Burkard spore traps coupled with qPCR detect *Pseudoperonospora cubensis* sporangia in Michigan fields

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Background

Cucurbit downy mildew (CDM), incited by oomycete pathogen *Pseudoperonospora cubensis*, is a destructive disease for Michigan's pickling cucumber industry. The pathogen arrives in the state annually via an influx of airborne sporangia. Since the re-emergence of the pathogen in the U.S. in 2004, intensive fungicide programs have been implemented by growers as previously resistant cucumber cultivars are now susceptible. In Michigan, spore traps are used to detect airborne *P. cubensis* sporangia and guide the initiation of fungicide sprays. However, *Psuedoperonospora humuli* sporangia, the causal agent of downy mildew on hops, cannot be distinguished from *P. cubensis* by morphological characteristics. *P. humuli* sporangia may occur earlier than *P. cubensis* as *P. humuli* overwinters in Michigan while *P. cubensis* does not. A multiplex qPCR assay was developed to detect and differentiate among *P. cubensis* clade 1 (primarily infects acorn squash, pumpkin, butternut squash, and watermelon), *P. cubensis* clade 2 (infects cucumbers and cantaloupe) (Fig. 1), and *P. humuli* (infects hops)[1,2,3].

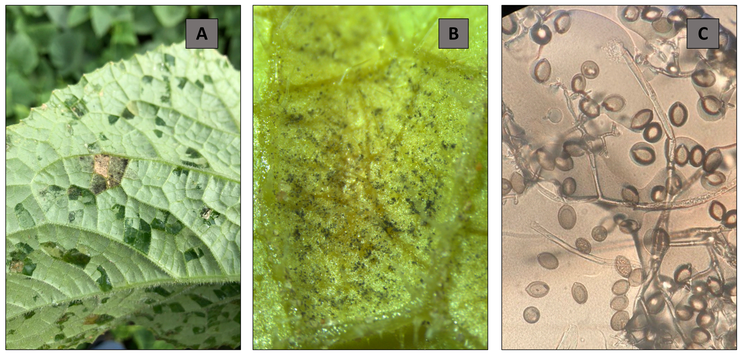


Figure 1: Cucurbit downy mildew symptoms on cucumber: (**A**) CDM lesion with water soaking and sporulation on cucumber leaf (**B**) Sporangia on cucumber leaf under dissecting microscope (Photo: N. Lukasko) (**C**) *P. cubensis* sporangia (measuring 14-25 µm wide by 20-40 µm long) under light microscope

Objective

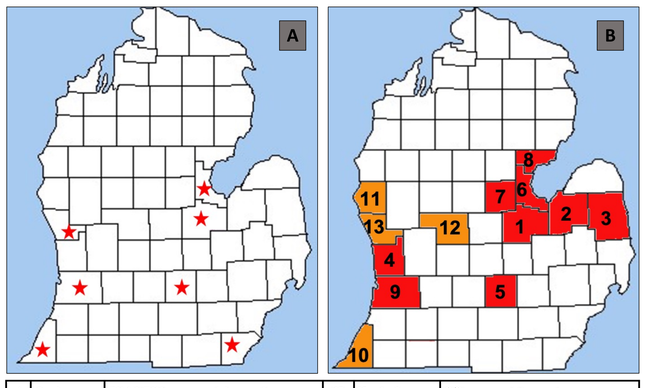
Detect airborne *P. cubensis* clade 2 sporangia in Michigan pickling cucumber production areas to alert growers to the presence of the pathogen so that fungicide sprays can be initiated.

Figure 2: Components of spore trapping (**A**) Burkard volumetric spore trap Photo credit: http://burkard.co.uk/product/7-day-recording-volumetric-spore-trap (**B**) Spore trap in field (**C**) Spore trapping reel (**D**) Spore trap tape mounted on slide and sporangia visible on slide at 200x magnification under light microscope.

Materials and Methods

Burkard spore traps (Fig. 2A) were placed in seven Michigan counties as an early detection tool for CDM. Spore traps were placed in commercial cucumber fields (Fig. 2B) in the west (Muskegon and Allegan counties) and east (Saginaw and Bay) production regions and in a commercial squash field in Monroe county (southeast). Additionally, spore traps were placed in research plots planted with both squash and cucumbers in Ingham (central) and Berrien (southwest) county locations (Fig. 3A). Spore trapping monitoring began May 17, 2021, in Allegan county and remaining spore traps were deployed as soon as a cucumber crop had germinated in that region. The Burkard spore trap takes in air at a steady rate and airborne particles, including sporangia, impact and stick to a tape mounted on a reel inside the spore trap which rotates 2 mm/hour over seven days (Fig. 2C). Spore trapping reels were collected weekly and divided into 24-hour time periods. The tape was split in half lengthwise. One half, representing seven days, was stained and mounted on a slide so sporangia could be observed by light microscopy (Fig. 2D). The second half, representing the same seven days, was processed in a DNA extraction and multiplex qPCR assay with primers and probes that detect and differentiate among *P. cubensis* clade 1 and 2 and *P. humuli* [1,2,3] . A positive or negative result for DNA of the pathogen(s) was recorded for each 24-hour period. Spore trapping data collection continued until mid-August when CDM on cucumbers was widespread in the state (Table 1, Fig. 3B, 3C). Monitoring continued until mid-October in Ingham, Berrien, and Monroe counties in an effort to detect *P. cubensis* clade 1 which infects crops that mature later into the fall.

Results



1	**Saginaw**	*7/14 & 7/19 commercial cucumber grower*	12	**Montcalm**	*8/9 commercial cucumber grower*
2	**Tuscola**	*7/19 commercial cucumber grower*	13	**Muskegon**	*8/9 commercial cucumber grower*
3	**Sanilac**	*7/19 commercial cucumber grower*			
4	**Ottawa**	*7/22 home owner, cucumber*			
5	**Ingham**	*7/23 research plot, cucumber; 9/29 commercial squash grower*			
6	**Bay**	*7/27 commercial cucumber grower*			
7	**Midland**	*7/27 commercial cucumber grower*			
8	**Arenac**	*7/27 commercial cucumber grower*			
9	**Allegan**	*7/28 commercial cucumber grower*			
10	**Berrien**	*8/4 research plot, cucumber*			
11	**Oceana**	*8/6 commercial cucumber grower*			

C

Figure 3: 2021 Spore trap locations and confirmed CDM symptoms in Michigan (**A**) 2021 spore trap locations (**B**) Counties in red indicate where CDM symptoms were first observed in July 2021 and orange indicates where symptoms were first observed in August 2021 (**C**) Details of confirmed CDM symptoms by county

References

[1]Bello, J. C., Sakalidis, M. L., Perla, D. E., and Hausbeck, M. K. 2021. Detection of airborne sporangia of *Pseudoperonospora cubensis* and *P. humuli* in Michigan using Burkard spore traps coupled to quantitative PCR. Plant Dis 105:1373-1381.
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[3]Crandall, S. G., Ramon, M. L., Burkhardt, A. K., Bello Rodriguez, J. C., Adair, N., Gent, D. H., Hausbeck, M. K., Quesada-Ocampo, L. M., and Martin, F. N. 2021. A multiplex TaqMan qPCR assay for detection and quantification of clade 1 and clade 2 isolates of *Pseudoperonospora cubensis* and *Pseudoperonospora humuli*. Plant Dis: 105: 3154-3161

Acknowledgments

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Results

Once spore trapping was initiated (May 17, 2021), DNA from sporangia of *P. cubensis* clade 2 was detected in Allegan county. *P. cubensis* clade 2 DNA was detected shortly thereafter in Monroe county (June 1) and was detected a total of five times before CDM symptoms on cucumber were observed. Early detection of *P. cubensis* clade 2 in the qPCR reaction was verified by observation of sporangia on the spore trap tape. *P. humuli* sporangia were first detected on May 18 in Bay county. CDM symptoms on cucumber were first observed in Saginaw county (July 14). Symptoms of CDM were observed across the state from mid July to early August (Fig. 3B, 3C). Frequency of *P. cubensis* clade 2 DNA in spore traps increased rapidly within 2-3 days of onset of CDM symptoms in that county (Table 1). DNA of *P. cubensis* clade 1 was detected on one occasion on October 11 in Ingham county, but disease caused by *P. cubensis* clade I was not observed in Michigan. However, *P. cubensis* clade 2 was observed to causing blighting of a pumpkin planting in one instance.

County	Date first *P. cubensis* [a] clade 2 sporangia detected	Date first *P. humuli* [b] sporangia detected	Date cucurbit downy mildew symptoms detected on cucumber
Allegan	17 May	21 May	28 July
Monroe	1 June	7 July	N/A[c]
Saginaw	14 June	Not detected	14 July
Bay	15 July	18 May	27 July
Berrien	19 July	Not detected	4 August
Muskegon	26 July	13 June	9 August
Ingham	26 July	9 June	23 July

[a] Cause of cucurbit downy mildew on cucumbers
[b] Cause of hop downy mildew
[c] Cucumbers not grown commercially in this region

Table 1: Date of first detection of *Pseudoperonospora* sporangia in Michigan counties monitored using Burkard spore traps, 2021 Date *P. cubensis* clade 2 DNA and *P. humuli* DNA were detected in qPCR detection assay and date CDM symptoms were observed on cucumber in counties where spore trap monitoring took place in 2021

Discussion

The molecular qPCR detection assay was successfully used for the early detection of *P. cubensis* clade 2 and is the second year that this technology was utilized to alert growers to an influx of the pathogen into their fields. Historically, we have relied on counting sporangia on the the spore trapping tape for early detection. The qPCR detection assay method is faster, less labor intensive, and can differentiate between sporangia of multiple *Pseudopernospora* pathogens. Both *P. cubensis* clade 2 and *P. humuli* were detected in early season monitoring (May-June) before CDM was observed in the state. This research is a collaboration between the Hausbeck Lab, extension educators, and commercial growers. Monitoring of airborne sporangia and scouting are used to alert growers to initiate fungicide sprays. Weekly qPCR results indicating which pathogens (*P. cubensis* clade 1 or 2, *P. humuli*) have been detected across Michigan's cucumber production regions were communicated via the website veggies.msu.edu, extension articles, and other outreach activities (Fig. 4). The website includes which pathogen(s) are detected in each county where monitoring occurs in a 24-hour period. Additionally, MSU extension fact sheets and MSU Extension News articles related to diagnosing and managing CDM are available (Fig. 4).

Figure 4: Website veggies.msu.edu: Spore trapping qPCR results are uploaded to the Hausbeck Lab website. Pathogen(s) detected in each 24-hour period in qPCR detection assay and locations where CDM symptoms have been observed are updated on website as results are available throughout the field season. Extension fact sheets and articles with information about cucurbit downy mildew are also available.