Monitoring Tomato Brown Rugose Fruit Virus in Greenhouses Using Bumblebees

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Abstract

The tobamovirus Tomato brown rugose fruit virus (ToBRFV) is a devastating pathogen of bell peppers and tomatoes. ToBRFV is a highly regulated virus around the world due to its severe ability to impact tomato production and marketability. Recently, two ToBRFV outbreaks were identified in Canada at commercial greenhouses in Ontario and Quebec. Phylogenetic analysis of samples collected from these greenhouses suggests that these were independent outbreaks. Due to the high transmission rate of ToBRFV, early detection of infection and regular monitoring is imperative for limiting spread. In tomato production greenhouses, bumblebees are commonly used as pollinators. Bees visit multiple plant individuals on one foraging trip and collect pollen to return to the hive. ToBRFV is present in pollen grains derived from infected plants, and monitoring for ToBRFV in greenhouses using bees can be more efficient than manual screening for symptoms. Commercial bumblebee hives were collected from ToBRFV infected greenhouses to evaluate different detection methods. RNA sequencing of bumblebees allowed for the detection of Tomato ringspot virus, Tobacco ringspot virus, Pepino mosaic virus, Tomato brown rugose fruit virus, and many others. These results suggest that the plants within the greenhouse may be infected with some of the viruses detected. ImmunoStrip tests can be conducted on leaf material to pinpoint specific plants which are infected. To determine the limits of detection through bee samples, an experimental system using Pepino mosaic virus is currently being developed. The use of bees as a sample material allows for quick and accurate detection of pathogens within agricultural systems and can be developed into an easy-to-use monitoring system for growers and researchers.

Introduction

ToBRFV belongs to the virgoviridae family and is a tobamovirus (CABI, 2020). This pathogen is commonly transmitted mechanically, and can be spread through pollen (Levitzky, et al., 2019). Symptoms of ToBRFV infection include wrinkling and mosaic of the leaves, necrotic spots, browned and undersized fruit, and even fruit abortion (Luria, et al., 2017). Due to its severity and its ability to affect tomato production and marketability, ToBRFV is a highly regulated virus around the world and has caused massive impacts on trade. ToBRFV outbreaks can be quite severe and leave fruit unmarketable. Countries such as the United States have restricted imports of tomatoes and peppers from all countries where the virus is present (APHIS, 2020). ToBRFV is not established in Canada and so it is extremely important to monitor greenhouse tomato production. Identifying pathogens after symptoms appear can be detrimental as considerable pathogen spread and transmission may have already occurred. Bees are a major transmission route for ToBRFV and other plant viruses. Bees can be collected and the extracted genetic material can be sequenced to identify the viruses present in the agricultural system they are pollinating. Two separate ToBRFV outbreaks were suspected in Canada in 2019, in commercial tomato-producing greenhouses in Ontario and Quebec. It is not clear if these outbreaks were somehow linked, or if they represented independent introductions into Canadian greenhouse tomato production. This further underlines the need for an improved monitoring system that can be used to detect viruses prior to establishment.

ToBRFV in Canada

- RNA was extracted from ToBRFV symptomatic plant material obtained from greenhouses and sequenced by Genome Quebec
- Illumina NovaSeq 6000 system was used with 50M paired-end sequences on ribosomal RNA depleted RNA
- Trimmed reads were assembled into contigs
- Contigs were BLASTed on the NCBI nucleotide collection database, filter by viral genomes to identify viruses present in the samples
- At least 8 ToBRFV contigs were obtained from each sample and had 99%-100% identity with the ToBRFV genome
- Phylogenetic analysis was conducted based on the 270-4633 nucleotide region of the ToBRFV genome
- These results indicated the two outbreaks were independent, outlining a need for better monitoring of pathogens

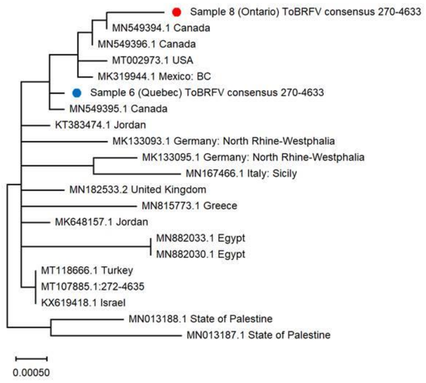
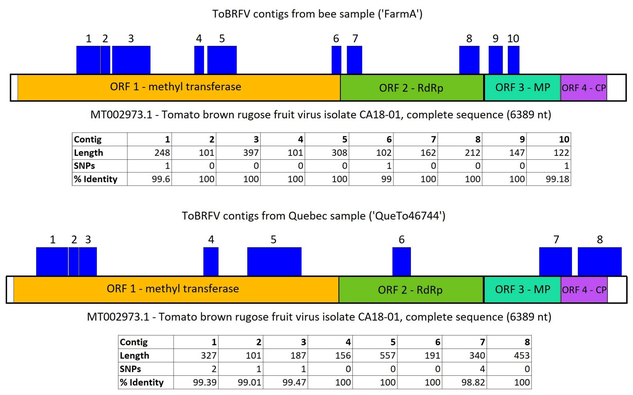


Figure 1: Phylogeny of ToBRFV sequences 270-4633 obtained from CFIA. Red and blue dots indicate sequences identified in Ontario and Quebec, respectively. Other Canadian isolates were submitted by CFIA.



Contig	1	2	3	4	5	6	7	8	9	10
Length	248	101	397	101	308	102	162	212	147	122
SNPs	1	0	0	0	0	1	0	0	0	1
% Identity	99.6	100	100	100	100	99	100	100	100	99.18

Contig	1	2	3	4	5	6	7	8
Length	327	101	187	156	557	191	340	453
SNPs	2	1	1	0	0	0	4	0
% Identity	99.39	99.01	99.47	100	100	100	98.82	100

Figure 2: Diagram of ToBRFV genome, indicating major protein domains, and coverage of contigs (blue) recovered thorough RNA sequencing. Tables indicate the similarity of the recovered contigs to the ToBRFV genome. Farm A contigs were recovered from bee samples, and Farm B contigs were recovered from tomato leaves.

RNA Sequencing of Bee Samples

- RNA was extracted from as little as 5 bees, which gave ample amounts of RNA for sequencing
- Next generation sequencing of the extracted RNA through an Illumina NovaSeq 6000 system with an S2 Flowcell produced 50M paired-end ribosomal RNA depleted reads
- Reads were assembled into contigs using Virtool (Rott, et al., 2017) and matches to the tomato and bee genomes were subtracted out
- Assembled contigs were BLASTed against the NCBI nucleotide collected database filtered by virus genomes to identify plant viruses present in the samples
- Over 40 plant and bee viruses were identified from the bee samples indicating the presence of these viruses in the bees and potentially in the agricultural system they are pollinating
- Viruses that are not known to infect tomatoes were also found in the bee samples such as Blackberry chlorotic ringspot virus and Peach rosette mosaic virus

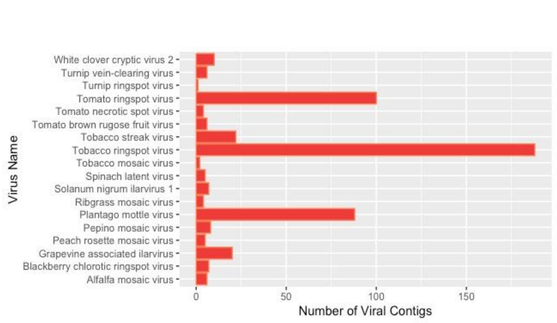


Figure 3: Viruses identified through bumblebees collected from greenhouses.

Major Plant Pathogens Identified

- Tobacco ringspot virus (TRSV) and Tomato ringspot virus (ToRSV) were both detected with over 100 contigs identified through bee samples
- Pepino mosaic virus and Tomato brown rugose fruit virus were both identified in the same sample
- These results illustrate the strengths of this monitoring approach, as multiple different viruses are detectable with the same test

Table 1: Major plant viruses identified from bee samples through NGS sequencing, and their identity to reference genomes on NCBI.

Virus	Contigs	Hit Length	Identity
Tobacco ringspot virus	188	140-2660	89.2-99.0%
Tomato ringspot virus	100	150-2534	80.1-99.4%
Tobacco streak virus	22	140-855	93.5-100%
Pepino mosaic virus	8	132-266	98.8-100%
Tomato brown rugose fruit virus	6	147-308	100%

Monitoring ToBRFV with Bees

- Developing an experimental system to explore dynamics of virus spread through bee related tomato pollination activities
- Can help determine the time between infection of the plant and detection in bee/pollen samples
- Evaluating the limits of detection of methods such as PCR testing or ImmunoStrips
- ImmunoStrip tests can also help pinpoint exactly which plants are infected with the virus in less than 30 minutes with only a few leaves
- This approach may be directly transferable to detection of ToBRFV in greenhouses

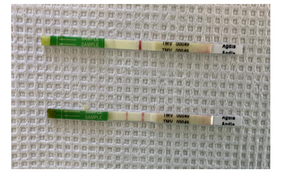


Figure 5: ImmunoStrip tests can identify the presence of a virus in a plant in less than 30 minutes while using only a few leaves. Two lines on the test indicate a positive result, whereas one line indicates negative.

Conclusion

- Two separate Canadian outbreaks of ToBRFV appear to be of independent origin, similar to results obtained by CFIA and submitted to NCBI
- In the absence of robust genetic resistance to ToBRFV, regular monitoring is the most effective tool for preventing the establishment of this virus in Canada
- Sequencing bee samples can detect pathogens present in the greenhouse while allowing for the testing of multiple individuals at once
- Monitoring of ToBRFV through bee and pollen samples could provide an early warning system for ToBRFV infection

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I would like to acknowledge the help and guidance of all collaborators and contributors to this project, including all members of the Griffiths Lab at AAFC Vineland, Dr. Jonathan Griffiths, Sandra McCutcheon and the growers who allowed us to collect samples from their greenhouses.