Learning to Live With ToBRFV

2023

Perspectives and Best Practices from North American Growers and Industry Experts.



This report was researched, written, and designed by IUNU team members. We'd especially like to acknowledge and thank all of the growers who completed our survey and contributed to the report. While we can't thank you by name due to the sensitive nature of the content of the report, we appreciate the contributions from each and every one of you. Thank you to the many researchers and scientists who also reviewed and added valuable information. In particular, we'd like to thank Erika Verrier, Karin Tifft, Alexis Franco, Travis Kroeker, Sukhi Pannu, Dr. Parm Randhawa, Dr. Michael Bledsoe, and Dr. Kai-Shu Ling for their efforts over the past year working to shed light on ToBRFV through this report.

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Introduction

Losses from Tomato Brown Rugose Fruit Virus (ToBRFV) continue to surge as the catastrophic virus impacts greenhouse operations worldwide. For more than 5 years, the industry has focused on developing strategies to combat this disease in every step of production. Despite detection in 2014 by seed companies, growers only became aware of the virus in 2018. Seasoned growers expect that managing risks is an inevitable part of their work, and tomato growers have long dealt with waves of crop risks. However, Tomato Brown Rugose Fruit Virus is a tsunami unlike any other.

Image 1.1 by IUNU

-Grower with 25 years of experience.

Since its discovery in 2014 in Jordan and Israel, ToBRFV has been detected in greenhouses across the world. In 2015, <u>a publication 1</u> deemed ToBRFV as a genetically unique tobamovirus after completing the genomic sequence. By 2018 the catastrophic virus had made its way to North America. It was first <u>discovered in Mexico 2</u> with similar strains detected subsequently in <u>California</u> ³. Today, ToBRFV has been reported in more than <u>35 different countries</u>⁴. Nearly every large scale grower in North America has been affected by ToBRFV and a tide of change in the way we grow has been left in its wake.

¹ https://link.springer.com/article/10.1007/s00705-015-2677-7 ² https://nextstrain.org/community/NPPO-NL/nextstrain-ToBRFV/20191231 ³ https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-11-18-1959-PDN ⁴ https://gd.eppo.int/taxon/TOBRFV/distribution The purpose of this report is to look at the risks of ToBRFV for tomato growers. Both quantitative and qualitative research was conducted. Our team surveyed a majority of the commercial tomato growers in North America to collect information on loss, prevention, and opportunities. Because of the sensitive nature of the virus, all data provided in the report is anonymized and no grower information is shared. Quotes that are not attributed come from growers.

At IUNU, we believe empowering growers starts with hearing their stories in the greenhouse and understanding the true impact of risks like ToBRFV. In addition to the survey, we listened to growers and industry experts about how ToBRFV is impacting their business, what resources they need, and how this virus has changed the industry. Our goal is to share these perspectives, engage in advocacy for growers, highlight industry progress, provide actionable tools, and determine critical next steps for growing in this new environment.

Learning to Live with ToBRFV

Greenhouse tomato growers are no stranger to ongoing pest and disease pressure. With each new risk, our industry evolves and eventually reaches a predictable trajectory of "learning to live with it."

Adapting to risk in a growing operation is a process for both humans and plants - together proceeding through *discovery*, *fear*, *research*, *planning*, *actualization*, *adaptation*, *management* and *progress*. Stakeholders across the industry have been hard at work since the *discovery* of ToBRFV, motivated by the *fear* of catastrophic loss to find solutions. *Research* has been conducted in all aspects of the tomato industry including seeds, diagnostics, propagation, growing, and sanitation, helping growers to *plan* and access resources for preventing and managing the risks. When growers are forced to *actualize* their management plan upon discovering ToBRFV in their crop they learn to *adapt* procedures and *manage* their processes over time. Feedback from the efficacy of these procedures in practice subsequently supports *progress* in helping the tomato industry "learn to live" with ToBRFV.

Tomato Crop Risk Timeline

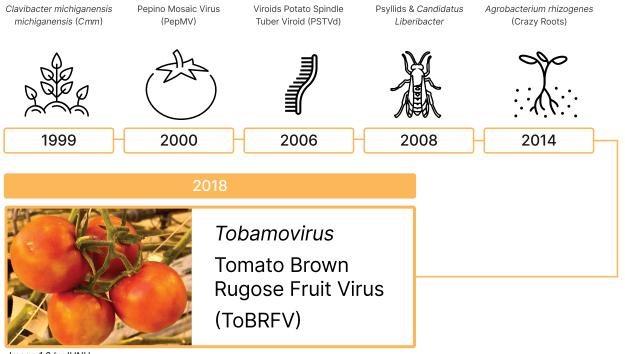


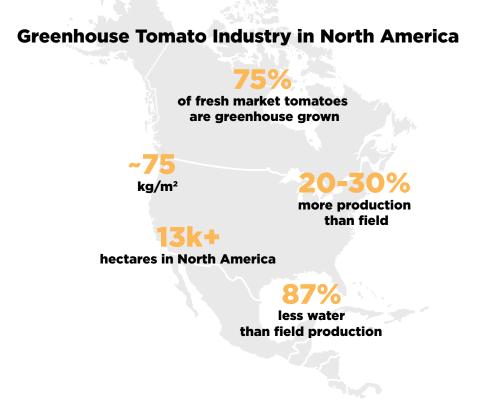
Image 1.2 by IUNU

"Let's face it, it's not going anywhere soon."
-Grower in the stage of actualizing his management plan upon initial discovery of ToBRFV in his crop.

Upon discovery in the US, large scale greenhouse tomato growers and experts met regularly to discuss challenges, identify needs, advocate for resources and learn from each other. Diseases like ToBRFV are so powerful they have the ability to catalyze collaboration even in a competitive and proprietary landscape. While we have seen the industry make significant progress towards managing ToBRFV, there is still work to be done. Growers are working hard to minimize losses through integrated approaches in biosecurity and management until disease resistance and resources for early detection become widely available. At the same time, they're becoming increasingly frustrated with what they feel is a "lack of progress and proactivity" by seed companies; they want more testing to circumvent contaminated seeds. "We're holding back [frustration]" explains one grower at the ToBRFV Research Symposium in Ontario.

How ToBRFV Spreads

ToBRFV can infect 100% of a greenhouse tomato crop, readily spread through contaminated seeds and mechanical contact which is inevitable in daily horticultural practices. A study of <u>spatiotemporal dispersion</u> <u>of ToBRFV</u> ⁵ in the greenhouse illustrates the rapid spread of this virus particularly via plant to plant contact in this system. The study highlights the need for starting clean with disease free seeds and plants with effective phytosanitary practices applied in the growing space. The impact of only a few infected plants in a system can result in total crop devastation.



5 https://www.mdpi.com/2073-4395/10/6/834/htm

How It Spreads

Across the Controlled Environment Agriculture (CEA) landscape, many crop risks exist; the challenge comes in learning to manage in an indoor environment where overall we expect to exclude risks.



In greenhouse tomatoes, worldwide seed distribution, intense labor and plant handling throughout long (sometimes overlapping, interplant) crop cycles play a role in the risk potential of certain pests and diseases.



Recirculated irrigation water and bees used for pollination and can also be vectors of this disease.



Currently there is no evidence that indicates transmission of ToBRFV by key greenhouse pests such as whitefly and aphids. In the greenhouse, ToBRFV losses translate to unmarketable fruit, reduced plant vigor and yield, premature crop death and plant removal in the infected area. Additional costs can be accumulated in labor, disposable materials (gloves, shoe covers, lab coats and tyvek), daily sanitation, greenhouse cleanout and additional seeds and plants.

Crop risks vary greatly from field and greenhouse growing environments. Due to the intense crop work needed to maintain greenhouse tomato crops, where each plant is touched 5-7 times per week, catastrophic loss from mechanical transmission is much more likely. The risk in field tomatoes is much less due to the reduced need for plant handling which predominantly occurs at the end of the crop cycle upon harvesting. Understanding the potential for risk in the greenhouse setting has been essential in understanding the need for policy and resources for managing ToBRFV globally.

Defining Catastrophic Risk in the Greenhouse

At such high infection rates, ToBRFV is causing massive product losses for growers. Despite this, as an industry we haven't yet defined what level of loss makes ToBRFV catastrophic. In our survey, we asked commercial growers across North America to define this level of major loss.

Of the growers we surveyed, more than 50% defined catastrophic loss as a cumulative loss of revenue of \$17 million in a given year. Just over 45% of growers indicated they have experienced this level of catastrophic losses from ToBRFV. A smaller subset of growers indicated that catastrophic loss should be defined as losing 6% or more due to the virus annually. Collectively, these growers have lost hundreds of millions of dollars in revenue; worldwide, losses amount to billions of dollars annually. We recommend defining a catastrophic loss as a loss amounting to the lesser of \$17 million or 6% of annual revenue (to account for scale of an operation) in a given year due to ToBRFV.

Product loss inside the greenhouse from the virus isn't the only contributing factor to the catastrophic revenue loss growers are experiencing. Quarantine policies at the border have caused significant losses due to spoilage while awaiting remote test results at the border. More recent onsite testing has helped to reduce the spoilage issues.

In 2019, the USDA initiated a <u>Federal Order for U.S.</u> <u>Imports of Tomato ⁶</u> as a response to intercepting a shipment of ToBRFV infected tomatoes from Mexico.

⁶ https://www.aphis.usda.gov/import_export/plants/plant_imports/federal_order/downloads/2019/DA-2019-28.pdf

The abrupt order to quarantine tomatoes at Mexican and Canadian borders based on "blotched fruit" symptoms resulted in losses in the millions of dollars to growers. Tomatoes that exhibited blotched ripening were held for several days while lab tests were conducted to determine the presence or absence of ToBRFV. Awaiting test results during the quarantine period resulted in catastrophic losses as shipments of tomatoes perished.

The quarantine policy in November 2019, shook the North American community of tomato growers and ignited collaboration, research, resource advocacy, and enhanced Integrated Pest and Disease Management (IPDM) programs. As with all crop risks, an integrated approach is needed and the first step is understanding the risk - or knowing the enemy.

Knowing the Enemy

ToBRFV, a member of the Tobamovirus genus of viral tomato disease which infects tomato and pepper crops. ToBRFV is a plant pathogen that poses no health risks to humans.





Image 2.1 by IUNU



Image 2.3 by IUNU



Image 2.5 by IUNU

Image 2.2 by IUNU



mage 2.4 by IUNU



Image 2.6 by IUNU

"The virus is a variant of the Tomato Mosaic Virus (ToMV), however ToBRFV is more challenging to the industry because it expresses on the fruit and not just the leaves like ToMV"

-Dr. Parm Randhawa - President at CSP Labs

Additionally, ToBRFV is not managed by the Tm-22 resistant gene unlike other Tobamoviruses, and delayed disease expression complicates the management of ToBRFV as one grower explains. "Once we see it in some of the plants, it's already too late. We know that the virus has already been here and has been moving throughout the crop for several weeks."

"Brown Rugose," or brown wrinkles, describes the damage observed on infected plant foliage and fruit. First symptoms typically appear on the leaf tips of new vegetative growth as mosaic patterns followed by characteristic rugose and thinning stem diameter. Fruit exhibits blotchiness upon ripening, similar to Pepino Mosaic Virus (PepMV) a virus that many growers have learned to manage with crop steering.

"It's like killer Pepino, the same symptoms are expressed at first but it becomes deadly to plants. The plants just get smaller and smaller until there is nothing left."

Detecting the Virus

ToBRFV is systemic, therefore it can be detected throughout the plant regardless of the point of infection once you begin to see symptoms in the leaves or fruit. Studies on how Tobamoviruses move throughout the plant date back to <u>The Movement of Tobacco Mosaic Virus Within the Plant, G. Samuel 1934</u>.

Understanding how the virus moves within the plant is critical to plant sampling efforts needed for early detection in the greenhouse. Regardless of where the virus enters the plant, it moves first to the roots then to the head of the plant within the first few days and then can be detected throughout the plant in subsequent weeks. Dr. Adrian Fox of Fera Science in the UK described his study at the ToBRFV Research Symposium - that if plant growth is generative or vegetative, the virus can be more readily detected in the reproductive parts or leaves respectively. Therefore, when sampling fruit bearing crops it is encouraged to sample sepals and fruit with foliage from the plant heads to aid in early detection.

In the greenhouse, growers report first signs of infection around 6-10 weeks after planting in the greenhouse when the plant is approaching or has recently achieved full fruit load.

"In the lab, symptoms can be seen approximately 2 weeks after inoculation and sometimes as early as 7 - 10 days."

-Dr. Kai-Shu Ling the leading USDA-ARS Research Plant Pathologist on this issue.

The Need for Resistance

With a lack of highly resistant (HR) ToBRFV varieties available, growers observe that environmental factors and variety play a critical role in disease expression once plants become infected. Based on trials they have conducted, seed company research, and conversations with other growers, some operations are transitioning to more resilient varieties and other cropping strategies.

ToBRFV poses other unique challenges upon infection, resurfacing disease risks that were once managed through resistance. General Tobamovirus resistance has long been bred into commercially available seed varieties, however ToBRFV has shown to be the gateway for other tobamovirus infection despite initial resistance, "Once a plant becomes infected with ToBRFV, it breaks down the Tm-22 resistance gene and "unlocks" susceptibility to other tobamoviruses by the tomato plant," says Sukhi Pannu, Director of Research and Business Development at CSP Labs.



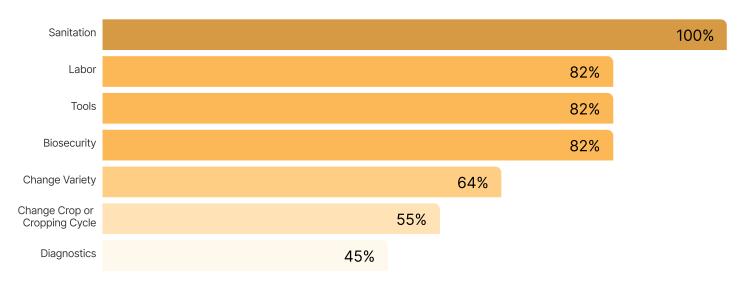
Image 3.1 by IUNU

⁷ https://web.archive.org/web/20181104083500/http://www.lib.kobe-u.ac.jp/repository/00228483.pdf

A critical "movement protein" has been identified as the key factor in ToBRFV's ability to overcome the Tm-22 resistance, which has otherwise worked for 60 years. According to Dr. Ziv Spiegelman of The Volcani Centre in Israel at the ToBRFV Research Symposium; this movement gene allows the virus to move through the plasmodesmata (the intercellular channels of the plant). This research is critical to understanding opportunities for resistance in the future alongside screening for resistance in wild cultivars. Additionally diagnostic experts are concerned that with so much focus on ToBRFV, an uptick is observed in the presence of Tomato Mottle Mosaic Virus (ToMMV, another tobamovirus) in seed samples from smaller seed companies. With the risk of ToBRFV, many other diseases we once learned to live with are back on the radar.

Managing Operations

Regardless of disease presence in their greenhouse, 100% of growers we surveyed have modified at least one aspect of their operation to prevent or manage the risk of ToBRFV. Growers have primarily focused on managing the disease through sanitation, labor, tools, and biosecurity. ToBRFV has forced the need for human, material and financial investments in biosecurity (exclusion) and risk management (containment) tactics to minimize losses until a solution is developed.



Adjustments Made to Manage or Prevent ToBRFV (% of respondents)

The greenhouse looks like a hospital. With our strong biosecurity procedures we focus on hygiene, hygiene, hygiene!" -Describes a Grower

Roadmap for the Future

Collectively, the industry has made progress in several areas needed to manage ToBRFV; as with all successful risk management programs an integrated approach within a grower's IPDM Toolbox is necessary. While there is still more work to be done to develop resistant cultivars and promote early detection, progress has been made in the area of diagnostics from seed to harvest as well as sanitation.

As always, growers continue to be resourceful and creative with their planting strategies and crop steering to promote a stronger, more resilient crop in the presence of catastrophic disease. In our work with growers and industry experts, we have gained an understanding of best practices for growers to consider in their biosecurity and management programs against ToBRFV specifically. The following section will provide an overview of the work being done in these various aspects and best practices we witness currently in the industry.

Diagnostics for Every Stage

Progress in the diagnosis of ToBRFV has been made in recent years, providing growers with tools for both onsite and off-site testing. Testing methods vary in accuracy, cost, timing, sensitivity, and function. With so many options, deciding which test to use can be challenging for operations who are interested in executing a robust biosecurity and disease management program. Given that the industry has zero tolerance for this disease, it is critical to choose diagnostic tools that are best suited for detecting the disease at various stages in production. Growers are maximizing onsite testing resources and validating with laboratory support to create custom sampling regimes that are proactive in detecting the disease prior to symptom expression.

Resource

We have worked with diagnostic experts throughout the United States and Canada to create a general Diagnostic Matrix comparing features of the many testing resources available on the market to support growers in their diagnostic decision making process.

<u>Access ToBRFV Diagnostic</u> <u>Matrix ⁸</u> "As with any other plant pathogen, there is no onesize-fits-all when it comes to diagnostic testing for ToBRFV. Each grower must assess their own unique risks and deploy testing strategies accordingly. Given the severity of disease caused by ToBRFV, however, growers have increasingly chosen to employ molecular methods, whether testing directly on-site with technologies like AmplifyRP or by sending samples to our Testing Services laboratory for molecular confirmation of positive ImmunoStrip results."

Coilin Walsh of Agdia

Testing accuracy relies on the representative nature of the sample as it relates to the whole population. Sample size and sample selection are key factors affecting confidence in the results. Diagnostic tools can help growers detect and circumvent ToBRFV at all stages of the production chain from seed to harvest.

"The industry has zero tolerance for this risk; testing the appropriate sample size is critical." -Sukhi Pannu of CSP Labs

⁸ https://bit.ly/IUNU-ToBRFV-Matrix

Seeds

90% of the growers we surveyed are interested in conducting additional seed testing beyond what's required by seed companies and the USDA/APHIS, as an added layer of caution.

Laboratories like California Seed and Plant(CSP) Lab who are accredited by the <u>National Seed Health System</u>⁹ support many growers with this work. Additional seed costs can be prohibitive for some growers since seed tests are destructive - seeds are ground into a solution and are no longer available for planting thereafter. Because of the additional cost, Sukhi remarks that "many growers are not testing enough seeds to make the testing worthwhile; small samples are not representative of the seed lot population resulting in low confidence levels."

Growers are encouraged to determine effective sample sizes using procedures from International Standards of Phytosanitary Measures (ISPM) aiming for a 99% confidence interval for each seed lot. Note that when purchasing seed varieties, several different lots may be used to complete an order. Each lot should be tested independently since it has been designated by the seed company as having been produced in a different location or at a different time. Lots are defined as identifiable, homogeneous, and of a certain origin group of seeds. Some growers even require the same lot to be retested if it was sent on a different day. Using a panel of PCR tests, Pepino Mosaic Virus and Pospiviroids can also be run on the same samples of seeds as ToBRFV and other Tobamoviruses. Seed testing can provide confidence to growers in their ability to move forward with disease free seeds in propagation.

Research shows that the virus predominately lives on the seed coat versus the embryo of the seed. There is hope that a nondestructive seed test can be developed which will not compromise the germination success of the seeds, but currently this does not exist making adequate seed testing unfeasible for some. This research does provide hope for seed disinfection as outlined by Dr. Nida Salem at the ToBRFV Research Symposium in Ontario, "Treatment of ToBRFV-contaminated seeds with 2% HCl for 30 min or 10% TSP for 3 h resulted in a 100% disinfection rate without significant decrease in seed germination." Growers should work with their seed companies to understand seed testing and seed treatment protocols and determine if additional steps should be taken to prevent risk.





Food for Thought

It's important to note that seed testing requirements vary, before a seed company can sell in any particular country, they must meet the seed testing requirements established by them including sample size. For example, sample size in most countries worldwide is 3,000 seeds, but in Australia it is 20,000.

Propagation

Growers should subsequently work with their propagation facility to understand when and how testing can be effectively performed on their crops to ensure a clean start. Growers we work with have modified their approach to grafting and pinching; while the approach is different among growers the aim is to grow vigorous short crops while reducing the risk of disease transmission and balancing seed expenses. For one grower this looks like 12 week pinched, non-grafted crops and for others this looks like 6 month single, grafted or non-grafted crops.

Grafting crops is still recommended to support plant vigor, and it is a key phase of propagation where

⁹ https://seedhealth.org/



excess plant material can be used for testing. For example, all rootstock tops and scion roots would normally be discarded - these could be used for composite sampling in the lab. Otherwise, ISPM procedures should be referred to for sample size and pattern from the propagation floor.

Environmental testing can be a useful tool in managing the risk of transmission from surfaces and irrigation water. Laboratories can send sample collection kits including environmental swabs that can test seeding machines, transplanting and spacing equipment as well as any other plant contact surfaces that could transmit the virus from plant to plant. With many

propagators using floor flooding and recirculating irrigation, drain water testing is a critical test of this system. Results from environmental testing can support propagation facilities in providing confidence to growers that their biosecurity practices are sufficient. Propagation facilities work hard to promote a safe and disease free environment for growers; ToBRFV management is front of mind as they work to manage the risk in their own operations.

Growers have expressed concern that additional seed and plant testing is futile. The potential for contamination during propagation is a real concern as few growers are participating in thorough diagnostic efforts at or before these stages of production. Biosecurity approaches are influenced by operational scale and relative risk tolerance among North American growers. "We can test all of our seeds at the highest level but if the grower's crop growing next to ours at propagation is contaminated then what good does it do us?" questioned one grower reflecting on his seed testing protocols.

Greenhouse Production

Visual inspection of all plant material on planting day is a helpful step to confirm that only healthy plants are introduced into the greenhouse, though as reviewed above, steps should be taken with seeds and propagated material to prevent introducing risks. The presence of symptoms alone is not suitable for detection of ToBRFV, since the virus can exist for several weeks and be triggered later.

Many growers choose to select samples at this time for testing onsite or offsite as an added layer of caution. Close monitoring of the crop leading up to full fruit load is essential, particularly in areas of the greenhouse where ToBRFV may have been established in previous crops. Experiencing recurrent infection in areas of the greenhouse forces growers to heighten their scouting and diagnostic efforts in these areas after replanting them. Many growers have designated one full-time employee specifically to ToBRFV scouting and regular plant sampling as well as environmental testing to ensure proper sanitation throughout the facility.



To appropriately manage the risk of diseases where expression is delayed, aggressive plant removal is necessary. Upon detection of ToBRFV infected plants in the greenhouse, several rows on either side of the infected plant are destroyed which equates to hundreds of plants and thousands of pounds in lost production. Testing in surrounding rows, with consideration to the flow of labor, to assess disease presence is subsequently conducted.

The need for accurate, efficient and early detection is key to prevent unnecessary losses. Growers should work with a qualified laboratory to understand and access diagnostic resources that best suit the needs of their operations. The development of on-site diagnostic tools such as ToBRFV specific immunostrips or AmplifyRP in recent years has improved efficiency in growers' response to ToBRFV and helped them slow transmission in their greenhouse.

Mapping disease presence and tracking the spread supports operations in managing the disease once it has been established. It is also helpful to note physiological responses to the diseases for each variety in the presence of disease and share this with seed suppliers. For example, growers have noted a decrease in average stem diameter as a health indicator in their crop and a first response to general stress or disease pressure.

Using our knowledge of how the virus moves throughout the plant and the greenhouse can help us determine a proactive method for sampling and diagnostics. Because the virus moves to the roots of the plant within days after inoculation occurs, drain water taken from each gutter may be used as an indicator of early disease presence. A study by ScientiaTerrae in Belgium shows ToBRFV can be detected in drain water before symptoms are seen and virus can be detected in the leaves. Growers are encouraged to use sampling of both drain water and plant material to be proactive in their disease detection in the greenhouse.

Sanitation

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Regardless of disease detection, sanitation is critical in preventing disease transmission through daily work and in the growing environment. "We treat the greenhouse like we have it. It's not a matter of IF we get Rugose, it's WHEN," stated another grower describing how they work to prevent widespread disease.

Growers are forced to investigate the effectiveness of every detail of their cleaning procedures for materials that enter the greenhouse right down to employee clothing and uniforms.

> We've asked our commercial laundry company to do studies on the effectiveness of killing ToBRFV on contaminated uniforms. We have determined that prolonged, high temperatures in the drying cycle are effective."

Regardless of the type of material from fabric to concrete, ToBRFV persistence makes eradication of the virus nearly impossible once it



Did You Know.

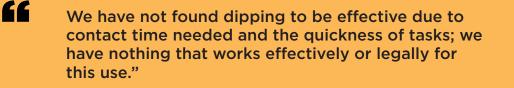
ToBRFV's high stability allows it to live on surfaces for 6 months or more and can remain infectious in organic debris for up to 20 years according to research at <u>Michigan State University</u>¹⁰.

¹⁰ https://www.canr.msu.edu/news/tobrfv-a-new-concern-for-tomato-and-pepper-producers#:~:text=There%20are%20reports%20of%20spread,is%20the%20 key%20to%20avoidance

has been introduced in the greenhouse. Exclusion, early detection, and aggressive extraction of plants in the infected area are essential to maintaining an environment with low viral load. One grower advises, "do not let the viral load build up. We can't get rid of it now in the east section of our greenhouse, it just keeps coming back."

Sanitizer efficacy is reliant on many factors; it is critical to ensure that the target organism can be killed, that concentrations maintain efficacy, and the application includes practical exposure time. Fortunately the work of Dr. Kai-Shu Ling, Lead Virologist on the issue at the USDA-ARS, has provided the industry with answers on which disinfectants are effective against ToBRFV in the greenhouse setting. His study, <u>Effective</u> <u>Disinfectants Against the Spread of Tobamoviruses</u>¹¹ highlights the efficacy of sanitizers like Virocid against ToBRFV.

Considerations for sanitation in the greenhouse include worker safety with regular handling in crop tasks, the need for short exposure times on tools and surfaces, and food safety. As always, product labels should be followed for the application. Preferred disinfectants like Virocid and Virkon are not permitted to have direct contact with food, a barrier for use within the rows of a ready to eat product like tomatoes.



This is a critical area where growers need resources to mitigate potential disease transmission from plant to plant. Studies from Dr. Fox at Fera Science showed survival of ToBRFV on hands and gloves beyond 2 hours. While Virocid and Virkon have proven to be effective at short exposure times, research to evaluate the safety of this application continues.

Growers are hopeful that updated labeling will support additional uses including tool dipping within the rows between plants. For now, some growers are assigning pre-disinfected tools, equipment and employees to designated areas along with frequent glove changes to reduce the potential spread. Greenhouses wrap trolleys and carts with removable plastic wrap to prevent contact with infected plants and remove and replace the covering on a regular basis along with frequent sanitation.

Growers are finding success with disinfection using these chemicals as part of their clean out toolbox, and though cleaning before sanitizing has always been the key to managing plant diseases, additional sanitation steps for ToBRFV are necessary. Due to the persistence of this virus, some greenhouses report taking additional steps to reduce the viral load on plant material by applying disinfectant to plant material prior to removal to decrease the viral load and potential for spread. Heat treating the greenhouse is another step in attempting to dry out and kill off pathogens before careful and detailed removal of the organics. Several additional phases of sanitation using chemicals or steam treating surfaces are involved in the cleanout process.

Even after a thorough clean out, some growers report detecting the virus in areas where the crop was heavily infected. What we've learned is the importance of keeping the viral load low during production through diligent removal of infected and surrounding plants upon detection, followed by detailed removal of organic debris and use of effective disinfectants on all surfaces.

It is also critical to focus on the regular disinfection of common areas such as breakrooms, bathrooms, locker rooms, offices and any area that employees may enter working in the greenhouse.

¹¹ https://virologyj.biomedcentral.com/track/pdf/10.1186/s12985-020-01479-8.pdf

Oftentimes these areas are overlooked and can be the source for recurrent disease transmission. Operations may use environmental testing to validate cleaning efficacy prior to replanting.

With shorter crop cycles and regularly scheduled cleanouts, we expect to see a shift in the overall success of IPDM programs. With a reset on all pests and diseases it may help growers to promote more efficient use of biological controls and reduced pesticide usage. It is possible that by embracing these clean starts with proactive approaches, such as early detection and detailed recordkeeping of all pests and diseases, growers may realize reduced expenses in their IPDM Programs. This may be one silver lining for growers and consumers alike.

Changes In the Growing System

After preparing a clean environment for plants, growers are focused on growing more resilient plants. More than 60% of the growers we surveyed changed varieties over their entire space due to the risk of ToBRFV. Growers are looking to seed companies and fellow growers for advice on choosing cultivars that show moderate resistance or decreased expression of the virus. With this, small trial areas or whole facilities are transitioned to these relatively higher performing varieties.

We are also seeing a shift from grafted, pinched (double head) plants to single stem plants. USDA-ARS Virologist, Dr. Kai-Shu Ling, still recommends the use of grafting as a strategy for growing more resilient plants. Growers are moving away from overlapping crops (interplant) to shorter monocrops with planned clean outs. All this equates to doubling the expenses for seeds and plants, along with gaps in production albeit planned.

Some growers have chosen to rotate facilities with non-susceptible vine crops like cucumbers to avoid disease pressure and unexpected loss. This is not an option for all facilities due to the differing needs between tomatoes and cucumbers particularly during harvesting and in the market. One Canadian cucumber grower reports a saturated market due to the shift in tomato acreage towards cucumbers as a result of ToBRFV.

Fortunately our packhouse is set up to also pack and ship cucumbers, we are grateful that we can switch to cucumbers and use crop rotation to manage the disease in compartments."

Seed Resistance

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Progress has been made to understand the performance and expression of ToBRFV within popular varieties of tomatoes.

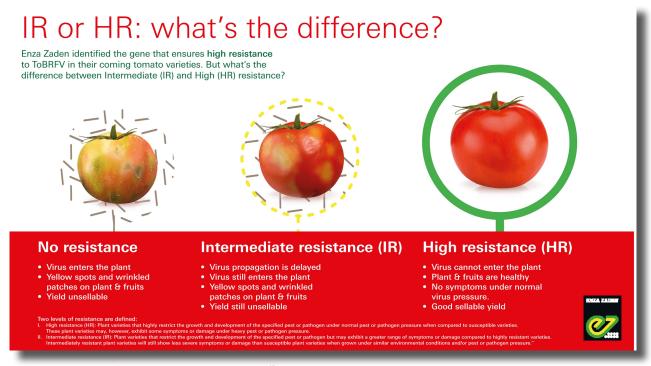
Trials were once executed as single rows, now I'm expediting trials on some varieties that I have heard perform well to move away from the ones we see are more susceptible." Still there are many variables that impact the expression of ToBRFV in these varieties with intermediate resistance including climate and other localized risks. Access to highly resistant varieties is foundational for the successful management of ToBRFV in the future. Resistance in both the scion and rootstock varieties will be necessary to reduce the risk in grafted crops. Seed companies are racing to develop highly resistant varieties and to promote commercial availability. This is the answer all growers are waiting for, though it is still at least 1-2 years away.

"Seed companies are doing what they can. Everyone is racing to develop resistant varieties. You can imagine how long it will take to incorporate resistance into all of their varieties."

- Dr. Parm Randhawa - President at CSP Labs

Growers believe that resistance is the most critical step in making progress towards managing this risk. "ToBRFV came from the seed companies, that's where we need to solve the issue and then with early detection," says an experienced grower.

The difference between the Intermediate Resistance (IR) and High Resistance (HR) is whether the plant reacts to the disease in the presence of ToBRFV. Varieties with high genetic resistance can be infected without expressing any symptoms or damage.



"IR or HR: what's the difference" Enza Zaden Nov 18 2022

In the face of persistent diseases like ToBRFV this level of resistance decreases the risk level from catastrophic to manageable. Recently, Enza Zaden <u>announced</u>¹³ that they have 7 highly resistant varieties adapted for Mexican growing conditions now commercially available. In parallel, the development of a plant virus vaccine is underway in Belgium and Canada using an attenuated strain of the virus. Initial trials not only show a reduction in symptom expression, but also an increase in fruit yield and quality. Growers will need to wait for the proper registration process to occur before it will be available for commercial use.

¹² https://www.enzazaden.com/infographic-ir-vs-hr-resistance-tomato-virus

¹³ https://www.hortidaily.com/article/9438994/new-tobrfv-resistant-varieties-presented-for-mexico/

Early Detection

With continued progress in the holistic management of ToBRFV, early detection is and will remain critical to reducing loss. Learning to live with the virus has taught us a lot about the timing and expression in the crop. With ToBRFV specifically, we are reminded of the importance of maintaining diligence in managing disease we once achieved control over.

With the help of technology, we can leverage early detection efforts, and reduce human error and bias. Many operations are challenged by labor and skill deficits, and may not be equipped to detect risks efficiently thereby increasing the risk of widespread infection.

Artificial Intelligence can make all the difference in these systems. At IUNU, we use computer vision to recognize reduced vigor and early symptoms of diseases in individual plants, map hot spots and alert growers to take action. As we learn to live with ToBRFV and add it to our scouting roster of crop risks, it will be critical to remain alert and amplify our efforts with technology.



Resource

By understanding the fundamentals of disease management and identifying opportunities for exclusion or elimination throughout the various stages of production you can develop a program to suit the needs of your operation.

Biosecurity & ToBRFV Management Checklist: From seed to harvest ¹⁴

Conclusion

It's been all hands on deck as the industry has navigated the waves of the ToBRFV catastrophe. We've made significant progress in various areas of understanding, excluding, and managing the virus from all angles of the industry and identified key areas for continuous improvement.

As we start to get our heads above water on this issue we admire the perseverance and resilience of growers and operations. Hearing grower stories and discussing challenges with experts in the industry empowers us to work harder to support them in their daily work and risk management. We're riding this wave with them by developing custom biosecurity programs, advocating for resources, building technology that aids in early detection and loss reduction, and making their important work of providing quality food a little bit easier

To learn how you can use LUNA AI to help detect ToBRFV or to talk to a IUNU Advisor about your IPDM programs, reach out to ToBRFV@IUNU.com.