An Early Alert System for Disease in Tomato

Ontario Tomato Research Institute (OTRI) 2023

Spornado
www.spornadosampler.com

Background

Spornado is a disease detection system that collects fungal spores from the air. Our easy-to-use solar powered air sampler captures spores from the air for diagnostic analysis. Samples are analyzed via qPCR to determine the presence of pathogenic spores that cause disease in the crop being tested. This data, which is generally found before symptoms exhibit can inform early IPM decisions for the best possible effect.

The Spornado holds a cassette that is changed either once or twice a week. The cassette is couriered to a laboratory for analysis. Results are emailed within 24 hours. The laboratory can test for any airborne fungal disease from the same cassette and can even perform tests for multiple diseases at the same time.

We received funding from Ontario Tomato Research Institute (OTRI) to implement a Tomato Air Monitoring Plan (TAMP) for target fungal diseases. The monitoring was done in collaboration with OMAFRA, who collected the samples twice a week, and provided results updates to Ontario tomato growers.

Monitoring Locations

A total of nine sites were monitored during the 2023 growing season.

1. Cedar Springs, ON
2. Union, ON
3. Harrow, ON
4. Rondeau, ON
5. Mitchell's Bay, ON
6. Leamington, ON
7. Simcoe, ON
8. Thamesville, ON
9. Port Lambton ON

The map below shows the general locations of Spornado deployment.

Crop / pathogen

During this trial tomato fields were monitored for Late Blight (*Phytophthora infestans*) and Early Blight (*Alternaria solani*) spores. Early blight persists in crop residue that is contaminated and can be transmitted through infected seeds and weed hosts. Spores remain prevalent throughout the entire growing season and can travel considerable distances through the air or be disseminated through splashing water. Early blight development is favored by temperatures ranging from 17-24°C (63-75°F) and prolonged periods of leaf wetness. Late blight prefers temperatures from 15- 21°C (59-70°F). Optimal conditions for disease development include cool nights and warm days, coupled with humid weather. Both diseases, if left uncontrolled, can be devastating with significant economic consequences.

Sampling details

Sampling occurred throughout the summer from June 23rd to September 1st. Samples were collected by an OMAFRA team member twice a week and shipped to Spornado's partner laboratory in Toronto, Ontario for molecular analysis. Samples were tested using real-time PCR (qPCR).

Analysis

Samples were analyzed by our partner laboratory, Sporometrics. Sporometrics is a Level 2 Biosafety laboratory, accredited to ISO/IEC 17025:2005 by the American Industrial Hygiene Association and to Good Manufacturing Practice by Health Canada.

DNA extraction

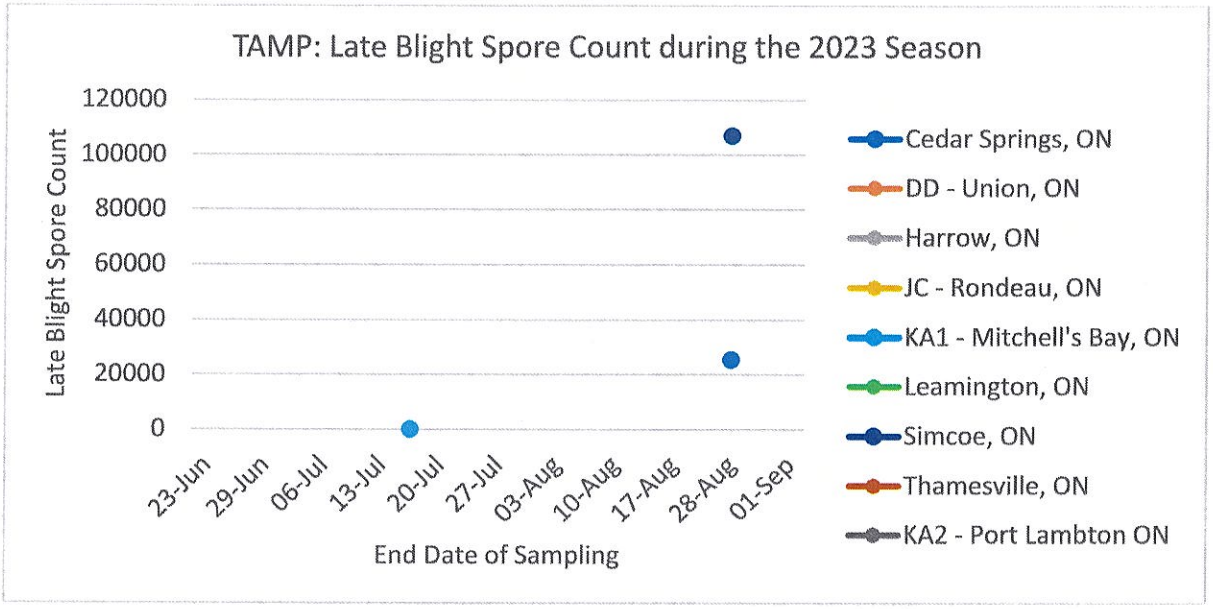
Spornado cassettes were opened with a metal cassette opener. Cassette membranes were removed from the cassettes pushing them with a ten-microliter sterile loop into the lysing matrix tube supplied by the commercial kit. The DNA was extracted as per manufacturer's instructions and eluted in 30-35µL of elution buffer (for clear to semi-clear cassettes) or 40-50µL (for cassettes having visible to heavy load of dirt or debris). Total DNA was extracted from all pure cultures and from the Spornado cassette membranes using the commercial FastDNA Spin kit for Soil (MP Biomedicals, USA). The concentration of the total DNA concentration was quantified using the Quantifluor dsDNA System (Promega, USA). DNA extracts were stored at -20°C until further analysis.

Quantitative PCR (qPCR) for pathogen detection

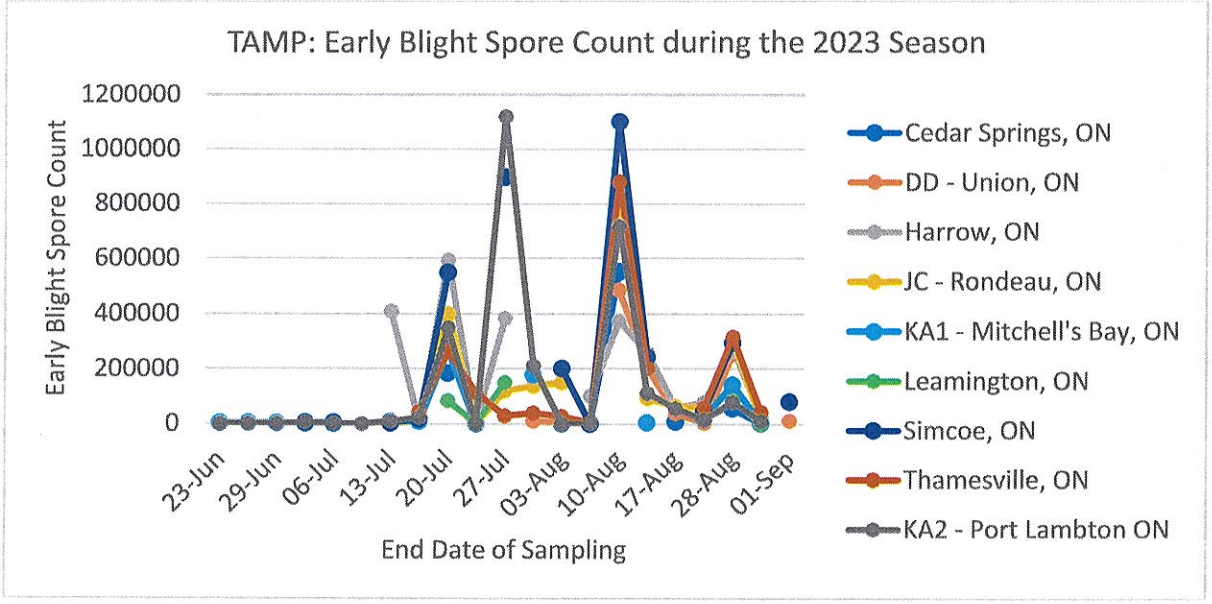
TaqMan qPCR assays have been optimized for the detection of *P. infestans* and *A. solani*. All qPCR reactions used 100 picograms of DNA template and were carried out in a CFX96 Touch Deep Well Real-Time PCR Detector (Biorad, CA, USA). Samples were considered positive when they yielded a cycle quantification value (Cq) corresponding to the PCR cycle number at which the fluorescence amplification curve crossed the threshold line. Standard curves were constructed by plotting DNA 10-fold serial dilutions with the corresponding Cq values with a fluorescence threshold set manually. The limits of detection were determined by performing 10-fold dilutions of total DNA extracted from pure cultures and reference DNA extracts ranging from 1 ng/µL to 1 fg/µL.

Results

A total of 305 samples were collected during the growing season and analyzed for both pathogens. The first samples found positive for late blight were collected during the week of July 17th at three locations (Union, Harrow, and Mitchell Bay). The number of spores identified on these samples were low and ranged from 3 – 12. A second set of positive samples were collected during the week of August 28th at two locations, Cedar Springs and Simcoe. These samples had high numbers of spores; 25,500 and 107,000, respectively.



Early blight was identified at two or more sites each week throughout the growing season. During the week of July 20th, it was identified at every location. The quantity of spores identified ranged from 23 to 1,120,000. The spore levels peaked during the week of July 27th and again August 10th. A graph showing the early blight spore counts is provided below.



The summary tables with testing data are appended to this report.

Discussion

The primary objectives of this disease monitoring was to provide the tomato growers of Ontario with a regional early alert of disease and data on levels of disease pressure. Weather-based disease models indicate if the weather is favourable for disease development, but don't factor in the presence of the pathogen. Spornado's air monitoring system fills this gap. Using air monitoring data on the presence of disease and weather can more precisely determine when and what to spray.

The first scouting report of late blight was around July 21, 2023, in the Simcoe County area. This was approximately 4 days after the first positive air sample. Overall, late blight disease pressure remained fairly low, with a few cases showing up in tomatoes in Norfolk and Elgin counties around July 28, 2023. It is likely that the disease started with asymptomatic potatoes saved for seed form 2022.

Early blight pressure in 2023 was relatively normal and disease symptoms were observed in tomatoes across growing regions but were not severe. The general fungicides used by many growers appear to be keeping this disease under control.

Appendix

LATE BLIGHT RESULTS

Date	Cedar Springs, ON	DD - Union, ON	Harrow, ON	JC - Rondeau, ON	KA1 - Mitchell's Bay, ON	Leamington, ON	Simcoe, ON	Thamesville, ON	KA2 - Port Lambton ON
23-Jun									
26-Jun									
29-Jun									
03-Jul									
06-Jul									
10-Jul									
13-Jul									
17-Jul		3	6		12				
20-Jul									
24-Jul									
27-Jul									
31-Jul									
03-Aug									
07-Aug									
10-Aug									
14-Aug									
17-Aug									
21-Aug									
28-Aug	25500						107000		
30-Aug									
01-Sep									

EARLY BLIGHT RESULTS

Row Labels	Cedar Springs, ON	DD - Union, ON	Harrow, ON	JC - Rondeau, ON	KA1 - Mitchell's Bay, ON	Leamington, ON	Simcoe, ON	Thamesville, ON	KA2 - Port Lambton ON
23-Jun				1140	1970				730
26-Jun				1367	2780				1250
29-Jun				681	1760				1390
03-Jul	1190	1720		1640	2210		4170		4560
06-Jul	223	410		591	29		4410		1230
10-Jul				73					26
13-Jul	1950	1280	410000	764	8300	602	4666		9220
17-Jul		15700	7550	9460	9400		17700	43700	22800
20-Jul	184000	313000	594000	401000	272000	84800	549000	265000	350000
24-Jul			4320	1130	75	48		114000	23
27-Jul			384000	122000		151000	897000	29500	1120000
31-Jul		10100		139000	178000			39800	211000
03-Aug	1070	6319		152000		9970	202000	27500	954
07-Aug	1310		103000			517	33	4320	1250
10-Aug	551000	486000	373000	819000			1100000	879000	719000
14-Aug		205000	257000	93500	4600	210000	244000	204000	113000
17-Aug	7670	38200	62500	66000					56700
21-Aug		4340	77300	62600	19500		45500	59400	14700
28-Aug	55800	254000	278000	304000	142000	85300	295000	317000	79500
30-Aug	1690		28200	15700	5170	347		43500	10500
01-Sep		11300					80800		