Project title:	Tomato Brown Rugose Fruit Virus (ToBRFV): Grower experience case studies
Project leader:	Dave Kaye, ADAS
Report:	Final report, November 2020
Previous report:	None
Key staff:	None
Location of project:	ADAS: Desk-based study.
Industry Representative:	N/A
Date project commenced:	1 August 2020
Expected completion date:	02 November 2020

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dave Kaye

Plant Pathologist

ADAS UK Ltd

Signature ..... Date .....

Report authorised by:

Angela Huckle

Associate Director

RSK ADAS Horticulture

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

The first reported outbreak of ToBRFV in the UK occurred in 2019, with five further outbreaks reported during 2020. On November 1st 2019, ToBRFV became subject to European quarantine measures (Q-status). The virus has now been confirmed on many of the world's largest production areas. Despite these outbreaks, information on how the virus spreads and interacts within the hi-tech production systems used by European growers is limited. More information is needed to improve disease avoidance and management strategies to support the UK industry.

Background

ToBRFV is a newly emerged and highly infectious virus of tomato and pepper. Emerging in Israel in 2014, ToBRFV spread rapidly, and is now present on many of the world's largest tomato production areas, including in the UK and the Netherlands.

The origins of ToBRFV are unclear. It is a tobamovirus, related to Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV), both of which have historically impacted tomato production. Breeding programmes successfully incorporated the *Tm-2*^{2} resistance gene into almost all commercial varieties providing protection against both ToMV and TMV.

ToBRFV breaks *Tm-2*^{2} resistance, meaning they are susceptible to infection. It is only once full resistance has been bred into commercial varieties that effective and reliable control will be achieved. Although considerable work is currently underway by breeding companies, at the time of reporting no commercial variety is fully resistant to ToBRFV. However, the seed company Bayer is currently trialling tolerant varieties, whilst Enza Zaden claim to have also identified a gene providing high levels of resistance against ToBRFV. This is a positive step, but tolerant varieties can still be infected with ToBRFV which can act as an inoculum source for other, non-tolerant varieties.

ToBRFV symptoms are known to vary by variety, geographical location, growing conditions, and abiotic stress. Symptoms may develop anywhere on the plant, including the fruit, leaves, stems, calyces etc. There are claims some varieties are asymptomatic, whilst others have been reported to die within weeks of symptom development. No chemical treatments are available to control any viral disease of tomatoes.

The impacts of ToBRFV on a production site can be catastrophic. During severe outbreaks, 100% infection rates can develop which can lead to loss of the entire crop. In reality crops are removed early when they become unprofitable, or to reduce build-up of inoculum on-site to maximise the likelihood of successful eradication.

As a response to ToBRFV many businesses updated their biosecurity and hygiene protocols after the first European outbreaks in 2018, with many investing in costly equipment e.g. low pressure steam sterilisers. Despite this, five further outbreaks developed in the UK in 2020. More work is needed to identify the sources of these infections, including addressing weak points in their processes. In addition to this, businesses need information and guidance ensuring best management and eradication practices are followed where outbreaks do occur.

Summary

Project aim: To undertake desk-based studies of the management of a ToBRFV outbreak in the UK from the perspective of three affected businesses, including how the virus was found, reported and subsequently managed.

Project objectives:

1. To inform better practice to growers, including improved management strategies for the avoidance of ToBRFV infections; strategies for reducing this impact where ToBRFV infections occur on site, and strategies for successfully eradicating the virus from infected sites, based on the experiences of three UK growers impacted by ToBRFV.
2. To contribute to informing best-practice for future surveillance activity undertaken by inspectors of the Plant Health and Seed Inspectorate (PHSI).

Due to the combined viral risks of ToBRFV and COVID-19 only one on site face-to-face visit was possible. Growers were contacted and the topics discussed via email, telephone and Microsoft Teams.

Overview of the outbreaks at Sites 1-3

Site 1

During 2019 the first UK ToBRFV developed at Site 1. Symptoms of ToBRFV developed in one of three varieties, in a 6.5 ha block shortly after planting, including nettling of the heads. Initially considered to be mild strain PepMV, ToBRFV was confirmed by PCR analysis after samples were sent to Fera Science Ltd and a diagnostic laboratory outside of the UK. Plants were removed shortly after confirmation and disposed of via landfill. The original source of the virus is unknown, but likely occurred at, or shortly after propagation.

The site was disinfected with a variety of disinfectant products known to be effective against ToBRFV, with a new crop grown at a different propagator. No outbreaks have developed in the subsequent crop and ToBRFV was declared eradicated with all statutory notices lifted from the business.

Site 2

Site 2 is a new-build expansion of a nearby parent business encompassing 8.0 ha, split over two 4.0 ha compartments (half NFT, half substrate). Construction delays at Site 2 during 2019 led to the introduction of plants before construction was completed.

Symptoms developed in the NFT block in all varieties before spreading to the substrate block and included nettling of heads. Similar to Site 1 it was originally considered to be symptoms of the mild strain PepMV following inoculation. ToBRFV was confirmed via the PHSI statutory surveillance programme. The source of infection remains unknown, however the contractors have been suggested as a possible source.

Unlike Site 1, Site 2 attempted to grow through the infection. Eventually the crop became uneconomical to continue. The crop was removed by staff on site and incinerated. A comprehensive clean-up process was undertaken and the block replanted. No outbreaks have been reported since replanting, but the site and packhouse remain under PHSI restrictions which Site 2 hope to have lifted.

Site 3

Site 3 is part of a much larger business where tomato production contributes only a minor source of their revenue. ToBRFV developed in 2020 in a NFT crop in a 1.2 ha glasshouse. No symptoms were visible before the virus was confirmed by the PHSI surveillance programme. The crop remained symptom free until an irrigation breakdown, and associated water stress, led to sudden symptom expression. Symptoms at Site 3 were the most extreme of any of the three sites and was the only site to develop rugose symptoms on fruit.

The origin of the virus on Site 3 remains unknown and site clean-up is underway. The crop will not be replanted until next February, with the production area re-fogged with disinfectant, shortly before arrival.

Financial Benefits

There are clear financial benefits to avoiding ToBRFV outbreaks occurring, and it is anticipated that most UK tomato production sites will have made adjustments to their businesses as a response to ToBRFV. Mitigation measures will vary dependent on the business, with some adaptations taken up more than others. These vary from simply

restricting entry of visitors onto sites, to maintaining and running low pressure steam systems to sterilise equipment. Some mitigation measures can be expensive, however for many these are likely to be cheaper than managing ToBRFV outbreaks, especially on larger businesses.

The financial implications of the outbreaks at the three sites included in this work has been considerable. At site 1 the entire crop was removed early, representing 11 weeks of lost production. At Sites 2 and 3 outbreaks developed later in the season, with overall reductions in yields exceeding 40%. Fortunately all three sites are components of much larger businesses and were supported. If this had not been the case the financial implications would have been crippling, with at least one of these businesses likely closing as a consequence.

Although yield loss represents the greatest financial impact to the affected businesses, other factors impact costs. Sites 1 and 2 replaced their crops leading to periods where production areas sat empty. Experienced staff are not easily replaced and all sites chose to keep their staff, despite little work for them to do. Sites 1 and 2 replaced their crops rapidly, but it was still several weeks of man hours, as well as other associated overheads, before fruit was harvested and revenue returned.

Disease avoidance is the best strategy and all businesses must assess their risks and act accordingly.

Action Points

The following lists the ‘best practice’ procedures based on the experiences and lessons learned by the three growers impacted by ToBRFV included in this report. Implementation of these practices may not be practically, or economically, viable for all sites, but demonstrate what can be done to effectively mitigate against ToBRFV infections developing, and how to better address infections which do occur.

ToBRFV avoidance

- Continue to update site biosecurity and hygiene protocols as new information becomes available, ensure staff a fully trained in these and check that they are being properly implemented.
- Ensure all staff and essential visitors/contractors etc. are aware of, and follow, site biosecurity/hygiene practices, including wearing clean clothing, disinfecting machinery and frequently washing/sterilising their hands
    - Assess your risks: ensure that visitors have not visited other production sites recently.
    - Restrict access to production areas unless essential and restrict what can be taken in e.g. mobile phones etc.

    - Provide personal protective equipment (PPE), including cotton oversuits, booties, hair coverings and disposable gloves, and ensure these are appropriately disposed of
    - Reconsider contractors, can the business do the task in house instead?
- Test young plants for ToBRFV before dispatch from the propagator and ensure all seed used is free of tobamoviruses, including ToBRFV.
- Restrict staff movement between, and within, production sites and avoid movement between packhouses and production sites. This is especially important where fruit is imported from countries known to have ToBRFV outbreaks e.g. Spain.
- Give 'ownership' of areas/rows to specific staff members to further control movement and restrict/slow spread of ToBRFV should outbreaks occur and identify potential entry points of ToBRFV.
- To reduce the potential increased risk caused by PHSI inspectors carrying out the sampling, in some instances staff sampling under the supervision of the PHSI may be considered and agreed. If inspectors do perform the sampling, supply them with PPE and appropriately dispose of these once they are finished on site.
- Provide laundered clothing and shoes to staff, including requesting that they wear PPE when working in production areas.
- Consider installing foot and hand sterilising machinery at the entrances to glasshouses and sites.
- Ensure feet/wheel dips are made available at entrances and frequently topped up with appropriate disinfectants e.g. Menno Florades (benzoic acid).
- Where possible, ensure that any construction, or significant maintenance work, is completed before the introduction of any plant material onto site, and consider disinfecting/fogging new, or any potentially contaminated areas, as an added precaution.

Identification and management of ToBRFV

- Develop a ToBRFV action plan in advance of infections occurring.
- Consider site/packhouse limitations as a result of the site and/or packhouse being placed on notice, including restrictions in the movement of staff, enhanced biosecurity/hygiene and finding alternative arrangements for selling loose fruit e.g. packaging, or diversifying into meal kits etc.
- Early identification provides the best opportunity to manage ToBRFV successfully, as well as identifying the source of the disease and weak points in biosecurity/hygiene procedures.

- Establish a monitoring schedule for your crops, do not wait or rely on the PHSI statutory surveillance programme for ToBRFV confirmation but continue to report any suspicions as soon as possible.
- If ToBRFV symptoms develop shortly after plant arrival, determine whether early removal, subsequent clean-up and replanting will provide the most economical approach, or offer the best opportunity to eradicate the virus.
- Six ToBRFV outbreaks have been reported in the UK. It is no longer safe to assume symptoms are not ToBRFV and therefore samples should be sent for ToBRFV analysis.
- Be critical of what appears to be physiological and nutritional disorders, especially following periods of plant stress. Consider sending samples for analysis as a precaution.
- Ensure staff are trained and can recognise ToBRFV symptoms, including nettled, deformed heads, stem lesions and uneven ripening etc. - rugose fruit symptoms are not expected to develop in all infections.
- Maintaining high levels of plant health/vigour may delay symptom onset. This should be prioritised where crops have been confirmed infected, but are currently asymptomatic.
- Plant stress (heat, water and light) have been linked to triggering symptom expression. Avoid unnecessary plant stress where possible.

Clean up and eradication of ToBRFV on sites

- Continue to monitor, and be aware of symptoms and patterns of spread, which may inform the likely sources of introductions/entry onto production areas.
- Remove infected crop debris, taking care not to spread plant debris to other production areas. Incinerate on site, or dispose of via deep burial or a biodigester. Where infected material is sent to landfill, place it in a covered skip and dispose of in a covered lorry to reduce the risk of further spread.
- Remove and replace as much as is feasible to make disinfection easier e.g. polythene, CO_2 lines etc.
- Vacuum production areas after sweeping to remove as much inoculum as possible, followed by jet washing, disinfectant use and fogging.
- Use disinfectants demonstrated to be effective against ToBRFV at their maximum rates e.g. Huwa-San (silver stabilised hydrogen peroxide), Unifect-G (glutaraldehyde), Jet 5 (peroxyacetic acid), Menno Florades and sodium hypochlorite.
- Clean all equipment e.g. bailers, trolleys etc. in the infected area before disinfecting. Consider placing equipment in a separate area following disinfection and carrying out a second disinfectant fogging. This should be followed by sampling to confirm disinfection was successful.

- Where production areas are left empty for long periods of time before replanting, consider a second disinfection process (e.g. site fogging), as an insurance policy. This would be recommended where infected plants have continued to be grown in nearby compartments.
- After disinfection swab high risk areas to confirm status of ToBRFV. Where ToBRFV continues to be detected, e.g. after using Unifect-G consider requesting a bioassay to confirm any residual ToBRFV is deactivated and no longer viable.
- Consider treating irrigation storage reservoirs and irrigation water for ToBRFV.
- Further guidance is available at the Plant Health ToBRFV portal, or the AHDB ToBRFV webpages.

SCIENCE SECTION

Introduction

Tomato brown rugose fruit virus (ToBRFV) is a recently emerged tobamovirus disease of tomato and pepper. First found in Israel in 2014, the virus spread rapidly, becoming widespread in Israel and Jordan. Following investigation the virus was classified as ToBRFV in 2015.

The geographical origin of ToBRFV remains unclear. It has been postulated that it originated from North Africa, and is an older virus than the closely related Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV), both of which also affect tomato crops (Aviv Dombrovsky, personal comment).

The spread of ToBRFV is likely the result of an unknown series of events which allowed it to spread from an original host species (e.g. solanaceous weed) into production plants. Since 2014, the virus has been reported as being present on many of the world's largest production areas. As of October 2020, ToBRFV has been confirmed in 16 countries, including the UK (Table 1).

Table 1. Confirmed worldwide geographical distribution of ToBRFV (October 2020).

Country/region	**Date first reported /identified**	**EPPO reference (where applicable)**
China	Apr-19	EPPO 2019/143
Cyprus	July-20	EPPO 2020/173
Czech Republic	Aug-20	EPPO 2020/223
France	Feb-20	EPPO 2020/037
Germany	Jul-18	EPPO 2019/012
Germany	Jul-20	EPPO 2020/199*
Greece	Aug-19	EPPO 2019/210
Israel	Oct-14	N/A
Italy	Oct-18	EPPO 2019/013
Jordan	Apr-15	N/A
Mexico	Sep-18	EPPO 2019/014
Poland	Mar-20	EPPO 2020/122
Spain	Oct-19	EPPO 2019/238
The Netherlands	Oct-19	EPPO 2019/209
Turkey	Jan-19	EPPO 2019/123
UK	Jul-19	EPPO 2019/163
USA	Sep-18	EPPO 2019/027

* ToBRFV was declared eradicated in Germany in 2019, but a new outbreak in an organic crop was identified in July 2020.

The occurrence of ToMV and TMV in crops on commercial production sites decreased with the move towards protected cropping, and with the adoption of improved phytosanitary measures. In addition, the incorporation of resistance genes including *Tm-2²,* provided protection to almost all commercial tomato varieties grown. Sweet pepper varieties containing the *L1, L3* and *L4* genes are also resistant to TMV and ToMV.

Unlike ToMV and TMV, ToBRFV breaks *Tm-2²* resistance in tomato. To date, no commercial variety has been demonstrated to be fully resistant to ToBRFV, and a race for full resistance is underway. Bayer is currently trialling two varieties which claim to have intermediate resistance to ToBRFV. Although a positive first step, plants with intermediate resistance are only tolerant to the virus. These varieties will continue to contain active ToBRFV if infected, which may act as an inoculum source for other plants. Enza Zaden claim to have identified a high level resistance gene. Plants with this gene did not develop any symptoms when infected with ToBRFV. However, it is unclear whether the virus can still replicate in these plants (at low levels) and if these plants can still act as an inoculum source. It will not be until full resistance has been developed that the risk posed by ToBRFV will be significantly reduced.

The *L* resistance genes in pepper continue to provide protection against ToBRFV, maintaining resistance for the majority of European pepper production. In regions where pepper varieties lacking the *L* genes are grown e.g. Mexico, the impact of ToBRFV on production can, and has been catastrophic. In Sicily in 2020, 85% of a pepper crop was confirmed infected with ToBRFV in a variety which did not harbour any *L* resistance genes (EPPO 2020/080). The year before, the same greenhouse was used for tomato production and had been removed due to ToBRFV infection.

Unlike other viruses of tomato and pepper, ToBRFV has no natural insect vector. The main route of virus transmission is via mechanical contact, with humans likely responsible for the majority of disease spread. Before ToBRFV was characterised it became widespread in Israel due to mechanical transmission (usually due to visits between sites), and after 2014 ToBRFV spread in Jordan in a similar way.

The virus is not present within the seed endosperm, but can be present on the seed coat. If the coat contains viable viral particles, this could infect developing seedlings, but is considered to be a rare occurrence (Aviv Dombrovsky, personal comment). If seeds are treated correctly, as in commercial practice (Samarah, Sulaiman et al. 2020), the likelihood of infections arising from seed is even further reduced.

The first outbreak reported in Europe was in Germany during 2018, at six sites as part of a grower collective. Following eradication measures ToBRFV was declared eradicated in Germany in 2019, however a new outbreak has been reported in July 2020. The first outbreaks in the Netherlands were reported in 2019 and the virus continues to be an issue, with a further 17 outbreaks reported in 2020.

As a response to the threat ToBRFV poses to UK tomato and pepper industries, AHDB Horticulture funded two fact-finding study tours to Europe and Israel in late 2019. Protected edible production sites and specific crop research institutes in the Netherlands, Germany and Israel, where ToBRFV has been found, reported and acted upon were visited. Information from the study tours will be included in this report where relevant, including drawing comparisons with the experiences and approaches taken by affected UK businesses.

As of October 2020, six outbreaks of ToBRFV had occurred on commercial production sites in England, five in 2020 and one in 2019. After 12 months of sampling by Plant Health and Seeds Inspectorate (PHSI) inspectors, and consistent negative test results, ToBRFV was declared eradicated at the 2019 outbreak site. The five 2020 ToBRFV outbreaks are currently under eradication measures.

Despite the best-efforts of growers, the wider industry, AHDB horticulture and researchers, the development of new outbreaks in 2020 demonstrate that more work is needed. Three production sites which have been impacted by ToBRFV have contributed their first-hand experiences for this work. This includes information on the nature of the virus; insights on the grower experience of sampling protocols, diagnostic test results and clean up etc. This knowledge sharing will ultimately improve industry resilience to ToBRFV. The outputs of this report will contribute to best practice for sites, as well as for future surveillance activity undertaken by inspectors of the PHSI.

A large amount of information was gathered, some of it anecdotal in nature. In situations where the reliability of this information is questionable, or even contradictory, it has not been included in this report. Where information has been included, but lacks robust scientific backing, this is noted in the text.

Results

Overviews of three UK businesses affected by ToBRFV outbreaks

Grower Site 1

The first UK ToBRFV outbreak occurred at Site 1 in 2019, but concern was already high within the industry following the 2018 outbreaks in Germany. As a consequence, the UK industry

was already proactively seeking information on the virus. Several businesses, including Site 1, reviewed and updated their hygiene/biosecurity protocols based on the information available at the time.

Symptoms of ToBRFV developed in plants in a 6.5 ha block shortly after planting. Three varieties, Piccolo, Roterno and Delisher were grown on substrate and infection was confirmed in all varieties following PCR analysis. However, only one variety, Piccolo developed any symptoms. It is the belief of Site 1 that 100% of plants may have been infected at this time. Symptoms were restricted to the leaves alone, as the plants were young and fruit development had not started. Leaves became deformed and twisted with the heads described as ‘nettled’, with narrowed, needle like leaves (Figure 1).

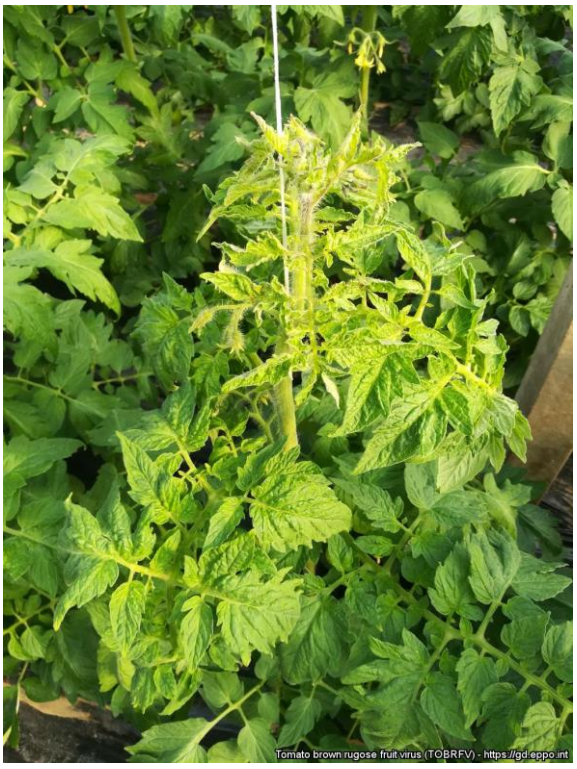


Figure 1. Early symptoms of virus expression on young tomato plant heads, including nettling of heads with narrower, needle-like leaves - Courtesy: Dr. Agr. Raffaele Giurato.

It is plausible that the Piccolo crop was infected before the other varieties, and that this variety acted as an inoculum source for mechanical spread to the others. Given more time it is anticipated that these varieties would also have developed symptoms. It is important to note that this is a theory, and variety specific susceptibilities, or general plant health/stress may explain why symptoms developed in the Piccolo crop initially.

Similar to several other outbreak sites in the UK and across Europe, this infection was originally attributed to PepMV infection which may have delayed action. All varieties grown at Site 1 had been inoculated with a PepMV mild strain and the symptoms were characteristic

of a strong response from a PepMV mild strain inoculation. The strength of this reaction is uncommon, but does occasionally happen and is attributed to environmental stress. Symptoms did not develop in clusters, but were seen across the entire crop (as would be anticipated for a crop showing symptoms after inoculation with PepMV).

This pattern of symptom distribution and progression supports the idea that the entire crop was infected at the same time. The early onset of symptoms also raises the possibility that the plants were infected at the propagation stage. However, young plants for several glasshouses at Site 1 were supplied by the same propagator. Symptoms did not develop in other glasshouses which suggests that may not be the case. The initial source of infection could also have been introduced after propagation, at any point from transportation of the young plants, to planting out.

Ultimately it is not known where the outbreak at Site 1 originated. The lag time between initial infection and symptom development makes this very challenging, especially at a time when less information on ToBRFV was available. Other factors considered include introductions from staff/visitors (contractors), equipment, machinery, trays, young plants etc.

Although not the initial source of infection, Site 1 has a recirculating irrigation system, reusing their spent irrigation solution and this may have contributed to the spread of the virus throughout the crop. The system has a disinfection process to treat the recirculating water and PCR analysis of irrigation samples taken after the disinfection process returned positive for ToBRFV. It is unclear if viral particles remain viable, or are inactivated by the disinfection process. Circulation of ToBRFV through the irrigation system may represent an additional transmission route and a bioassay is needed to confirm if the virus remains infective. If ToBRFV remained viable, this may explain why infection was believed to be uniform. This should be considered a research priority and investigated further, including the impact of alternative water disinfection methods on ToBRFV including, UV and chemical disinfection. At Site 1, the recycling storage reservoirs and the irrigation water are disinfected before use.

The presence of ToBRFV at Site 1 was initially confirmed after symptomatic tissue samples were sent to a laboratory outside of the UK, for analysis. Note, the PHSI surveillance programme did not start until 2020, the year after the outbreak at Site 1. After confirmation of virus presence, the Animal and Plant Health Agency (APHA) was notified and inspectors took further samples to confirm infection. Wearing personal protective equipment (PPE) supplied by the site, and following site specific hygiene/biosecurity protocols, PHSI officers sampled 100 plants from their base (at shoulder height), reaffirming the presence of the virus in all varieties and placing the business on notice. Notice restriction as a consequence of ToBRFV infections on production sites/packhouses are located in Appendix 2. Two other production

areas were sampled at this time, but each returned negative. In addition, Site 1 collected 20 samples from the heads of plants which were sent to another laboratory outside the UK for confirmation.

After the initial positive result, access to the infected house was restricted and all work on the crop stopped. Internal discussions within the business led to the decision to remove the crop early, rather than attempting to grow through it. This was a business decision and not one mandated by PHSI. Over time ToBRFV replicates within living plant tissue, increasing the inoculum load. Early removal increased the likelihood of fully eradicating the virus. This was aided by the fact that staff interaction with the crop (i.e. deleafing and harvesting) and associated crop debris were low.

As a consequence of this outbreak being the first in the UK, minimal information and advice was available from PHSI to support Site 1, however some guidance was provided. Hygiene and site biosecurity protocols were reviewed and assistance based on the information known provided. Information was also sought from other businesses/organisations in the UK and Europe, including growers and the AHDB ToBRFV steering group.

The irrigation system was switched off two days prior to removal as standard. Clean-up was performed by contractors, who followed site biosecurity protocols and were not allowed access to the other production areas. The plants were removed and disposed of via landfill.

Little information was available on the effectiveness of different disinfection products in eliminating ToBRFV. As no specific guidance was available within the UK at this time, assistance was sought from areas already impacted by ToBRFV, including Germany and Israel. Products, rates and their method of application were chosen based on this information, literature and other recommendations. The disinfection programme used by Site 1 following the outbreak is commercially sensitive and unable to be shared in this report, but follows protocols which have been successfully used to eradicate cucumber mosaic virus. Several common products were used at maximum rates, to achieve effective control.

The disinfection process used to eliminate ToBRFV from Site 1 in 2019 may be considered to be extreme, but was viewed as necessary by the business. It was an economic decision based on the perceived impact on the business from further infections. Following the successful eradication of ToBRFV, this process will be replicated in the event of future outbreaks. This will not become the standard clean up procedure for this site in the absence of ToBRFV outbreaks, but some components/products have been incorporated into an updated protocol for routine clean up where they were seen to add benefit.

The costs of dealing with the ToBRFV outbreak at Site 1 were significant. In addition to the 11 weeks of lost production time, the site had to pay for a replacement crop to be propagated,

as well as the extra costs associated with crop removal and site disinfection. If the crop had been removed later, it would likely have cost the business more, as well as risking spreading the virus to other houses. The business believes that identifying ToBRFV early and removing the crop helped reduce their overall losses.

As a precaution, the replacement crop was grown at a different propagator from where the previous plants were sourced. Samples were taken and confirmed free of ToBRFV infection by this propagator (at the request of Site 1), before any plants were dispatched. ToBRFV sampling at this stage accrues additional costs, ~£65 per sample processed (not including sampling time by propagator staff etc.). Sites may require several samples be collected and processed for each variety, and at multiple times and this may not be fiscally practical for smaller businesses. All seed lots produced by commercial seed houses will have been tested for, and confirmed free of, seed borne pathogens including tobamovirus (TMV, ToMV and ToBRFV) Confirmation of this is included on the seed certificates supplied to propagators. Once site disinfection was completed, the new plants were planted out within two days.

Following planting of the replacement crop the site was visited by PHSI several times. Samples for analysis were collected by PHSI at the base of the plants (at shoulder height), as well as by the business. After 12 months of negative tests from sampled leaf tissue, the virus was declared eradicated and all notice restrictions lifted. Site 1 was included in the PHSI statutory surveillance programme during 2020 and no further outbreaks have been reported.

Despite the presence of other tomato production areas at Site 1, the outbreak remained restricted to one house. This absence of spread can be considered a positive outcome, implying that overall site hygiene and biosecurity practices were sufficient to prevent spread. Although these processes were adequate, they continue to be reviewed and updated. Laundered clothing and shoes are now provided for staff and feet/hand disinfection machines e.g. Hygiene Lock, have been installed at the entrance to site buildings and glasshouses. All staff are required to wear gloves, booties and coverall suits prior to entry into production sites and hands must be disinfected before any interaction with plants.

The original source of the infection remains a mystery and potential routes of entry onto site need to be reviewed/identified to prevent introductions in the future. In the Netherlands, Nextstrain, an open source bio-informatic tool has been used to identify the diversity and spread of ToBRFV, based on isolates collected since 2014 (van de Vossenberg, Visser et al. 2020). The Dutch NPPO continues to add sequence data, improving this tool. Isolates of the ToBRFV strain which caused the infection at Site 1 are available. Sequence data for this can be included into the Nextstrain build which might provide information on the origin and distribution of this isolate.

Grower Site 2

Two of the five additional ToBRFV outbreaks, which developed during 2020 occurred at Site 2.

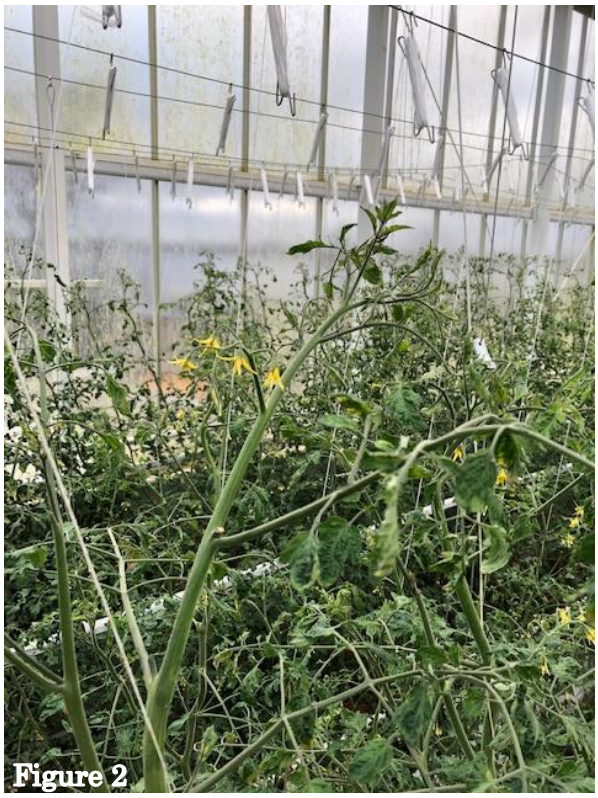
Site 2 is a new-build expansion of a nearby, but geographically isolated, parent business encompassing 8 ha, split over two 4 ha compartments. Construction delays at Site 2 during 2019 led to the business making the decision to introduce plants onto the site before construction was completed.

The first 4 ha compartment was planted in mid-July 2019 and was grown in a nutrient film technique (NFT) system. Three varieties including Piccolo, Arlinta and Roterno were grown. The second 4 ha compartment was planted in September and included the varieties Piccolo and Yelorita, grown on substrate media. Similar to Site 1, Site 2 also chose to inoculate their crop with a PepMV mild strain.

As a consequence of the construction delay, site biosecurity and hygiene was impacted and the presence of contractors and machinery/equipment further impacted the ability of Site 2 to maintain high standards. By the time the young plants were introduced most of the construction was already complete, with only some tasks, including the installation of the LED top lights remaining.

A degree of inter-lighting was already available to the plants. However, the impact of the low light levels on the crop was apparent as autumn progressed. As the plants reached the height of the inter lights, leaf distortion symptoms, similar to that described at Site 1, developed in the Arlinta crop in the NFT compartment. These arose in the first couple of rows and were initially attributed as a physiological reaction to the increase in light levels (light shock). Over time symptoms spread to the entire crop.

As with most physiological shock disorders of plants (heat stress, water stress etc.), it was anticipated that these symptoms would diminish as the plants adapted to the new conditions. However, these symptoms persisted (*Figures 2* and *3*). Samples were collected and sent to the supplier of the mild PepMV strain used at this site for analysis, and mild strain PepMV infection was confirmed. Symptoms had now become severe and were impacting yields. At this time, the substrate block remained symptom free. Advisers had seen the crop and it was determined that ToBRFV was not suspected due to the continuing growth in the crop. All accounts of ToBRFV at this stage stated that you would see plants dying if infected. Biosecurity measures were enhanced and movement between the two blocks was controlled to try to limit spread to the substrate block.



Figures 2 (NFT blocs) and 3 (substrate block) - Leaf distortion symptoms (nettled heads) as a consequence of ToBRFV infection at Site 2 - (February 2020).

The exact origins of ToBRFV on Site 2 remain unknown and have been debated by the growers on-site, and discussed with other industry members. The general consensus is that the introduction of ToBRFV infection likely arose as a result of site construction. If this is the case, these circumstances are unusual and would not normally develop on an established production site. With the availability of the Nextstrain build it is also possible to include sequence data from isolates at Site 2 to gain insights on the origin of this virus based on sequence similarities with other isolates.

The larger business, of which Site 2 is a component of, did not develop ToBRFV at its other production areas. Similar to Site 1, this suggests that biosecurity/site hygiene measures were sufficient for the business as a whole, but were compromised by the presence of contractors/construction materials. It is believed that the contractors were responsible for the outbreak, but what is not clear is if they introduced the virus onto site, or it was able to gain entry because of their activities. It should be noted that this is based on several assumptions and the exact origin of the virus remains unknown.

The pattern of symptoms implies that the outbreak developed in the NFT block before spreading to the substrate block. This is likely the case, but without diagnostic results from this time, this cannot be confirmed. Transmission between plants was likely via mechanical means e.g. movement of staff and equipment. If the contractors were responsible for the

ToBRFV outbreak it underlies the importance of knowing who is coming onto sites and where they have worked/visited recently.

Many grower holdings in the UK and Europe now request that visitors arrive on sites at the start of the day, ensuring that they have not visited other production sites, or visitors are asked to declare if they had recently visited other production sites. If visitors are considered to pose even a slight risk they may be denied entry. Only individuals deemed essential, or risk free, should be allowed onto production sites. This would have been difficult with the construction situation at Site 2, but it is possible that if contractors and their equipment had been disinfected prior to entry that this outbreak could have been avoided.

Despite the ToBRFV outbreak in the NFT block, the plants continued to grow. Symptoms were restricted to the leaves, but stem diameter reduced as general plant health diminished. Although developing fruit remained free of wrinkling/rugose symptoms, many failed to ripen fully, retaining an orange colour. Ripening issues are another symptom of PepMV infections highlighting how differentiating viral species by symptoms alone is difficult. In heavily infected plants fruit set was reduced, or fruit was aborted shortly after setting.

Light levels and temperatures were reduced in an attempt to steer the crop towards a vegetative growth pattern to increase the leaf area. This was successful to a degree and leaf area and stem diameter increased improving plant condition. Unfortunately, despite improved growth, fruit set remained impacted and fruit abortion continued.

Site 2 was visited by PHSI in March 2020. Due to the highly persistent, and easily transmissible nature of ToBRFV, this site was concerned about the risks associated with the presence of PHSI inspectors on site. This business, along with most of the industry, closed their doors to all non-essential visitors in 2019. PHSI inspectors were considered high risk because they visit other production areas, potentially and unknowingly spreading the virus.

Under guidance from the PHSI officers, staff at Site 2 performed the sampling process themselves. Fifty leaf samples were taken from each variety (five varieties, spread over two blocks), with gloves changed between each variety samples. Samples were taken at shoulder height and placed in pre-prepared bags. At this time only plants in the NFT block were visibly symptomatic of viral infection.

Results were delivered within four days of sampling. ToBRFV was confirmed present in both the NFT and substrate compartments, despite the substrate compartment being free of symptoms. With ToBRFV confirmed a second round of sampling was performed by PHSI directly, under grower supervision. Samples were taken as before, with gloves changed between each variety. In addition, samples were also collected by the grower and sent to a laboratory outside the UK for independent confirmation. The sampling procedure

recommended by this laboratory differed from the PHSI sampling protocol and samples were collected at the heads of the plants (the same procedure used by Site 1). Gloves were replaced more frequently, after each plant was sampled to prevent cross contamination to uninfected samples.

Results from the second round of sampling from both laboratories reconfirmed the earlier results with high levels of ToBRFV detected by PCR (corresponding to a low CT value) by Fera Science Ltd. Lower levels of ToBRFV (higher CT scores) were reported by the non-UK laboratory and this discrepancy is likely a consequence of the primers used by the different laboratories, or differences in the inoculum levels in the leaf material sampled. At this time nettling symptoms were starting to develop in the heads of plants growing in the substrate. PHSI placed Site 2 on notice (see appendix 2 for details) and discussed clean-up plans with the business. Little guidance was able to be provided on how to manage the infected crop, a consequence of the limited information which was available.

Fruit from Site 2, and its parent company, is processed at an off-site central packhouse facility. Fruit is supplied in crates which are returned to the production site of origin, never shared between different production areas. As a consequence of the statutory notice, Site 2 was unable to sell loose fruit and all fruit was packaged in cardboard trays with plastic wrappers, before being sent to supermarkets in clean trays. These processes were considered by PHSI to be sufficient to reduce the transmission risk of ToBRFV and fruit prepared in this way were permitted to be sold. As standard for Site 2, all returning crates were passed through an automatic tray washer using hot water (under pressure) and the disinfectant Huwa-San (silver stabilised hydrogen peroxide) applied at the manufacturers recommended rates (6% solution). This has been demonstrated to be effective against ToBRFV on hard plastic (AHDB project PE 033), dependent on the rate used. At 12.5% (the recommended concentration for glasshouse disinfection, this was effective after 1 hour of contact time. When applied at 3% for one hour, Huwa-San was not effective against ToBRFV (PE 033a). It is unknown if the tray disinfection process used at Site 2 is sufficient to eliminate the virus. In addition to the rate used, effectiveness will depend on the exposure time, temperature of disinfectant solution, how long the trays remain ‘wet’, the cleanliness of the trays and the duration of time they are washed for. Studies (AHDB project PE 033) demonstrated that handwashing for extended times (in excess of 60 seconds) may reduce ToBRFV inoculum levels (to below detectable limits), even in the absence of disinfectant. This was not consistent, but the cleaning process of the tray wash is likely to be more vigorous than simple hand washing, and this may be sufficient, even if the disinfectant rate is lower than that shown to be effective. The only way to establish this would be to place trays inoculated with ToBRFV through this

process on site, however this would likely pose an unacceptable risk to any business which hopes to have eradicated ToBRFV from their business.

Despite best efforts to grow through the infection by improving plant health, including steering the crops more vegetatively, the decision was eventually made to pull out both compartments. The NFT block was removed on 12 May (43 weeks after planting) before the substrate block was taken out on 22 June (48 weeks after planting). Some plants were still producing marketable fruit, but continuing production would have led to a further increase of ToBRFV inoculum on Site 2 and there was no economic value in continuing. At removal roughly 60% of plants (across all varieties) were symptomatic of ToBRFV infection. However, it is anticipated that all plants could have been infected by this time.

Clean-up at Site 2 included the use of several disinfectant products at the maximum rates recommended by the manufacturer. This included Jet 5 (1:125), Unifect G (1:25 rate) and sodium hypochlorite (rate used unknown).This was followed with fogging using Jet 5 (1:12 rate, with 1 litre of Jet 5 per 200m^3 of space) and was carried out by staff at Site 2, not contractors.

The crop was carefully removed using a conveyor system rolling the stems into bales. Shredding of plant material was not practiced. As an isolated site, an exemption license was granted for the bales to be incinerated on-site. This avoided the need to store infected material and sending it to landfill, which risks further spread. After bailing, the conveyor was cleaned in the compartment using an air jet to remove any dried on material, including debris located in hard to reach places. This was followed up with treating with a chemical disinfectant e.g. Virkon S (1:100). The conveyor was moved to a warehouse, fogged again with Jet 5 (1:125) and swabbed and sent for lab analysis for ToBRFV to confirm disinfection was successful. All other critical equipment was sterilised as described above, with non-critical equipment disposed of via deep burial, before being replaced with new.

After plant removal the site architecture and equipment were cleaned down thoroughly. The lights were cleaned and covered with new bags to protect them from damage during the rest of the disinfection process. The rows were swept thoroughly to remove as much plant debris as possible before being vacuumed to remove the rest. The CO_2 lines were removed and the glass and gutters jet washed, before any disinfectants were applied. After which both compartments were fogged with Jet 5 (1:125).

The NFT block was left empty for two months. This block was fogged with hydrogen peroxide once, whilst the substrate block was fogged twice. This was a consequence of cropping continuing in the substrate block as the NFT block was cleaned. As a precaution before any new young plants were allowed onto Site 2, the compartments were swabbed and sent for

laboratory analysis to check for the presence of ToBRFV. This was not at the request of PHSI, but was to give peace of mind to the business. During the outbreak PHSI provided no specific guidance on how the crops at Site 2 should be monitored for ToBRFV symptoms, but did require monthly updates on the situation from Site 2. Businesses are advised to have in place a continuous monitoring strategy to pick up symptoms or subtle changes observed in the crop.

Unexpectedly, following disinfection none of the swab samples sent for laboratory analysis by real time RT qPCR were negative for ToBRFV. This included two negative controls, one was used to swab brand new polythene at this site, whilst the other remained unopened. These results were false positives and attributed to contamination during transit. Other negative controls from the same batch of swabs (which were not sent to Site 2) were processed and returned negative for ToBRFV.

The positive ToBRFV results from the disinfected areas was very concerning as this implied that their efforts had been insufficient to eliminate ToBRFV. The disinfection process utilised a variety of disinfectant products, one of which was Unifect G (glutaraldehyde). In addition to their use as a disinfectant, glutaraldehydes are used as a biological fixative, preserving materials. It is possible that this was responsible for the positive results, but research is required to confirm if this is the case. Site 2 went through successive rounds of real time RT qPCR testing, sampling from the same ten locations each time, and although negative results never occurred, the quantity of the virus was found to reduce (corresponding with increased CT values).

Not knowing if the virus had been eradicated from Site 2 was frustrating. Site 2 believed that everything that could have done to eradicate ToBRFV had been done and the gradual reduction in CT values was seen as strong evidence for this. A new crop was propagated by the original UK propagator who supplied the business and these were due to arrive whilst the site was still receiving positive test results. The plants were confirmed free of ToBRFV before dispatch.

PCR analysis is unable to distinguish between viable and non-viable virus particles, unless the genetic material is sufficiently degraded. The nucleic acid preserving properties of glutaraldehyde may have extended the period that non-viable virus particles tested positive. As a consequence of the positive ToBRFV results, a bioassay using indicator plants was run to investigate the infectivity of returned swabs. The propagator was contacted and requested to hold the plants for a further 10 days to allow time for this to be completed. The day before the plants were due to arrive, the bioassay confirmed that no viable virus was present. Swabs saved from earlier sampling dates (pre site disinfection) were tested also and gave positive

results, validating the test. The new NFT crop was planted out on the 22 July 2020, with the substrate block planted two weeks later.

The cropping cycle was effectively reset, with similar quantities of the original varieties planted in 2019. Site 1 continues to be monitored closely with samples taken regularly for ToBRFV analysis by the grower. To date (October 2020) no plant has tested positive for ToBRFV. Usually, ToBRFV will be declared eradicated from the site if the new crop planted in the Spring is sampled at an appropriate growth stage and tests negative for ToBRFV. PHSI notices will be then lifted.

Notices placed on businesses can be restrictive, limiting the ability of affected businesses to fully operate and will not be lifted from a site until the new crop is negative for ToBRFV when tested.

Grower Site 3

Site 3 is part of a much larger business where tomato production contributes only a minor source of their revenue. The tomato area was split between one soil grown (not organic) house (2.0 ha) and one NFT house (1.2 ha), both grown under old glass. A different cherry tomato variety, DRC564, was grown at Site 3 compared with Sites 1 and 2 and this crop was not artificially inoculated with a mild strain of PepMV. Although ToBRFV had already impacted the UK by this time (Site 1), the decision to reduce the area designated to tomato production was unrelated to this.

Both houses were planted in February 2020 with young plants propagated by a UK propagator. In mid-April 2020, Site 3 was visited by PHSI as part of the statutory surveillance programme, with samples collected and sent off for analysis. Inspectors arrived first thing on a Monday, confirming that they had not been to other production businesses for at least 24 hours. Due to recently imposed coronavirus safety measures, the production areas were made free of staff allowing PHSI to sample unsupervised. The inspectors were supplied with PPE, rather than use their own. This included shoe coverings, cotton oversuits, booties and gloves. Inspectors were requested not to take items into the glasshouse apart from essential items including sample bags.

Results were returned to Site 3 within days and confirmed a medium to high level of ToBRFV infection at the NFT site (corresponding to a medium to low CT score). Fortunately ToBRFV was not detected in the soil grown crop grown at the other production site.

As ToBRFV may also affect capsicum species (Panno, Caruso et al. 2020) and this business also cultivated chilli in a separate glasshouse geographically isolated from the tomato production area. Samples were taken from the chilli plants (Habanero, Dorset Naga, Carolina

Reaper, Jalapeno, Birds Eye and Cayenne) in May, all of which returned negative. It is anticipated that these cultivars contained the *L1, L3* or *L4* resistance genes which protect most capsicum species against ToBRFV.

After the positive test results, PHSI returned to Site 3 to discuss the options available to the business. As with Sites 1 and 2, production was able to continue at the growers discretion, but a notice was put on the packhouse and production area (see appendix 2), and loose fruit was no longer permitted to be sold. This was an issue for the business as some fruit had always been sold in this way. As an alternative, fruit which would have been sold loose was packaged and sold in 'recipe boxes' e.g. HelloFresh, Mindful Chef etc. Fruit was also packaged and sent for processing.

Unlike the other sites, no ToBRFV symptoms were present in the affected glasshouse when the virus was confirmed. Symptoms did not develop for some time, and Site 3 continued to cultivate their crop which appeared healthy and vigorous. Despite this, additional biosecurity measures were put in place. Special efforts were made to explain the situation to staff, and movement between sites was restricted to prevent further spread. Workers were required to wear fresh, clean clothing and footwear was also provided. Foot/wheel dips containing Menno Florades (benzoic acid at label rates) were placed at all entrances and used by staff, as well as machinery/equipment e.g. forklift trucks. Hand washing became mandatory before entering any production area and hand sanitiser was made freely available.

Attempts to identify the source of the outbreak at Site 3 have been unsuccessful. Many sources were proposed including the introduction of infected plants from the propagator, or from visitors contaminated with ToBRFV. Although possessing only a small production area at Site 3, the business imports a large quantity of fruit from Spain and the Netherlands to their packhouses, one of which was on the site of the infected plants. These countries are both known to have ToBRFV infected production areas and it is possible that an inoculum source was introduced from infected fruit imported by the business. As a mitigation measure packhouse staff are now prohibited from entering production areas, with other staff movement carefully controlled. Trays and equipment which move between packhouse and infected production areas were not routinely washed/disinfected, but this is likely to be implemented moving into the next season. It was also suggested that the virus may have infected the site at the end of 2019 and subsequently carried over to the 2020 crop. This is possible, however no visible symptoms developed in the crop, suggesting if introduction did happen, it occurred towards the end of the growing season, and that site disinfection was insufficient to eradicate this. All potential sources of infection have been, and will continue to be, scrutinised and site biosecurity protocols routinely updated to further mitigate against introductions to future crops.

In May an underground irrigation pipe burst which contributed to an irrigation system failure in June, a significant problem for any NFT crop. This occurred on a weekend where on-site staff numbers were low and the issue was not spotted for several hours before being repaired. Weather conditions were hot during this period and the plants were significantly affected.

Physiological symptoms of heat stress developed over the next few days and progressed rapidly. These included sun scorch of the canopy and blossom end rot (BER) development in fruit. ToBRFV symptoms also developed at this time, suggesting the heat and water stress from the irrigation issue was the trigger for symptom expression. Lesions developed on leaves, with leaf tips turning yellow and brown from tissue death. Symptoms developed in the middle of the stems before moving towards the canopy (Figure 4). This is interesting as it differs from the symptoms seen at Sites 1 and 2 where nettling of the heads and a reduced canopy size was the most significant early symptom.

As symptoms spread, truss and fruit size decreased and fruit set was reduced, with some fruit abortion occurring. Some developing fruit, or fruit which did not abort, suffered ripening issues, or displayed ‘typical’ rugose symptoms, not seen at the other site which had a fruiting crop (site 2). By mid-June 2020 volumes were down on initial projections and continued to reduce. Once symptom progression reached the top of the canopy, thin nettled heads developed. The cherry variety (DRC564) (grown at Site 3) was a generative variety. Similar to Site 2, the grower considered steering the plants vegetatively as an option to improve plant condition. However, the rapid onset and progression of symptoms made this impossible.

Figure 4 (left). ToBRFV symptoms developing from the middle of the stem towards the canopy following irrigation failure at Site 3.

Figure 5 (right). Rugose fruit symptoms in severely affected plants – Site 3.

ToBRFV symptoms initially developed in patches, rather than uniformly across the crop. This sporadic spread further supports the argument that the infection was not introduced during propagation as it would be anticipated that all plants would be similarly infected.

Although initially patchy, a pattern of symptoms did develop. The business at Site 3 uses a biomass boiler to supply heat to the affected house. Heat is fed into one side and then distributed across the house leading to one side being hotter than the other. The distribution of ToBRFV roughly corresponded with this heat gradient, and greater symptom severity was linked to the warmer side. This was a temporary observation as symptoms continued to develop. By the time the crop was ended two months early (September 2020), 100% of plants were visibly infected, with several occurrences of plant death. At this time some marketable fruit was still being produced, but continuing the crop was uneconomical. Over the 2020 season it was anticipated that marketable yields were down by as much as 50%. If Site 3 had not been part of a much larger business it would have found itself in severe financial hardship, or closure.

After the final harvest the glasshouse was closed. As is standard, the irrigation system was switched off before the crop was removed to use up the remaining NFT solution in the system. The crop was removed by staff at Site 3 and similar to Site 2 was incinerated on site.

At the time of writing Site 3 was still planning their site disinfection process, with it due at the end of 2020. Unlike Sites 1 and 2, Site 3 is waiting until spring 2021 to replant their crop. As a consequence of this they intend to disinfect the site thoroughly this year, and then again in early 2021. This second lighter touch disinfection will be done as an insurance policy as they recognise the need to eradicate ToBRFV completely before introducing new plants.

Single use troughs, floor plastics and any single use plastic will be removed, disposed of by landfill, with some materials incinerated on site. All site architecture, including, but not limited to the paths and rails, will be pressure washed with water before disinfecting using a variety of disinfection products with different modes of action. Huwa-San, Unifect G, Menno Florades and a quaternary ammonium compound (QAC) will be used at recommended rates. This will be followed by a fogging with Jet 5. In addition to the site architecture all machinery and equipment will be disinfected and access to the cleaned site limited until preparation begins for the 2021 season.

The site will continue to be swab tested for ToBRFV, however the use of the glutaraldehyde (Unifect G) may result in a similar experience to Site 2 where ToBRFV was detected after disinfection. A bioassay may be required to confirm eradication.

Discussion

The experiences of Sites 1-3 have been reviewed, with similarities and lessons learned highlighted. Where appropriate information gathered during the study tours to Europe and Israel has been included. This has been split into five sections;

1. ToBRFV origins
2. ToBRFV identification, symptoms and management
3. PHSI sampling and guidance
4. Clean up
5. Future management

ToBRFV Origins

The main transmission routes for entry onto UK production areas is believed to be workers/visitors, infected seed and young plants infected at propagation (grown outside of the UK). Contaminated fruit, either from imported fruit packed onsite or from workers lunch boxes, also poses a risk, however fruit produced from outside production areas is almost always banned.

The lag time between initial infection and symptom development, and the ease at which ToBRFV is mechanically transmitted, makes identification of the initial source of the virus difficult. One thing that is common between all impacted businesses is that ToBRFV was able to establish itself on sites despite the biosecurity and hygiene procedures put in place to prevent this from happening. Another thing common for all sites is that none are 100% certain as to the actual source of the virus on their sites. The best management approach is always disease avoidance and more research is required to identify weak points in site hygiene and biosecurity to prevent further introductions occurring.

The early development of symptoms across the whole crop at Site 1 suggests that ToBRFV originated at the propagator, however this same propagator supplied plants for several other production areas at Site 1 and ToBRFV was not detected in these plants. Infection may also have occurred during transit e.g. from an inoculum source in the delivery lorries, or via contact with infected machinery/staff. This is also considered unlikely as symptoms were relatively uniform and would be expected to be more sporadic if infected in this manner. Other possible sources of inoculum are the compartment at Site 1 itself (carry over from the previous crop), or staff/visitors. The true source of ToBRFV at Site 1 will remain unknown. However, as symptoms developed early and rapidly, it can be concluded with a high degree of certainty that infection occurred at, or very shortly after, propagation.

The plants delivered to Site 2 were unlikely to have been infected at propagation as symptom development did not occur until they were established. In addition, no outbreaks developed on the main site which was supplied by the same propagator. The situation at Site 2 was unusual in that it was a construction site when plants were introduced. As a new build, carry over of ToBRFV from the previous season was impossible and it is the belief of the business that a contractor, or other visitors were responsible for the infection. Symptoms were initially seen down the first few rows of the NFT block supporting the theory that people/or equipment were the cause. This is the same pattern of infection which developed at several of the German outbreak sites during 2018 where the virus was spread by visitors walking down the first few rows. Symptoms initially developed in the NFT block implying that this was infected first, before being spread to the rest of the production area via mechanical transmission.

At Site 3, tomato production is a smaller component of the overall business output therefore the standards of hygiene and biosecurity may not have been as high as those practiced by growers exclusively growing protected crops. Infection was confirmed by the PHSI surveillance survey, and almost all plants became symptomatic of severe infection following irrigation failure. This indicates a uniform level of infection, however the pattern of symptom severity within the glasshouse followed the heat gradient from the heating system, with more severe symptoms initially observed in warmer areas of the glasshouse. The source of

ToBRFV at Site 3 is unknown but was likely introduced by staff or visitors and subsequently spread by staff and/or machinery. Since the outbreak, site hygiene and biosecurity procedures have been enhanced.

Introductions of diseases onto sites from propagated plants is a major concern for any grower, and one which is taken very seriously. Significant efforts are made by propagators to mitigate against spreading diseases to production sites. As a consequence of ToBRFV, propagators, like the rest of the industry, have restricted entry onto sites to essential visitors only and have increased biosecurity measures in place. Seed producers follow International Seed Testing Association (ISTA) practices for determining the viability of seed transmissible viruses and an ISTA protocol for the detection of ToBRFV in tomato and pepper seed is now available (developed by the International Seed Health initiative (ISHI). All commercial seed supplied to propagators should be confirmed free of seed borne diseases before use. In addition to ISTA, most seed producers are a member of, or follow other seed quality certifications e.g. Good Seed and Plant Practices (GSPP). Some propagators are reluctant to propagate trial varieties, as the number of seeds tested may be fewer than the 3000 recommended by most diagnostic labs. The limit of detection will vary with the number of seeds sampled (Table 2). No seed test will give 100% certainty of seed cleanliness, but smaller sample may pose additional risk to their businesses as the virus is less likely to be detected.

Table 2. The quantity of seeds required for determining ToBRFV infection to 0.1 and 0.3% in seed.

Sample size (seeds)	**Detection limits (95%) confidence**
3000	0.1% infection
1000	0.3% infection

As a consequence of this concern, significantly fewer trial varieties were grown in 2020. Many propagators now sample young plants for ToBRFV on behalf of their customers as an added assurance, or are requested by growers. The costs associated with this are dependent on the amount of plants being propagated, the number of varieties and the number of sampling times requested. These costs will be passed on to the growers, which are easily absorbed by larger production sites, but may pose a significant economic impact to smaller growers. Some growers may choose to not test at this stage, risking introducing ToBRFV onto their sites which could otherwise be avoided.

The experiences of the three sites underlie the importance of managing who is entering onto production areas. It was imperative for the functioning of Site 2 for construction to be completed, and the presence of contractors was unavoidable. In hindsight, additional disinfection processes could have been implemented which may have prevented this

outbreak from occurring. However, mitigation measures cost money as well as taking additional time. A balance needs to be found between necessary measures and the perceived risk ToBRFV poses to businesses. This needs to be considered carefully as outbreaks can be extremely expensive and disruptive. With only one outbreak reported in 2019, production areas may have considered their risk to be low. However, with the five ToBRFV outbreaks reported in 2020, businesses should implement more rigorous biosecurity and hygiene plans for the 20/21 season.

NFT systems work by re-circulating a stream of water containing dissolved nutrients required for plant growth down a channel containing the bare roots of plants. With ToBRFV so easily transmitted mechanically it is logical to assume that the presence of the virus in the hydroponic solution could lead to an almost instantaneous infection of the plants within the system. At site 2 the NFT solution was sampled and confirmed to contain ToBRFV particles. Despite this rapid spread, uniform infection throughout the crop did not occur at site 2 as would be expected if this route of transmission was significant. The underlying biology behind why this is the case is unknown, and highlights the need to conduct research in this area to better understand the epidemiology of the virus, including how it attaches to and invades root/stem tissues in NFT systems.

Regardless of the source of infection, staff and equipment will spread the virus within and between houses, as well as act as an inoculum source infecting any other sites visited if this is not prevented. The measures put in place at Sites 1 - 3 were sufficient to prevent infection spreading to the rest of the business which is positive and reinforces the issue that more effort should be placed on disease avoidance.

ToBRFV identification, symptoms and management

Differentiating viral diseases of tomato based on symptoms is difficult. Early symptoms are easily missed or attributed to other factors including nutritional deficiencies or environmental stress. Similar symptoms can develop in plants which are infected with different (e.g. PepMV), or similar viral species (e.g. TMV and ToMV). This is especially true when dealing with newly emerging viruses such as ToBRFV, when it is logical to assume any issues are from a different virus or disorder. Without the use of diagnostic tools e.g. real time RT qPCR and ELISA, misdiagnosis can easily occur. Table 3 includes a summary of the symptoms seen at Sites 1-3.

Table 3. A summary of the symptoms experienced in the infected varieties grown at Sites 1-3.

Site	Variety	Growing system	Symptoms	Notes
1	Delisher	Substrate	No symptoms	Symptoms developed shortly
1	Roterno	Substrate	No symptoms	

1	Piccolo	Substrate	Deformed twisted and nettled heads with needle like leaves	after arrival and plants were removed before fruit set. All plants believed to be infected
2	Arlinta	NFT	Deformed twisted and nettled heads with needle like leaves; reduced fruit and truss size; fruit abortion; weaker stems with reduced stem diameter. Symptoms were identical regardless of variety or growing system	Symptoms occurred in a mature crop, initially in the NFT block, followed by the substrate block. Two thirds of the crop was symptomatic by crop removal
2	Piccolo	NFT & substrate		
2	Roterno	NFT		
2	Yellorita	Substrate		
3	DRC564	NFT	Development of nettled heads, leaf lesions and chlorosis, leading to tissue death; formation of lesions on the centre of stems moving towards the canopy; reduced fruit and truss size; fruit abortion; rugose symptoms on fruit and plant death.	Symptoms developed rapidly after an irrigation breakdown. 100% of crops were symptomatic by crop removal.

With the development of TMV and ToMV resistance genes, the main viral issue occurring on tomato production sites has been PepMV. Symptoms of PepMV include chlorotic leaf spotting as well as fruit ripening issues, including fruit marbling, all of which have been used to describe symptoms of ToBRFV.

Similar to many large commercial production sites in the UK, and Europe, Sites 1 and 2 inoculated their crops with a PepMV mild strain. Introduction of this mild strain, known as cross protection, is in effect a vaccination strategy. Although mild strains can still have a small impact on plants, it is an insurance policy to prevent significant crop losses which might occur if an outbreak from an aggressive wild strain occurred on site.

Under some periods of plant stress, PepMV symptoms can develop in mild strain inoculated plants. With no ToBRFV outbreak reported in the UK at the time, it was logical that Site 1 assumed their symptoms were due to this inoculation. Similarly the outbreak at Site 2 was attributed to mild strain PepMV infections when symptoms initially attributed to the physiological stress of the inter lighting persisted.

In some crops e.g. carrots, mixed viral infections have been linked with greater disease severity. As a newly emerged virus, interactions between ToBRFV and other viruses are not well understood. Research into mixed infections of ToBRFV and the wild CH2 strain of PepMV have been documented in Israel (and is present in the UK, alongside the EU and US1 strains). Plants infected with both viruses were found to contain increased PepMV titres compared to plants which were infected with PepMV alone (Klap, Luria et al. 2020). This implies that plants

infected with ToBRFV and inoculated with mild strain PepMV could exhibit more significant symptoms. At Site 1 and Site 2, symptoms were originally attributed to severe mild strain PepMV infections which supports this claim. In house, laboratory-based research by a producer of a mild strain of PepMV found no negative interaction between ToBRFV and PepMV, however this has not been independently verified under commercial conditions.

Site 3 did not inoculate their crop with a mild strain PepMV, so did not experience a combined infection. However, due to the irrigation failure, symptoms developed rapidly. Further research on mixed infections with ToBRFV and mild and wild type PepMV strains (including the CH2, EU and US1 strains) could identify if there is a risk to production sites by inoculating their crops. Interactions between ToBRFV, and the newly identified Southern Tomato Virus (STV) could also be investigated.

Once a plant has become infected with ToBRFV, the virus will replicate and spread within the plant. Mechanical spread to other plants is inevitable if infected plants are not isolated or removed. Early identification of ToBRFV (or any pathogen) is essential for best management.

Site 1 did not have the advantage of the PHSI statutory surveillance programme which identified the presence of ToBRFV at Sites 2 and 3. In 2020 the programme demonstrated its worth, especially where ToBRFV infections were found on sites before symptoms developed (e.g. Site 3). Site 2 grew an overwintered crop and it is anticipated that this site would have benefitted from being sampled at a younger stage, ideally shortly after planting out. Despite the presence of the programme, businesses should not be reliant on this alone for sampling and confirmation of ToBRFV infection. With ToBRFV having now developed on several UK production sites, businesses should send suspicious samples for testing, even in circumstances where the issue is believed to be caused by another virus e.g. PepMV.

Smaller production sites can have reduced hygiene and biosecurity standards compared with larger commercial sites as a consequence of smaller production areas and fewer staff. Smaller sites are still at risk of becoming infected and the impact of ToBRFV to them will often be more significant.

The lower standards of hygiene and biosecurity procedures at Site 3 relative to the other 2 sites have been implicated as a contributing factor to ToBRFV becoming introduced onto this site. Without the statutory surveillance system, sending samples in for testing can be expensive which might prevent small sites from identifying ToBRFV infections. Compensation is not available to UK businesses affected by ToBRFV and an outbreak at any business could spread rapidly and lead to closure if allowed to run unchecked. Although significantly more information is now available on recognising and managing ToBRFV outbreaks, more could

be done to support smaller growers to improve their procedures directly. Any outbreak at any site risks spreading the ToBRFV further to the detriment of all.

Until recently, information and images of the effects of ToBRFV on European production systems has been limited. In many cases the 'typical' ToBRFV symptoms described from outbreaks in countries such as Israel did not manifest on every site, or in every variety. Site 1 took a cautious approach removing infected plants before fruit development, when symptoms were still restricted to leaves of just one variety. As a consequence little information on symptoms was available from this site, however one variety (Piccolo) which was also symptomatic at Site 1 became symptomatic at Site 2. Site 2 took the opposite approach and tried to grow through the infection. Despite growing plants for a longer period of time, at no point did the infected varieties display any rugose symptoms on fruit, but foliar and some ripening symptoms did develop. All varieties at Site 2 responded similarly, and no clear differences were reported between them, regardless if they were grown in NFT or substrate.

The original German ToBRFV outbreaks occurred in 2018 in a substrate system comparable to that used at Sites 1 and 2. These sites also inoculated their crop with a PepMV mild strain. Unlike the outbreaks in the UK there were clear varietal differences in symptom expression between the varieties in German outbreaks and this has also been observed in Israel. Some varieties show symptoms on the leaves alone, others only on the fruit and some on both leaves and fruit. The fact that no symptom differences were noted between UK varieties at Site 2 suggests that these are not necessarily varietal or growing system related.

One variety, Juanita, was severely impacted at the German site visited as part of the AHDB ToBRFV study tours and was dead within six weeks of visible symptom development. This variety had performed poorly over the entire season, including before ToBRFV infection was believed to have occurred. The impact of the virus on this variety was not considered to be varietal susceptibility, but as a response of lower plant health compared with the other varieties on site. If this is the case it underlies the importance of maintaining high levels of plant health and vigour to delay symptoms. This may have been the case at Site 3 where the healthy crop showed no symptoms until the irrigation failure.

One symptom that was always consistent between the affected UK and German sites was reduced leaf area as a consequence of the 'nettling' of heads. This increase in the amount of 'visible sky' in the canopy was what triggered the German plant protection advisor who originally identified ToBRFV in Germany to have the crop tested for ToBRFV. This is an important symptom to be aware of and one which is evident to an experienced grower.

There is strong evidence that plant stress acts as a trigger for symptom expression e.g. extremes in temperature, light or water stress. In Israel symptoms are more severe during the

very hot summer months which leads to a reduction in the total truss number. Careful planting of two crops a year and a 'hands-off' approach has enabled growers there to increase total number of trusses by a small degree, but not to the number seen before ToBRFV emerged. In contrast very cold temperatures have been linked to triggering ToBRFV symptom expression in crops in Turkey.

During the 2018 outbreaks in Germany, symptoms developed in the autumn at most of the sites impacted, spreading from the original infected site which had shown symptoms in July. The stress associated with the reduced day length is believed to have triggered visible symptom development. In the Netherlands most of the 2019 outbreaks were also reported in autumn months providing further evidence that lower light levels may act as a trigger. It should be noted that although the Dutch outbreaks were reported in the autumn, sites may have previously been infected with visible symptoms apparent and not confirmed as ToBRFV until they legally had to be reported in the autumn of 2019.

At Site 2 day length was increasing when symptoms first manifested. Symptoms arose around the time the plants reached the inter-lighting, as well as with the switching on of the LED top lights. This increase in light (light shock), in combination with the PepMV mixed strain was initially considered to be the problem. However, it is possible that light stress triggered ToBRFV symptoms to develop in the plants which were already infected.

ToBRFV symptoms had not developed at Site 3 before the virus was confirmed by PHSI. Plants continued to grow well until the irrigation failure. Following this, symptoms developed rapidly and were the most severe of the three sites covered in this case study, with rugose marbling of the fruit, nettling of the heads and in some cases plant death. These symptoms provide further support for plant stress acting as a trigger for symptom expression, in this case water stress. The degree of stress is likely proportional to the symptoms seen, explaining why Site 3 was affected so badly compared with the other two sites, as well as why symptoms are reported as being more severe in the relatively less favourable growing conditions in places such as Israel. This site grew a variety (DRC564) not grown at Sites 1 or 2, and it is impossible to state if the rugose symptoms arose from the high degree of water stress, or if this was a varietal trait. It is plausible that if Sites 1 and 2 were subjected to similar levels of stress to Site 3 that rugose symptoms may have developed in most/all varieties.

As a response to the reduced canopy area and stem diameters, the crop at Site 2 was steered towards a vegetative growth pattern by reducing temperatures and light levels. Steering in this way had some success in increasing leaf area and stem diameter, but this was not enough to prevent fruit abortion and there was no subsequent uplift in yield. Unlike the main stem, newly developing side shoots appeared strong. New heads were selected and these

were twisted back into the crop as an experiment, but this provided no benefit. It is unknown if either of these strategies would have worked in plants with a lower inoculum load. If this was the case it would only likely offer a short term solution, as ToBRFV would continue to replicate in the plants over time.

PHSI sampling and guidance

The virus was identified at Site 1 after samples were confirmed by a diagnostic laboratory outside of the UK. ToBRFV was confirmed at Sites 2 and 3 after sampling as part of the PHSI surveillance programme.

In all cases local PHSI inspectors arrived on sites having not visited any other tomato production areas recently. Each wore PPE provided by the site, but could supply their own if required. Leaf material was sampled at shoulder height and bagged, with gloves changed between varieties. Samples were sent for real time RT qPCR analysis and results retuned within 7 days by phone call from the PHSI inspector immediately after ToBRFV was confirmed.

Interpretation of the results was viewed as confusing, with some businesses disappointed in the degree of explanation provided to them. Results were provided as either strong, medium, weak positive or absent, with no further explanation provided. One site requested the raw data from the results in the form of PCR CT values.

A standardised ToBRFV diagnostic test is not currently available. Laboratories validate their own assays, using two different primers, which they may have developed themselves. Different laboratories use a different number of cycles in their tests which results in a different range of CT values for the different levels of detection. In order to guarantee the quality and validity of the results from private laboratories or national authorities, and to comply with regulations, validation tests are performed by diagnostic laboratories as part of the Valitest project. This was recently completed for ToBRFV and informs diagnostic laboratories on the performance of their validation test in relation to others, to support them and improve their practices, ensuring that diagnostic tests are continually refined. Although differences may exist between exact number of cycles and CT value ranges between laboratories, the process used should be robust.

Real time RT qPCR is the current gold standard test for ToBRFV detection and is able to detect a smaller fragment of ToBRFV DNA (70-100 nucleotides long), than conventional PCR. Two ToBRFV primers (targeting different segments of the ToBRFV genome) are always used to confirm a positive signal, ensuring the test is robust and avoid false positives. Many

laboratories will use the ToBRFV ISHI primers. However, some diagnostics laboratories are concerned about one of the two ISHI primers and use an alternative primer instead.

Different laboratories run real time RT qPCR tests for a different number of cycles, corresponding with the negative CT value for that laboratory. Fera run up to 40 cycles before stopping the test if no positive signal has been detected, whilst other labs may stop at 32, as is the case for the non-UK laboratory used by Sites 1 and 2.

CT values must be interpreted carefully and within context. High or low CT values are easy to interpret indicating a strong presence or absence of the target signal, however inconclusive (previously referred to a “weak positive”) scores (CT values of around 30-35) are difficult to interpret. Real time RT qPCR is a powerful tool that can identify very low levels of virus in a sample, given enough cycles. The transmissible nature and persistence of ToBRFV makes eradication difficult, and despite best efforts, diagnostic laboratories will struggle to avoid contamination of equipment and surfaces. Despite wearing disposable PPE and replacing bench covers, very low levels of ToBRFV can persist, which may lead to the contamination of testing equipment. The extremely high sensitivity of this equipment means that ToBRFV contamination, even at very low levels, can be detected and lead to an inconclusive result being returned. Where inconclusive results are returned the test is repeated, with new subsamples processed from the original samples collected. If the target signal for both primers is identified again, an inconclusive result is recorded. Under circumstances of inconclusive results, PHSI inspectors will revisit, resample and retest to confirm if ToBRFV is present on site.

Exact side by side comparisons between the results from the UK and non-UK laboratories are not possible, as it is unknown which primers were used by the non-UK laboratories. These are likely to be robust, but businesses would feel reassured if provided with additional information assisting with the interpretation of PCR results. Diagnostic laboratories could also be requested to provide information on their performance in the ToBRFV Valitest programme.

The sampling protocols provided by the laboratory outside of the UK also differed from the sampling procedure followed by PHSI. PHSI required sampling at fruit truss height, whilst the non-UK laboratory requested that samples be collected from the heads of the plants. The optimal tissue to sample for ToBRFV confirmation is unknown. Traditionally leaf samples for viral analysis have been taken from young plant tissue near to the head of the plant, but this takes more time. Both sampling procedures used detected the presence of ToBRFV in the case of site 2, but based on existing knowledge sampling of younger tissue may pick up low level infections which would not be detected in older tissue. An AHDB project, PE 034, is currently underway which will help inform optimum sampling procedures.

Once a site was confirmed as positive, inspectors returned and reviewed site specific hygiene and biosecurity processes as well as putting sites and pack houses on notice. Very little guidance was available to Site 1 on managing ToBRFV, with only a small amount more available to Sites 2 and 3. This is not a criticism of APHA or Defra, but a recognition of the newness of the virus, and the low number of outbreaks from which to learn lessons from.

Both Site 2 and 3 wished for more clarification and guidance on what measures to put in place, but understand that this takes time to identify. More information on the virus is now available in the DEFRA Tomato brown rugose fruit virus Plant Pest Factsheet and Defra ToBRFV contingency plan, as well as from the AHDB ToBRFV web pages which are updated regularly and lessons learned from these case studies will feed into further guidance.

As a consequence of the limited information available from APHA / Defra, many production businesses have sought information from alternative sources, including disinfectant companies. These businesses recognise the need for this information to be independently verified, as is currently underway in AHDB project PE 033a. This process takes time, with some businesses investing in costly equipment before efficacy is validated.

Twelve months after the final positive result, ToBRFV was declared eradicated from Site 1 and all notices and restrictions lifted. Notices can place significant restrictions on businesses. Site 2 is concerned that the use of glutaraldehyde disinfectants may result in positive swab testing of the structures at Site 2 (despite what they believe to be a full eradication of viable ToBRFV) and that this may impact the lifting of restrictions. Site 2 is working with PHSI to get this lifted early, including having additional tissue samples sent for analysis.

At site 3 the notice prevented the sale of loose fruit which meant rapid and costly modifications had to be implemented at their packaging facility in order to continue to market their fruit. Site 3 was unaware of the restrictions associated with being placed on notice. This information would be useful to all sites, enabling them to plan contingencies in advance of infections developing to improve their overall management strategies

Clean up

An effective clean up process is essential, and experience from Site 1, as well as the original six German outbreak sites, has shown that eradication of the virus is possible. AHDB-funded research has demonstrated the virus is persistent, remaining active on multiple surfaces for long periods of time. Use of the correct disinfectants and processes is essential to ensure viable ToBRFV does not remain to infect subsequent crops.

Crop removal needs to be considered carefully and planned in advance to prevent further spread of the virus on-site. Site 2 chose to remove the crop themselves, whereas Site 1 hired

contractors. As contractors have been identified as a potential source of infection (Site 2) it is important to understand where contractors have been, and their movement on site needs to be controlled. Under no circumstances should they enter onto other production areas and they should be supervised if necessary. This is especially true when removing infected material, where other unaffected production areas continue to grow.

Site 2 bailed their crops as they were removed from the glasshouse by conveyor. As a precaution this conveyor was isolated, cleaned and fogged after use. Bailing has the benefit of keeping the infectious material as contained as possible. This was then burned on-site destroying the virus. Incineration is not possible for many sites leaving deep burial and landfill as the other available options. If sending infected material off-site for disposal (as was the case for Site 1) ensure it is stored in a covered skip to reduce the risk of it spreading to other nearby production areas. This will also prevent it from infecting wild hosts and potentially establishing itself on site. It is inadvisable to shred material infected with ToBRFV.

In addition to crop debris all sites removed and replaced as much as possible, including irrigation and CO_2 lines, polythene, and troughs etc. Air jets were used to remove stuck on plant materials from the site architecture, with this removed by sweeping and vacuuming. Only disinfectants demonstrated to be effective against ToBRFV should be used, and at their maximum rates as informed by the manufacturer and independent research trials. In addition to the site architecture all equipment e.g. bailers, trolleys etc. were washed down in the infected glasshouse before disinfecting and removing. Consider placing in a separate area and re-fogging, followed by taking swabs to send for laboratory analysis to confirm if disinfection was successful.

Future and updated management

Most commercial production sites, including all the sites included in this case study, continue to update their hygiene and biosecurity practices as new information on ToBRFV emerges. Biosecurity has been reviewed and weak spots identified, including those identified from the UK outbreaks. Information on the optimal disinfection products is now available and this is being used to plan improved clean-up programmes. This information is being used for treating both the site architecture, as well as for equipment and machinery, with the latter cleaned more frequently.

With concerns of staff transmitting ToBRFV, Site 2 now supplies laundered staff uniforms. This is becoming increasingly common with sites in the Netherlands, Germany and Israel using this as a ToBRFV management tool. Staff change on arrival, washing and disinfecting their hands before entering production areas. Gloves are worn which are replaced frequently. After shifts these clothes are washed in a washing machine, using standard detergent. Staff

uniforms would ideally be washed at temperatures which are known to deactivate the virus, but if the clothing is made from polymers, as is the case at site 2, these temperatures are not suitable. Although not confirmed to deactivate ToBRFV, the washing and rinsing process is considered by this site to be sufficient.

Disinfection stations have been placed at all entrances on to sites 1 and 2, plus entrances to each compartment for foot and hand disinfection. Gloves are worn at all times in the crop and changed after each row at Site 2. Nitrile gloves were originally used, however shortages as a consequence of the coronavirus pandemic has meant that these have been downgraded to vinyl. These gloves are thinner and tear more easily, but frequent hand washing and hand disinfection should mitigate against this issue.

Other staff measures have been put in place which will reduce the risk of introducing and spreading ToBRFV. Break times have been staggered and mobile phones banned from use within production areas. More senior management are allowed to take mobile phones onto production areas, but these must be disinfected and sealed in a clean new plastic bag. Movement of staff which was already in place remains and will continue to restrict the spread of ToBRFV on sites.

As a new site, Site 2 hired a significant amount of inexperienced staff and were reliant on agency workers at certain times which made management more difficult. Most staff at all three sites are now seasoned and aware of ToBRFV and its impacts which will make monitoring for, and managing outbreaks easier.

The disinfection process at Site 1 was enough to eliminate the virus, and it is hoped that the processes used at Sites 2 and 3 will also be sufficient. Despite outbreaks occurring, the virus has been eradicated from several businesses in multiple countries. The clean-up process used at Site 1 and 2 were considered to be extreme and above and beyond what would be used in a ‘normal’ production year. Both of these sites will continue to use elements of this improved procedure, but will not follow it exactly in non-outbreak crop change overs as it is considered unnecessary and is expensive. Site 3 is currently undergoing clean-up and the effectiveness of this will not be known until after the next crop is planted.

Case study: best practice guidance

The following lists the best practice guidance identified from the experiences and lessons learned by the three businesses interviewed in this case study. Not all suggestions are relevant to all production sites, and many will not be practically or economically viable. However, it is anticipated that these points will improve ToBRFV avoidance and management strategies.

ToBRFV avoidance

- Continue to update site biosecurity and hygiene protocols as new information becomes available. Monitor the implementation and understanding of these with staff.
- Ensure all visitors/contractors etc. are aware of, and follow, site biosecurity/hygiene practices, including wearing clean clothing, disinfecting machinery and frequently washing/sterilising their hands
    - Restrict access to production areas unless essential and restrict what can be taken in e.g. mobile phones etc.
    - Ensure that visitors have not visited other production sites recently.
    - Provide personal protective equipment (PPE), including cotton oversuits, booties, hair coverings and disposable gloves.
    - Reconsider contractors, can the business do the task in house instead?
- Test young plants for ToBRFV before dispatch from the propagator and ensure all seed used is free of tobamoviruses, including ToBRFV through adherence to industry schemes and plant passporting requirements.
- Restrict staff movement between, and within, production sites and avoid movement between packhouses and production sites. This is especially important where fruit is imported from countries known to have ToBRFV outbreaks e.g. Spain.
- Give ‘ownership’ of areas/rows to specific staff members to further control movement and restrict/slow the spread of ToBRFV should outbreaks occur and identify potential entry points of ToBRFV.
- To reduce the potential increased risk caused by PHSI inspectors carrying out the sampling, in some instances staff sampling under the supervision of the PHSI may be considered and agreed. If inspectors do perform the sampling, supply them with PPE and appropriately dispose of these once they are finished on site.
- Provide laundered clothing and shoes to staff, and instil in staff to regularly wash/sanitise hands and wear and replace gloves.
- Consider installing foot and hand sterilising machinery at the entrances to glasshouses and sites.
- Ensure feet/wheel dips are made available at entrances and frequently topped up with appropriate disinfectants e.g. Menno Florades (benzoic acid).
- Where possible, ensure that any construction, or significant maintenance work, is completed before the introduction of any plant material onto site, and consider disinfecting/fogging new, or any potentially contaminated areas, as an added precaution.

Identification and management of ToBRFV

- Develop a ToBRFV action plan in advance of infections occurring.
- Consider site/packhouse limitations as a result of the site being placed on notice, including identifying alternative arrangements for selling loose fruit e.g. packaging, or diversifying into meal kits etc.
- Early identification provides the best opportunity to manage and contain ToBRFV successfully, as well as identifying the source of the disease and weak points in biosecurity/hygiene procedures.
- Establish a monitoring schedule for your crops, do not wait or rely on the PHSI statutory surveillance programme for ToBRFV confirmation but continue to report any suspicions as soon as possible.
- If ToBRFV symptoms develop shortly after plant arrival, determine whether early removal, subsequent clean-up and replanting will provide the most economical approach, or offer the best opportunity to eradicate the virus.
- Six ToBRFV outbreaks have been reported in the UK to date. It is no longer safe to assume symptoms seen are not ToBRFV and samples should be sent to a laboratory to test for ToBRFV.
- Be critical of what appears to be physiological and nutritional disorders, especially following periods of plant stress. Consider sending samples for testing as a precaution.
- Ensure staff are trained and can recognise ToBRFV symptoms, including nettled, deformed heads, stem lesions and uneven ripening etc. - rugose fruit symptoms are not expected to develop in all infections.
- Maintaining high levels of plant health/vigour may delay symptom onset. This should be prioritised where crops have been confirmed infected, but are currently asymptomatic.
- Plant stress (heat, water and light) have been linked to triggering symptom expression. Avoid unnecessary plant stress where possible.

Clean up and eradication of ToBRFV on sites

- Continue to monitor, and be aware of symptoms and patterns of spread, which may inform the likely sources of introductions/entry onto production areas.
- Remove infected crop debris, taking care not to spread plant debris to other production areas. Infected crop debris can be either incinerated on site, disposed of via deep burial, biodigester, or sent to landfill. Where infected material is sent to landfill, place it in a covered skip and disposed of in a covered lorry to reduce risk of further spread.

- Remove and replace as much as is feasible to make disinfection easier e.g. polythene, CO_2 lines etc.
- Vacuum production areas after sweeping to remove as much inoculum as possible, followed by jet washing, disinfectant use and fogging.
- Use disinfectants demonstrated to be effective against ToBRFV at their maximum rates.
- Clean all equipment e.g. bailers, trolleys etc. in the infected area before disinfecting. Consider placing equipment in a separate area following disinfection and carrying out a second disinfectant fogging. This should be followed by sampling by taking swabs for laboratory analysis to confirm if disinfection was successful.
- Where production areas are left empty for long periods of time before replanting, consider a second disinfection process (e.g. fogging), as an insurance policy. This would be recommended where infected plants have continued to be grown in nearby compartments.
- After disinfection swab high risk areas to confirm eradication of ToBRFV. Where ToBRFV continues to be detected, e.g. after using Unifect-G consider requesting a bioassay to confirm any residual ToBRFV is deactivated and no longer viable.
- Consider treating irrigation storage reservoirs and irrigation water for ToBRFV.
- Removal of PHSI statutory notices will be reviewed on on a case by case basis, once ToBRFV is believed to have been successfully eradicated from an affected business and tests from the new crop are negative. See Appendix 2
- Further guidance is available at the Plant Health ToBRFV portal, or the AHDB ToBRFV webpages.

Future research priorities based on the experiences of the three businesses

- The implications of combined ToBRFV infections with other viruses are not understood and should be investigated to determine their impact. Mixed ToBRFV infections with mild strain PepMV, 'wild strain' PepMV and Southern tomato virus (STV) could be examined further.
- It is currently unclear if the symptoms observed in particular varieties is a consequence of varietal susceptibilities, plant health or symptom severity. At the three sites studied in this work, only one variety was grown at more than one business. Unfortunately this was removed at Site 1 before any significant symptoms developed and true comparisons were not possible. Controlled investigations of varietal susceptibilities, including the impact of high plant health vigour and the role of stress in triggering symptom expression would be valuable to understanding the impacts of the virus.

- ToBRFV particles have been identified within the nutrient solution of NFT systems, as well as within the irrigation solution of crops grown in artificial substrate e.g. rockwool, or coir media.

  It is logical to assume that the presence of ToBRFV in both of these solutions would rapidly lead to uniform infections developing once contaminated, however this does not appear to occur. It is unknown why this is the case and further research may provide insights into ToBRFV attachment and inform on its epidemiology.
- ToBRFV has also been found after chemical disinfection of irrigation water. It is not known if these virus particles remain viable, or are deactivated after the disinfection process. If they continue to be viable, the efficacy of various water treatment methods, e.g. chemical, UV should be investigated.
- A consequence of ToBRFV being ubiquitous in soil grown crops in Israel meant that cultivation methods there have changed. This includes careful planting, minimising plant contact and taking of additional heads. As an experiment, Site 2 took extra heads, twisting them back into the main crop. The new heads grew well when compared with the main stem, but this ultimately gave no benefit, with fruit still aborting. It is possible that these additional heads would have been more successful if they were established before visible symptom development and this could be investigated.
- Investigate the effectiveness of commercial tray cleaning equipment against trays contaminated with ToBRFV using disinfectants e.g. Huwa-San at commercial rates.

Adaptations to PHSI sampling, interaction and reporting based on the experiences of the three businesses

- Different sampling protocols were used for sampling by PHSI and a European diagnostic laboratory (sampling at shoulder height vs. the head of the plants). Although both these techniques returned positive results, they may not have done in situations where virus levels were low. Research identifying the optimal sampling procedure, including the most appropriate sampling location, would reduce the risk of false negative results.
- Although PHSI surveillance identified ToBRFV on Site 2, the virus is suspected to have already been present for some time as it was an overwintered crop. The surveillance programme ran in the spring of 2020, but sites with overwintered crops would benefit from sampling within a few weeks/month following planting.
- Interpretation of the results from PHSI were limited with several growers wishing for a greater level of interpretation, including CT values for comparison with other laboratory test results. Although a positive is still a positive, more information and the CT values

would reassure businesses and may assist with the development of management strategies.

Knowledge and Technology Transfer

- A presentation at the British Tomato Growers Association 2020 conference (available online until 24 Dec 2020).
- Discussion of project and outcomes at Tomato Study Group (TSG), and Tomato Working Party (TWP) meetings.
- AHDB podcast (tbc.)

Additional information

- AHDB ToBRFV webpages
- Plant Health ToBRFV portal
- EPPO Global Database: *Tomato brown rugose fruit virus* website

Appendices

Appendix 1. List of questions/topics discussed with the representatives from the three businesses included in these case studies.

ToBRFV origins:

- How and when was ToBRFV first detected on site? Routine crop monitoring by grower/crop workers or PHSI statutory surveillance?
- Based on the above, what is considered to be the likely entry point of the virus onto site? Young plants, machinery, trays, staff (contractors) etc.?
- Where infections have been identified in multiple houses/compartments on one site, do you consider these to be independent infections or local transmission from a single infection point?
- What practices have you put in place to reduce mechanical spread by workers/visitors and equipment?
- How do you think the disease spread from the initial infection, e.g. equipment, irrigation, staff, bees?
- Were there patterns to the distribution of infection or locations of symptom hotspots?

ToBRFV crop impacts:

- Where infection was identified by PHSI statutory surveillance in asymptomatic plants, did these plants remain asymptomatic in the long term, or before removal? If not, how

long did this take from detection to symptom development? What do you consider triggered the switch to symptom expression?

- How have different varieties (and root stocks) responded to infection and what symptoms developed (leaves, stems, truss, flower and fruit)?
- Are there any varieties which you would not consider growing based on your experiences with ToBRFV infections? Which varieties responded best to infection?
- What proportion of the plants became infected per affected compartment/house?
- Were consistent symptoms observed where the same variety is infected across multiple locations on site?
- Do different growing methods impact symptom development and/or severity, e.g. substrate vs. NFT vs. organic production?
- What impact do different environmental conditions/stresses have on symptom development? Were symptoms triggered following extreme conditions?
- Have any agronomic inputs affected symptom development? Do you believe that plant health/nutrition is linked to symptom development?
- Do poorer performing or diseased plants exhibit more severe symptoms? Do symptoms appear earlier in these plants?
- If applied, do you consider the use of a mild strain of PepMV to have impacted symptom development?
- Has irrigation management been altered as a result of ToBRFV on site?

<u>Clear-up:</u>

- How did you manage the infection once it was detected and what measures did you put in place to limit further spread of infection once it was confirmed? How effective do you consider these practices to have been?
- Where infected crops have been removed, how has this been done in a way that will minimise risk of further spreading ToBRFV on site?
- How have you disposed of your infected plant material, e.g. deep burial, composting, incineration, other?
- Where did you store/dispose of your infected material, on site or off site?
- What is your clean-up and crop turnaround procedure for ToBRFV eradication? What chemical disinfectants have you used, how have you applied them and for how long?
- How long did you leave before replanting after clean-up?
- Where the clean-up procedure has been enhanced to eradicate ToBRFV infection, would you continue to use this procedure in seasons where ToBRFV has not developed?

- Did you do your own clean-up, or contract it out?
- What testing is being done to confirm the elimination of ToBRFV from the affected houses, e.g. type, location, frequency etc.?

Business impacts:

- What impact has the infection had on fruit quality, yield and marketable yield (shelf life)?
- What is the likely reduction in yield expected to be over the entire 2020 season? Will it impact the start of the 2021 season?
- What are the associated costs for crop loss and clean-up?
- What impact has ToBRFV infection had on your ability to sell marketable fruit?
- If marketing infected fruit, how have you minimised the risk of spreading ToBRFV in the supply chain?

PHSI sampling:

- Overview of the step-by-step process for sampling by PHSI.
- How many glasshouses were sampled?
- How many samples were collected per house/compartment? What plant tissues were collected and where were these collected from, e.g. young/old leaves, stems, fruit, etc.?
- Did plant health inspectors follow on-site biosecurity measures? Did they use PPE, and was this their own or supplied by sites? Did they follow rules to prevent cross-contamination of the samples they collected? Had they visited other production sites recently?
- What is the grower perspective on the quality of work carried out by PHSI?
- Where infections were detected, what additional swabbing/testing was carried out by PHSI and/or the grower?
- Do you believe any changes need to be made to how PHSI collect samples?
- Were results clear to understand, e.g. weak positive vs. strong positive? How did your interpretation of the results impact on your decision making?

Staff:

- What changes to processes have you had to put in place to reduce the spread of ToBRFV on site by staff, e.g. restricted access, supply of fresh clean clothing, etc.?
- What hygiene/biosecurity measures have been put in place (compared with what was in place before)?

- Where staff members live in accommodation on-site, have any changes been made? If so, how have these changes been implemented? How well are they followed?
- The coronavirus outbreak will have impacted staff operations, and to some extent Brexit may have limited the availability of experienced staff. Has this negatively impacted your ability to manage, or limit ToBRFV infections?
- What changes to staff training have you put in place?

Future:

- What are your plans for any subsequent cropping at affected sites?
- Are you planning any larger site adaptations for the next season, e.g. purchase of low pressure tray sterilisers?
- What changes would you like made to sampling and testing in 2021?
- What lessons have you learned and what changes have you made?
- What do you wish you knew now that you did not know at the start of the season?
- What are the most important changes you will make ahead of the 2021 season?

Appendix 2. ToBRFV notice restrictions placed on production sites and packhouses.

Once Tomato Brown Rugose Fruit Virus (ToBRFV) has been confirmed by laboratory diagnosis on leaf samples, the infected tomato production glasshouses and associated pack houses are placed under a statutory plant health notice (SPHN). The notice can be lifted and ToBRFV declared eradicated if it has not been found following inspection and sampling of the new crop after an appropriate host crop-free period

Notice restrictions may include the following:

- Movement of harvested fruit is restricted, usually to the local pack house for retail and fruit may not be moved from the premises without permission from PHSI.
- Movement of plants and plant material, including leaves, stem and waste fruit etc. must remain on site and be destroyed by deep burial, composting or incineration.
- Information relating to tomato production must be supplied to PHSI if requested.
- Enhanced biosecurity processes, supplied by PHSI and company specific protocols must be adhered to prevent further spread, including:
    - Restriction in staff movement to uninfected premises, unless wearing clean clothing and appropriate PPE, including disinfected footwear.

- Visitors must be made aware of the problem and entry to the site restricted. If entry onto production areas is essential, visitors must wear PPE and requested to avoid visiting other production sites for at least 24 hours.
- Equipment must only be moved if it has been cleaned down with suitable disinfectant.
- Movement of trays/crates between the production area and packhouse should be identified and used for no other purpose, and appropriate biosecurity measures taken.
- Foot cleaning and hand cleaning stations must be placed at the entry and exit of every glasshouse (with appropriate disinfectant) to prevent transmission.
- Machinery, including lorries, traveling between the infected, and any other production, or packaging site must be disinfected each day.

Special thanks

This work would not be possible without the support of the three businesses impacted by ToBRFV who were happy to openly discuss their experiences for this report.

REFERENCES

Klap, C., et al. (2020). "Tomato Brown Rugose Fruit Virus Contributes to Enhanced Pepino Mosaic Virus Titers in Tomato Plants." Viruses **12**(8).

Panno, S., et al. (2020). "First report of Tomato brown rugose fruit virus infecting sweet pepper in Italy." New Disease Reports **41**: 20.

Samarah, N., et al. (2020). "Disinfection treatments eliminated tomato brown rugose fruit virus in tomato seeds." European Journal of Plant Pathology.

van de Vossenberg, B. T. L. H., et al. (2020). "Real-time tracking of Tomato brown rugose fruit virus (ToBRFV) outbreaks in the Netherlands using Nextstrain." PLOS ONE **15**(10): e0234671.

