Margery. Ornamental Plant Diseases. Certified Nursery and Landscape Professionals Training, New York State Nursery and Landscape Association, Holtsville, NY. February 11, 2016
78. Daughtrey, Margery. Research on Impatiens Downy Mildew. Wageningen Glastuinbouw, Wageningen University/DLO Foundation. Bleiswijk, The Netherlands. January 25, 2016.
79. Daughtrey, Margery. Rounding Up Rusts and Battling Blights. Professional Turf and Landscape Conference, New York State Turf and Landscape Association, Yonkers, NY. January 13, 2016
80. Harlan, B. and M. Hausbeck, 'Downy mildew of impatiens,'Greenhouse Session, Great Lakes Fruit, Vegetable and Farm Market Expo/Michigan Greenhouse Growers Expo, Grand Rapids, MI, Dec 2014.
81. Harlan, B. and M. Hausbeck, 'Epidemiology and Management of Impatiens Downy Mildew in the United States,'GreenSys2015 International Symposium on New Technologies and Management for Greenhouses, Plant Protection Session, Evora, Portugal, July 2015.
82. Harlan, B. and M. Hausbeck. Downy mildew of impatiens. Floriculture Growers' Meeting, Allendale, MI, Mar 2014.
83. Harlan, B., and Hausbeck, M. 2015. Epidemiology and management of impatiens downy mildew in the United States. GreenSys2015 International Symposium on New Technologies and Management for Greenhouses, Plant Protection Session, Evora, Portugal, July.
84. Harlan, B., and Hausbeck, M. 2015. Epidemiology and management of impatiens downy mildew in the United States. GreenSys2015 International Symposium on New Technologies and Management for Greenhouses, Plant Protection Session, Evora, Portugal, July.
85. Harlan, B., and Hausbeck, M. 2015. Greenhouse Disease Control Update. Great Lakes Greenhouse Expo, Grand Rapids, MI, December.
86. Harlan, B., and Hausbeck, M. 2015. MSU Floriculture Research Update – Impact of Downy Mildew Mitigation Strategies on Growth and Development of Bedding Impatiens. Great Lakes Greenhouse Expo, MSU Research Floriculture Update Session, Grand Rapids, MI, December.
87. Harlan, B., and Hausbeck, M. 2017. Managing greenhouse pathogens: Starting clean and finishing healthy. Ag Action Meeting, Kalamazoo, 13 Jan.
88. Harlan, B., and Hausbeck, M. 2017. Vegetable diseases and control strategies. Michigan Agribusiness Association Meeting Lansing, 11 Jan.
89. Hausbeck M. K. and Quesada-Ocampo L. M. Downy mildew and fungicide resistance - What works? Chicago, IL, October 2013.
90. Hausbeck, M. 'Fun with plant pathology,' DeWitt Millenium Garden Club, DeWitt, MI, 4 Sep 2014.

91. Hausbeck, M. 'Progress in cucumber disease control,'Pickling Cucumber Session, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec 2014.
92. Hausbeck, M. 'Research advances in controlling fruit rot and downy mildew,' Pickle Packers International Inc. Spring Meeting, Dearborn, MI, 22-24 Apr 2014.
93. Hausbeck, M. 2015. Downy mildew, *Pseudoperonospora cubensis*. Emerging Diseases Seminar, Michigan State University, East Lansing, MI, Apr.
94. Hausbeck, M. 2015. Managing fungal leaf and stem pathogens. GrowPro Series, Pittsburgh, PA, Sep.
95. Hausbeck, M. 2015. Managing fungal leaf and stem pathogens. GrowPro Series, Pittsburgh, PA, Sep.
96. Hausbeck, M. 2016. Downy mildew research and recommendations. Saginaw Branch/Women's National Farm and Garden Association Meeting, Saginaw, MI, 14 Jun.
97. Hausbeck, M. 2017. A smorgasbord of vegetable diseases is on today's menu. MSU Extension and AgBioResearch State Council Meeting, Lansing, MI, Mar.
98. Hausbeck, M. and L. Quesada, 'Downy mildew and fungicide resistance – what works?' Pickle Packers International, Inc. Annual Meeting, Chicago, IL, Oct 2013.
99. Hausbeck, M. K. and L.M. Quesada-Ocampo. Downy mildew and fungicide resistance - What works?. Chicago, IL, October 2013.
100. Hausbeck, M. Progress in cucumber disease control. Growers' Meeting, Bay City, MI, Feb 2014.
101. Hausbeck, M., and B. Harlan. Downy mildew of impatiens. Floriculture Growers' Meeting, Kalamazoo, MI, Feb 2014.
102. Hausbeck, M., and B. Harlan. Downy mildew of impatiens. Floriculture Growers' Meeting, Grand Rapids, MI, Jan 2014.
103. Hausbeck, M., and B. Harlan. Downy mildew of impatiens. Floriculture Growers' Meeting, Comstock, MI Jan 2014.
104. Hausbeck, M., and B. Harlan. New tools and advances to battle the mildews. Horticulture Growers' Short Course, Pacific Agriculture Show, Abbotsford, British Columbia, Canada, Jan 2014.Hausbeck, M. Rots and blights of vegetables. Horticulture Growers' Short Course, Pacific Agriculture Show, Abbotsford, British Columbia, Canada, Jan 2014.
105. Hausbeck, M., and Goldenhar, K. 2017. Updates on cucurbit downy mildew in the upper Midwestern region. Wisconsin Fresh Fruit and Vegetable Conference, Wisconsin Dells, WI, 23 Jan.
106. Hausbeck, M., and Harlan, B. 2015. Flower diseases in the greenhouse. Greenhouse Growers' Meeting, Dalton, OH, Jan.
107. Hausbeck, M., and Harlan, B. 2016. Downy mildew of impatiens (and other diseases). Costa Greenhouse Growers Meeting, Ft. Lauderdale, FL, Sep.
108. Hausbeck, M., and Harlan, B. 2016. Rots and spots: Keep them out of your greenhouse. Greenhouse Growers' Meeting, Kalamazoo, MI, 6 Oct.
109. Hausbeck, M., and Harlan, B. 2016. Rots and spots: Keep them out of your greenhouse. Winfield Academy Meeting, Minneapolis, MN, 3-4 Oct.
110. Dudek, T., and Hausbeck, M. 2016. MSU research brings healthy impatiens back to home gardeners. MSU Extension News for Agriculture-Floriculture: 29 Feb.
111. Hausbeck, M., and Harlan, B. 2016. Rots and spots: Keep them out of your greenhouse. Greenhouse Growers' Meeting, Charlotte, NC, Oct.
112. Hausbeck, M., and Harlan, B. 2016. Rots and spots: Keep them out of your greenhouse. Greenhouse Growers' Meeting, Asheville, NC, Oct.
113. Hausbeck, M.K., L.M. Quesada-Ocampo and R.P. Naegele, 'Downy mildew populations: Changing with the times,'Great Lakes Pickle Group Meeting, Grand Rapids, MI, Dec 2014.
114. Meadows I., Mauney C., and Quesada-Ocampo L. M. 2016. Agent training on disease diagnostics and management in vegetable crops. Extension Conference. Raleigh, NC, November 2016.
115. Palmateer, A.J. 2013. Downy mildew of impatiens. Miami Green Bytes (Spring Edition). Miami Dade County Extension. UF IFAS.

116. Palmateer, A.J. 2013. Impatiens downy mildew. Greater Plains Diagnostic Network Webinar Series. Invited Speaker.
117. Palmateer, A.J. 2013. Impatiens downy mildew: a challenge for the ornamental plant industry. December 11th, Michigan Greenhouse Expo, Grand Rapids, MI. Proceedings Paper and Invited Speaker.
118. Palmateer, A.J. 2013. Recent outbreak of impatiens downy mildew in the United States. April 30th, Central Plant Board, Manhattan, KS. Invited Speaker.
119. Palmateer, A.J. 2013. Update on managing downy mildew of impatiens in the nursery and landscape. November 22nd, Lee County Extension, Ft. Myers, FL. Invited Speaker.
120. Palmateer, A.J. 2013. Update on managing downy mildew of impatiens in the nursery and landscape. November 20th, Palm Beach County Extension, West Palm Beach, FL. Invited Speaker.
121. Palmateer, A.J. 2013. Update on managing downy mildew of impatiens in the nursery and landscape. September 4th, Naples Botanical Garden, Naples, FL. Invited Speaker.
122. Palmateer, A.J. 2014. Ornamental plant disease update and management for greenhouse, nursery and landscapes. March 18th, Tropical Research & Education Center, University of Florida, Homestead, FL. Presentation and tour of impatiens downy mildew nursery and landscape demonstration trials. 38 participants.
123. Palmateer, A.J. 2014. Update on diseases affecting ornamental nursery crops and landscapes in Florida. March 11th, Collier County Chapter of the Florida Nursery Growers and Landscape Association, Naples, FL. Presentation. 62 participants.
124. Palmateer, A.J. 2014. Update on managing impatiens downy mildew in the nursery and landscape. March 21st, Florida Nursery Growers & Landscape Association tour for landscape architects, Tropical Research & Education Center, University of Florida, Homestead, FL. Presentation. 50 participants.
125. Palmateer, A.J. Contrast and Control Strategies for Foliar and Crown/Root Diseases. BASF Ornamental Meeting. Mobile, AL, June 28, 2016.
126. Palmateer, A.J. Contrast and Control Strategies for Foliar and Crown/Root Diseases. BASF Ornamental Meeting. McMinnville, TN, June, 30, 2016.
127. Palmer, C.L. 2014. Impatiens Downy Mildew: Description of a Devastating Disease & Hope for the Future. NJ Nursery & Landscape Association NJ Plants Annual Trade Show. January 2014.
128. Quesada-Ocampo L. M. 2016. Diagnosis and management of cucurbit diseases. Cucurbit field day. Cleveland, NC, September 2016.
129. Quesada-Ocampo L. M. 2016Disease identification on vegetables. Certified Crop Advisor Training. Smithfield, NC, December 2016.
130. Quesada-Ocampo L. M. 2016Fungicides and host resistance for cucurbit downy mildew management. 31st Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2016.
131. Quesada-Ocampo L. M. 2016Management of cucumber downy mildew using fungicides and host resistance. Pickle Packers International Annual Meeting. Charleston, SC, October 2016.
132. Quesada-Ocampo L. M. and Hausbeck M. K. Improving detection and control of cucurbit downy mildew. Pickle Packers International Annual Meeting. Chicago, IL, October 2013.
133. Quesada-Ocampo L. M. Control options for cucurbit downy mildew. 29th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2014.
134. Quesada-Ocampo L. M. Cucurbit downy mildew management, diagnostics, and pathogen populations. Pickle Packers International Spring Meeting. Raleigh, NC, April 2016.
135. Quesada-Ocampo L. M. Downy mildew and Phytophthora control in cucurbits. NC Watermelon Convention. Wrightsville Beach, NC, March 2016.
136. Quesada-Ocampo L. M. Downy mildew updates for cucurbits. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, January 2016.
137. Quesada-Ocampo L. M. Expanding your toolbox for cucurbit downy mildew detection and control. Pickle Packers International Annual Meeting. Orlando, FL, October 2014.

138. Quesada-Ocampo L. M. Identification and management of cucurbit diseases. North Carolina State University Extension Conference. Raleigh, NC, November 2013.
139. Quesada-Ocampo L. M. Improving detection and control of vegetable diseases in North Carolina. The Sainsbury Laboratory, Norwich, England, July 2014.
140. Quesada-Ocampo L. M. Integrating field and genomic strategies to keep North Carolina vegetables healthy. AgBiome, Durham, NC, February 2016.
141. Quesada-Ocampo L. M. Management of cucurbit downy mildew and tomato late blight. Winter Vegetable Conference. Asheville, NC, February 2015.
142. Quesada-Ocampo L. M. Managing downy mildew on hops in the south. South Atlantic Hops Conference. Richmond, VA, March 2016.
143. Quesada-Ocampo L. M. Organic management of foliar vegetable diseases in high tunnels. High Tunnel Production Workshop. Pittsboro, NC, March 2014
144. Quesada-Ocampo, L. M. 2015. Diagnostics and management of cucurbit downy mildew. Pickle Packers International Annual Meeting. Fort Worth, TX, October.
145. Quesada-Ocampo, L. M. 2015. Diagnostics and management of cucurbit downy mildew. Pickle Packers International Annual Meeting. Fort Worth, TX, October.
146. Quesada-Ocampo, L. M. 2015. Management of cucurbit downy mildew and tomato late blight. Winter Vegetable Conference. Asheville, NC, February.
147. Quesada-Ocampo, L.M. and M. K. Hausbeck. Improving detection and control of cucurbit downy mildew. Pickle Packers International Annual Meeting. Chicago, IL, October 2013.
148. Quesada-Ocampo, L.M. and M. L. Adams. Control of cucurbit diseases. Cucurbit field day. Laurel Springs, NC, September 2013.
149. Quesada-Ocampo, L.M. and M. L. Adams. Cucurbit downy mildew: Prepare, predict, prevent. Tomato and vegetable field day. Mills River, NC, August 2013.
150. Quesada-Ocampo, L.M. and M. L. Adams. Fungicide resistance of cucurbit downy mildew in the field. Cucurbit downy mildew field day. Clinton, NC, October 2013.
151. Quesada-Ocampo, L.M. and M. L. Adams. Management of cucurbit downy mildew. Mountain Research Station field day. Waynesville, NC, July 2013.
152. Quesada-Ocampo, L.M. Identification and management of cucurbit diseases. North Carolina State University Extension Conference. Raleigh, NC, November 2013.
153. Quesada-Ocampo, L.M. Updated recommendations for management of cucurbit and sweetpotato diseases. Southeastern Extension Vegetable Workers Annual Meeting. Mills River, NC, August 2013.
154. Rivera, Y and Crouch, JA (2014) Downy Mildews on Ornamental Crops. 2014 Invasive Species Program. March 25, 2014, Baltimore County Center for Maryland Agriculture, Cockeysville, MD.
155. Rivera, Y and Crouch, JA. (2014) Downy Mildews on Ornamental Crops. Central Jersey Turf & Ornamental Institute. March 6, 2014, Manalapan, N.J.
156. Rivera, Y. 2014. Downy mildews on ornamental crops. Central Jersey Turf and Ornamental Institute, Rutgers University/NJAES, March 6, 2014.
157. Rivera, Y. 2014. Impatiens downy mildew and other disease management issues. New Jersey Nursery and Landscape Association June 18, 2014.
158. Rivera, Y., Malapi-Wight, M., Ismaiel, A. and Crouch, JA. (2014) Application of droplet digital PCR technology for *in situ* genotypic analysis of plant pathogen populations. Proceedings of the 23rd Annual Rutgers Turfgrass Symposium, B. Fitzgerald and T. Molnar, eds., page 50.
159. Shishkoff, N. 2019. Impatiens downy mildew: what to do now, what to expect in the future. Presentation at the Indiana Green Expo, Jan 9-11.
160. Wallace E. C. and Quesada-Ocampo L. M. (2014) *Pseudoperonospora cubensis* populations in North Carolina: Microsatellite screening in commercial and non-commercial hosts. NCSU Masters Sympossium, Raleigh, NC, November 2014.
161. Wallace E. C. and Quesada-Ocampo L. M. Non-commercial cucurbits and the downy mildew epidemic. 29th Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2014.

162. Withers S. and Quesada-Ocampo L. M. Improving detection and control of cucurbit downy mildew. 28th annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2013.
163. Withers, S. and L.M. Quesada-Ocampo. 2013. Improving detection and control of cucurbit downy mildew. 28th annual Southeast Vegetable and Fruit Expo. Myrtle Beach, SC, December 2013.

Trade Journal Articles/Trade Proceedings

1. Bird, G., Bishop, B., Grafius, E., Hausbeck, M., Jess, L., Kirk, W., Pett, W., and Warner, F. 1990-2014. Insect, Disease and Nematode Control for Commercial Vegetables. Michigan State University Extension Bulletin E312.
2. Daughtrey, M. 2014. Defying downy mildews. Greenhouse Management (June) 34:26, 43-48. http://www.greenhousemag.com/digital/201406/files/26.html
3. Daughtrey, M. 2015. Dodging downy mildews, pages 123-131 *in* Gill, Stanton, Palmateer, Aaron, and Palmer, Cristi, eds. Proceedings, SAF 2015 Pest and Production Management Conference, Feb. 19-21, Orlando, FL. 137 pp.
4. Daughtrey, M. 2015. Lessons from 2014 for 2015. GrowerTalks 78(10). Ball Publishing, W. Chicago, IL http://www.ballpublishing.com/GrowerTalks/ViewArticle.aspx?articleid=21320
5. Daughtrey, M. 2017. Still not out of the woods: IDM 2017. GrowerTalks 80(12):70-71.
6. Hausbeck, M. 2014. Downy mildew confirmed on cucumbers in Gratiot County July 11. Michigan State University Extension News for Agriculture - Vegetables: Jul 11.
7. Hausbeck, M. 2014. Downy mildew watch: Fungicides recommended for cucumber disease control. Michigan State University Extension News for Agriculture - Vegetables: Jul 2.
8. Hausbeck, M.K., and Goldenhar, K. 2016. Downy mildew prevention and control. Pages 3-9 in: Pickling Cucumber Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online.
9. Hausbeck, M.K., and Harlan, B. 2014. Chemical disease controls for the greenhouse industry. Michigan State University Extension Bulletin E-2750 (poster) (major revision).
10. Hausbeck, M.K., and Harlan, B. 2016. Get ahead in the greenhouse disease control game. Pages 2-9 in: Greenhouse Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo/Michigan Greenhouse Growers Expo, Grand Rapids, MI, Dec. Online.
11. Naegele, R.P., and Hausbeck, M.K. 2014. Progress in cucumber disease control. Pages 5-8 in: Pickling Cucumber Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online.
12. Palmateer, A.J. 2013. Managing impatiens downy mildew in the landscape. Chase News Volume 1(5).
13. Palmateer, A.J. 2013. Use of phosphonates to "cure" downy mildew of impatiens in a landscape. Chase News Volume 1(6).
14. Palmer, C. and Daughtrey, M. 2014. Impatiens: Is there life after downy mildew? AmericanHort CONNECT, 2014(2), p. 3, 8-9.

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1. Catlin, N. and Daughtrey, M., 2014. Extension outreach on coping with impatiens downy mildew. Cornell University, 2013 Annual Research Report, Long Island Horticultural Research & Extension Center Annual Report, Page 37-38.Hausbeck, M., and B. Harlan. Downy mildew of impatiens. Floriculture Growers' Meeting, Allendale, MI, Mar 2014.
2. Catlin, N., Daughtrey, M., Getter, K, and Welch, D. 2014. Determining the impacts of impatiens downy mildew on the greenhouse industry. Cornell University, 2013 Annual Research Report, Long Island Horticultural Research & Extension Center Annual Report, Page 37.
3. Daughtrey, M. and Hyatt, L. 2014. Orvego tested for downy mildew control on impatiens. Cornell University, 2013 Annual Research Report, Long Island Horticultural Research & Extension Center Annual Report, Page 41.

4. Daughtrey, M. and Hyatt, L. 2015. Effect of fungicide drenches on oospore formation in *Impatiens walleriana* infected by downy mildew. Pages 46-47 In Long Island Horticultural Research & Extension Center Annual Report. http://www.longislandhort.cornell.edu/pdfs/2014_annual_reports/floriculture-greenhouse.pdf
5. Daughtrey, M. and Hyatt, L. 2015. Management of downy mildew on impatiens via controlled-release in 4-inch pots. Pages 42-43 In Long Island Horticultural Research & Extension Center Annual Report. http://www.longislandhort.cornell.edu/pdfs/2014_annual_reports/floriculture-greenhouse.pdf
6. Daughtrey, M. and Hyatt, L. 2015. Management of downy mildew on impatiens via controlled-release in hanging basket containers. Pages 43-44 In Long Island Horticultural Research & Extension Center Annual Report. http://www.longislandhort.cornell.edu/pdfs/2014_annual_reports/floriculture-greenhouse.pdf
7. Daughtrey, M. and Hyatt, L. 2015. Management of impatiens downy mildew with experimental fungicides. Pages 44-45. In Long Island Horticultural Research & Extension Center Annual Report. http://www.longislandhort.cornell.edu/pdfs/2014_annual_reports/floriculture-greenhouse.pdf
8. Daughtrey, M. and Hyatt, L. 2015. Management of impatiens downy mildew with experimental fungicides. Pages 43-44. In Long Island Horticultural Research & Extension Center Annual Report. http://www.longislandhort.cornell.edu/pdfs/2014_annual_reports/floriculture-greenhouse.pdf
9. Daughtrey, M., Keach, J., Hyatt, L., Bridgen, M., and Salgado, C. 2016. Downy mildew on impatiens: susceptibility of different *Impatiens* species. 2015 Annual Report Long Island Horticultural Research and Extension Center, Cornell University, p. 41. https://cuaes.cals.cornell.edu/sites/cuaes.cals.cornell.edu/files/shared/documents/2015LIHREC_complete_annual_report_sm.pdf
10. Hausbeck, M. 2015. Downy mildew on cucumbers detected in key areas of Michigan. Michigan State University Extension News for Agriculture-Vegetables. July 7.
11. Hausbeck, M.K. 2013. Impatiens downy mildew: http://veggies.msu.edu/Research/FS_ImpatiensDM.pdf
12. Hausbeck, M.K. 2013. Management of cucurbit downy mildew for gardeners: http://veggies.msu.edu/Research/FS_HomeGardenCucurbit_DM_8-1-2011.pdf
13. Hausbeck, M.K., and Harlan, B. 2014. Controlling downy mildew on impatiens. Pages 2-5 in: Greenhouse Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online.
14. Hausbeck, M.K., and Harlan, B. 2015. Greenhouse disease control update. Greenhouse Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online.
15. Hausbeck, M.K., and Harlan, B.R. 2016 Greenhouse Impatiens Downy Mildew Recommendations.
16. Keach, J., Bridgen, M. and Daughtrey, M. 2017. Breeding for downy mildew resistance in *I. wallleriana*. 2016 Annual Report, Cornell University Long Island Horticultural Research & Extension Center, Riverhead, NY. Daughtrey, M., Hyatt, L. and Rychlik, P. 2017. Observations on overwintering of impatiens downy mildew. 2016 Annual Report, Cornell University Long Island Horticultural Research & Extension Center, Riverhead, NY. https://cuaes.cals.cornell.edu/sites/cuaes.cals.cornell.edu/files/shared/documents/LIHREC_2016AnnualReport_comp.pdfS
17. Kemble J., Quesada-Ocampo L. M., Lewis Ivey M., Jennings K. M., and Walgenbach J. F., Eds. (2015) Southeastern US 2015 Vegetable Crop Handbook.
18. Kemble, J., Quesada-Ocampo, L. M., Lewis Ivey, M., Jennings, K. M., and Walgenbach J. F., eds. 2015. Southeastern US 2015 Vegetable Crop Handbook.
19. Quesada-Ocampo L. M. 2015. Basil downy mildew reported in North Carolina. Extension Plant Pathology Portal. July 7.
20. Quesada-Ocampo L. M., Ed, (2015) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.
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22. Quesada-Ocampo, L. M. 2013. Basil downy mildew: http://content.ces.ncsu.edu/basil-downy-mildew/
23. Quesada-Ocampo, L. M. 2013. Cucurbit downy mildew: http://content.ces.ncsu.edu/publication/cucurbit-downy-mildew/
24. Quesada-Ocampo, L. M. 2013. Hop downy mildew: http://content.ces.ncsu.edu/hop-downy-mildew/
25. Quesada-Ocampo, L. M., ed. 2015. Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.
26. Sanagorski, B. Schall, A.J. Palmateer and N.A. Peres. 2013. Impatiens Downy Mildew. Gainesville, FL: IFAS Communications. PP309.

Field days

Adams M. L. and Quesada-Ocampo L. M. Management of cucurbit downy mildew. Mountain Research Station field day. Waynesville, NC, July 2014.

Quesada-Ocampo L. M. and Adams M. L. Control of cucurbit diseases. Cucurbit field day. Laurel Springs, NC, September 2013.

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Quesada-Ocampo L. M. and Adams M. L. Fungicide control options for cucurbit downy mildew in the field. Cucurbit downy mildew field day. Clinton, NC, October 2014.

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News release:

Calabro, J. 2016. Downy mildew research collaboration: Key findings revealed. The Michigan Landscape 59(4):32. This article is a summarization of key findings from 2015 APHIS research.

Popular Press

Lane, Tahree. (2014) A Beautiful Mystery: Experts Can't Say If Downy Mildew Will Strike Impatiens Again This Year. Toledo Blade, May 4, 2014 (Daughtrey quoted in).

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Research Meeting

This research team developed a research meeting to summarize findings for APHIS. Most members of this team were able to be present and provide presentations highlighting key elements and scientific advancements.

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