Project title:	Tomato Brown Rugose Fruit Virus (ToBRFV) fact-finding trip to Israel and Europe
Project leader:	Dave Kaye, ADAS
Report:	Final report, December 2019
Previous report:	None
Key staff:	Emily Lawrence, ADAS
Location of project:	ADAS Boxworth, protected edible production sites, and specific crop research institutes, in the Netherlands, Germany and Israel
Industry Representative:	Dr Phil Morley, TGA, APS group (?)
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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

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GROWER SUMMARY

1. Headline

Tomato brown rugose fruit virus (ToBRFV) is an emerging virus threat to tomato and pepper production worldwide and on November 1st 2019 the virus became subject to European quarantine measures (Q-status). More information on the epidemiology, disease avoidance and management strategies are urgently needed to support the tomato and pepper industries.

2. Background

Tomato brown rugose fruit virus (ToBRFV) is a highly infectious, emerging tobamovirus of tomato and pepper. Emerging in Israel in 2014, ToBRFV has the potential to become one of the major factors limiting global tomato production, and is now present on several of the world's largest tomato production areas.

The exact geographical origins of the virus is unclear, although it is believed to be older than the similar tobamoviruses, Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV) which have historically impacted tomato production in the UK.

No chemical treatment is available to control viral diseases in plants. ToMV and TMV transmission are currently controlled by cultivation of tomato plants containing the *Tm-2²* gene which is present in almost all European commercial varieties. ToBRFV breaks the *Tm-2²* resistance. To date, no commercially available variety has demonstrated resistance to ToBRFV. Symptoms vary by variety and growing conditions e.g. temperatures or day length. Some varieties show symptoms on fruit (Figure 1), some on the leaves, stems, calyces etc., with many on all. Some varieties are asymptomatic, whilst highly susceptible varieties can be dead in as little as six weeks after visible symptoms develop.

Figure 1. Tomato fruit of var. Ikram infected with ToBRFV. Note marbling symptoms typical of ToBRFV infections with this variety – Dave Kaye, RSK ADAS Ltd.

European sweet pepper varieties do not contain the *Tm-2*^{2} gene, but instead the *L*^{4} gene. This provides protection against ToBRFV in pepper and no outbreaks on commercial sites have been reported in Europe. The *L*^{4} gene is not incorporated in pepper varieties grown in Mexico and the impact of ToBRFV on production there has been catastrophic.

As of December 2019, ToBRFV has been confirmed to be present in twelve countries, seven of which are in Europe, although it has now been eradicated from the UK, Germany and the USA. Initial spread was sporadic, but the virus is most likely present in other countries, but has not yet been identified, or reported.

3. Summary

Project aim: To undertake a fact-finding study tour of tomato and pepper production sites and specific crop research institutes, in Israel and Europe, where Tomato Brown Rugose Fruit Virus (ToBRFV) has been found, reported and acted on.

Project objective: To identify management strategies for the avoidance of ToBRFV infections, and improved strategies for reducing this impact where ToBRFV infections occur on site, including successfully eradicating the virus from infected sites.

Two fact-finding study tours of protected edible production sites, and specific crop research institutes, in the Netherlands, Germany and Israel, were completed. Seed producers,

propagators, growers, consultants, researchers and diagnostic laboratories were contacted and visited during October (Europe) and November (Israel).

It was anticipated that direct face-to-face contact would facilitate honest, open discussions between the project lead and the individuals/sites visited. Not only would these discussions provide insights into ToBRFV management and avoidance strategies, it would also establish a platform for continued information exchange.

Europe

The first official outbreak of ToBRFV in the Netherlands was reported the week before the study tour. The location of this site remained anonymous and was not visited. Since this time, ToBRFV has been reported on several other grower sites, with more under suspicion of infection. Despite best efforts, no growers in the Netherlands were able to be visited during this work. Seed producers, a propagator, crop consultant and several research laboratories, including Naktuinbouw were visited.

In 2018 an outbreak of ToBRFV developed on several grower sites in Germany as part of a collective, the virus was successfully eradicated, and is no longer present in Germany. The site where the first outbreak was reported in Europe was visited and the situation discussed with the grower and the crop consultant involved in the initial diagnosis. Landwirtschaftskammer (German Chamber of Agriculture), and the Julius Kühn institute were also visited.

Israel

Unlike Europe, ToBRFV is endemic in Israel and most growers are waiting for resistance to be developed. Repeat cropping, grown intensively in the same soil, has meant that soil now contains significant levels of ToBRFV infected material, which is available to infect newly planted crops. Unlike the ToBRFV avoidance strategy in Europe, Israel follows a delay strategy to maximise the amount of marketable fruit produced, before the plants succumb to the virus. This includes careful planting to delay infection from sources within the soil, allowing plants to become more established before infection occurs. Limited contact with young plants after planting also delays infection via mechanical transmission. Although not a delay strategy, most growers now grow two crop cycles per year. For most this is more cost-effective than one crop which is unlikely to be economical, or survive, an entire season.

Initially crop loses for some sites were as high as 100%, however careful variety selection and modifications to grower practices have reduced losses associated with ToBRFV to around 20%.

Representatives from two of the largest propagation sites in Israel were met, in addition three visits to local tomato growers in the arid West Negev region where 70% of tomato production takes place. Two seed producers, a diagnostic laboratory and research laboratories at the Volcani institute were visited.

General observations

ToBRFV is not vectored via insects, but is efficiently spread via mechanical transmission. The main route of infection is by contact with clothing/hands, equipment (including trolleys) and trays containing infectious ToBRFV particles. If seed producers and propagators supply healthy, uninfected plants, and strict rules on who/what is allowed onto production sites are adhered to then outbreaks should be avoided. Most sites allow limited access with only essential visits permitted, preferably first thing in the morning so ensuring other sites have not already been visited that day. It is stipulated that all staff and visitors should wear clean clothing, with some sites considering supplying fresh, laundered clothing for staff to work in. Clothing capable of being laundered at around 95°C is being researched alongside viricidal detergents. Restrictions on the movement of staff on-site can help to reduce the risk of spreading ToBRFV if an infection was to occur.

Existing hygiene protocols, and emergency ToBRFV protocols have been updated by seed producers, propagators and growers as a response to ToBRFV, and these will continue to be updated as new information becomes available.

ELISA, (RT) and qPCR are used to identify ToBRFV infection in seed and plant material, but bioassays are required to confirm infectivity of ToBRFV. Cross-reactivity with other tobamoviruses (ToMV and TMV) is common with current ELISA tests, however ToBRFV specific tests are in development by several research institutes/laboratories. Multiple ELISA protocols are available. Identification of a ‘gold standard’ ToBRFV protocol, recommended for use by all laboratories is currently in development. All propagators and main crop growers should request certificates confirming all seeds are confirmed free of ToBRFV and sufficient seeds were tested, before planting. Trial varieties pose a significant risk to propagators and growers as limited seed availability often mean far fewer than the recommended 3000 seeds are tested, and so trial varieties should not be grown unless suitable seed certificates are supplied.

Returned trays are considered one of the greatest risks of spreading ToBRFV between sites. Some sites now using automated machines to disinfect trays, whilst others have switched to single use trays. Many European and Israeli sites use Virkon S (a viricidal product containing a stabilised blend of peroxygen compounds, surfactant, and organic acids) to clean down equipment/machinery e.g. knives and trolleys. Virkon S is not considered food safe in the UK

and can cause phytotoxic effects on fruit and leave detectable residues. Menno Florades (benzoic acid) is also used and certificates of crop-safety can be obtained in the UK. Water treatment must be sufficient to eliminate the virus from recirculated water e.g. high level UV and hydrogen peroxide.

Fruit infected with ToBRFV is likely being sold in UK supermarkets, especially with the recent confirmation of the disease in Spain. Some sites which import fruit from abroad, in addition to selling their own fruit, are now reconsidering whether to continue this practice.

From November 1st 2019, ToBRFV gained Q-status in Europe and all ToBRFV outbreaks are legally required to be reported to relevant plant health organisations e.g. Defra. All recommendations should be followed to reduce the risk of further spreading ToBRFV on site, and to other UK production sites.

Seed production

ToBRFV is not present within the seed endosperm, but infections may develop from inoculum sources on the seed coat, but this latter route of transmission is considered to be a very rare occurrence. Many producers are members of accredited bodies, following best practice guidelines or well defined protocols produced by e.g. Good Seed and Plant Practice (GSPP), International Seed Testing Association (ISTA) and International Seed Health Initiative (ISHI). Seed producers process and treat seeds to eliminate seed transmissible pathogens, including tobamoviruses. All seed batches are confirmed to be free of infectious tobamoviruses before sale, using diagnostic tests including ELISA and RT-qPCR, followed by a bioassay if necessary.

Some seed producers are considering moving, or have already moved, production from areas believed to be at a high risk of developing ToBRFV infections, and access to parent stock is tightly controlled. Many have divided seed production areas into blocks, so allowing any potential future affected areas to be isolated and production to continue.

Resistance breeding is underway by the majority of large seed producers. The Volcani Centre has developed a ToBRFV resistant root stock, especially useful in Israel, where infections often develop from the soil. It should be noted that this rootstock will not prevent mechanical transmission between infected plants during cropping. Another seed producer believes they have also developed varietal resistance and this will be tested over the next few seasons. It is unknown if this resistance is different to that developed by the Volcani Centre.

Propagation

In Europe and Israel, any plants infected with ToBRFV at propagation will not develop visible symptoms until after planting at main production sites. Therefore, seed testing certificates

provide the only assurance available to both propagators and growers that the plants are clean. Any seed grown must have a corresponding certificate, including information on the diagnostic tests used, including the number of seeds sampled. Some propagators will provide growers with information on their plant production procedures so providing additional peace of mind.

All propagators will have altered their practices as a response to ToBRFV and likely developed emergency ToBRFV protocols. These will be updated as new information becomes available. All visits, including customer visits are restricted and advised against, unless essential. In mainland Europe, where customers wish to inspect their plants they will not be allowed onto production areas, but their plants will be brought to them. Propagators have split production on sites, enabling the isolation of areas containing suspicious plants, but allowing the rest of production to continue. The Q-status of ToBRFV in Europe has meant that all suspicious outbreaks must be checked by and reported to relevant authorities.

Plant wounding is considered a key entry point for early infection. Israel follows careful planting practices to delay the occurrence of infection, including the use of organic, biodegradable films surrounding the plug so preventing direct contact between wounded roots and infected soil at planting. This practice is unlikely to be of benefit to UK production, however, this could be used to protect against potential soil borne diseases in organic production.

Main Crop production

Growers have to trust that the plants delivered are free of seed transmissible infections, including ToBRFV. If in doubt, samples can be taken at delivery and sent for testing, but it will be some time before a result is returned. During this time the virus could be mechanically spread to other plants/houses. The most efficient practices for sampling planted crops are currently being investigated.

Some German and Dutch growers sterilise their knives overnight in disinfectants, including Virkon S or Menno Florades. One Dutch grower uses a two knife practice, re-sterilising each blade whilst the other is in use, whilst a German grower provides a pre-sterilised knife for use down each individual row. Some sites choose to spray trolleys using a knapsack containing disinfectant between rows, but this is unlikely to be fully effective. Other production sites monitor staff electronically through digital monitoring software, ensuring that sufficient time is spent disinfecting equipment after working each row. Trolley cleaning machines are used by some and likely represent the most effective method of disinfection, so long as the disinfectant has activity against ToBRFV and is used at its recommended rate.

Dependent on the variety, initial health of the plant pre-infection, and the time of the year, it is possible to continue to produce a degree of marketable fruit despite infection, but any outbreak in Europe must still be reported. Varietal differences in the severity of ToBRFV have been seen in both Europe and Israel, with some varieties asymptomatic of ToBRFV infection. Asymptomatic varieties still contain viable inoculum and will provide an inoculum source to further spread ToBRFV. The true susceptibilities of the affected varieties during the German outbreak is unclear. The severity of symptoms was confounded by poor plant vigour in the varieties worst affected. Nutrient deficient plants, as a consequence of using desalinated water in Israel, show enhanced symptom expression. Therefore, excellent growing conditions and high levels of plant health may reduce symptom expression. Where infections occur, other plant stresses have been implicated as a major trigger of symptom expression. ToBRFV symptoms in the Netherlands developed at a similar time to Germany in 2018, were believed to be a consequence of shorter day lengths. Heat and cold stress will exacerbate symptoms. In Israel symptoms are most severe at the height of midsummer, whilst symptoms are greatest in Turkey when crops are exposed to very cold temperatures (~4°C).

Bumblebees have been demonstrated to spread ToBRFV, but will only spread in houses where ToBRFV is already present, and likely already widespread. In cases where a suspicious plant is found, hives should be closed until the plants has been tested and results returned. Efforts should be focused on reducing the risk of mechanically spreading the virus by the routes considered highest risk, staff, machinery and equioment.

Limited studies show mixed infections of ToBRFV and mild strain PepMV do not appear to be more severe than plants infected with either virus alone, but the impact of mixed infections with ToBRFV and wild strains of PepMV is unknown. Mild strain ToBRFV ‘vaccines’ are in development, and may provide cross-protection to ToBRFV, but these are several years away from being registered for use. Plants infected with multiple viruses e.g. Tomato yellow leaf curl virus (TYLCV) or the Paprika mild mottle virus isolate from Japan (PaMMV-J), in addition to ToBRFV will suffer more than plants infected with ToBRFV alone.

Effective disease management and comprehensive site clean-down, using disinfectant products identified to be effective against tobamoviruses, led to Germany being officially declared ToBRFV free in July 2019. The infected German sites now replace their coir each year, rather than reusing it year-on-year. Boxes and drip pegs are also replaced. Solarisation for one month can be used to reduce inoculum levels in Israel, but this is not suitable for protected crops in the UK. No effective means of managing ToBRFV have been identified for organic producers in the UK, apart from using ToBRFV resistant varieties once available.

During clean-down all contractor equipment should be disinfected before entry on-site, including any fabric rolls used to remove plant material. Contractors should wear clean clothing not worn to other production sites. ToBRFV infected material must be incinerated in Germany, but composted in the Netherlands. The requirements for dealing with infected materials in the UK are unknown. Slabs/plant material should be disposed of in a covered truck and not stored outside the site for long periods of time. Waste water which may contain infectious ToBRFV should be flushed into the sewage system, rather than released locally where it may enter watercourses and permanently establish in weed species nearby. Weed species can act as alternative hosts of ToBRFV and these need to be identified in the UK.

4. Financial Benefits

Despite the costs associated with mitigating against ToBRFV outbreaks occurring on sites, the financial benefits from preventing ToBRFV, or reducing the impact of a ToBRFV infection, are significant.

- Outbreaks at seed production sites, especially on parental material, could put seed production of several popular varieties at risk, limiting availability for future seasons.
- A confirmed ToBRFV infection at propagation could lead to the quarantine of part of,, or an entire, propagation site, resulting in significant loss of income to the propagator. The follow-on financial implications of these plants not reaching main production sites would also lead to considerable financial repercussions.
- Due to the Q-status of the virus, infections on production sites may lead to the removal of entire crops, requiring sites to be cleaned down and fresh plants sourced. Sourcing of new plants takes time, leaving empty glasshouses not producing fruit during times of peak production.

5. Action Points

The following lists the ‘best practice’ procedures identified from the two study tours. Implementation of these practices may not be practically, or economically, viable for all sites, but demonstrates what can be done to effectively mitigate against ToBRFV infections developing, and how to better address infections which do occur on sites.

<u>Pre-infection</u>

- Review and update site hygiene protocols to include new ToBRFV management practices, and train all staff on ToBRFV symptoms, including on what to do if an outbreak occurs.

- Consider installing foot and hand cleaning machines at the entrances to each site. These must be maintained, ensuring that the active ingredients used are at recommended rates and have activity against ToBRFV. Alcohol hand gel has no activity against ToBRFV.
- Maintain strict biosecurity practices and stop all site visits unless essential. Where visits are required, request they take place first thing in the morning. No tomato products should be brought onto, or consumed on-site by staff or visitors.
- Ensure visitors read and understand site hygiene protocols, including wearing protective clothing at all times, ideally changing into this before leaving their vehicles. Where sites are visited by individuals frequently, shoes can be purchased to remain on-site and these should be routinely disinfected.
- Consider supplying fresh, laundered clothing for staff to wear during shifts which is then replaced each day. Normal hand washing (30 seconds) is insufficient to deactivate ToBRFV on hands and staff should wear non-absorbent, e.g. vinyl, gloves at all times, replacing them often, and frequently re-sterilising with an appropriate disinfectant.
- Limit staff movement between production areas on each site, do not move equipment or machinery between areas. Identify and designate high and low risk areas, and control movement between these areas.
- Ensure seed health certificates are sourced for each variety grown, especially trial varieties. Check the diagnostic tests were performed by an accredited laboratory, and that a sufficient number of seeds was tested (3000).
- During grafting, change blades after each new tray, or more frequently if possible.
- Ensure trucks are disinfected with a suitable disinfectant e.g. Virkon S, or Menno Florades, before arrival on-site and again before use.
- Monitor crops frequently for disease symptoms, be aware of typical ToBRFV patterns of infection (symptoms spreading along rows), and if a plant looks suspicious have it tested.
- Focus on maximising plant health to suppress symptom onset should infections occur.
- Use the German one knife per row, or Dutch two knife method to ensure disinfected knives are used at all times.
- Clean and disinfect all equipment with an effective, crop-safe disinfectant between each row, or use a trolley cleaning machine.
- Avoid excessively stressing plants and take care to avoid unnecessary wounding which may act as an entry point for infection to occur.
- Ensure crates are cleaned using a crop-safe disinfectant, or alternative method e.g. low pressure steam treatment, before they are used.
- Where water is recirculated, the sterilisation process should be checked to ensure it is sufficient to eliminate infectious ToBRFV.

- Ensure all waste water enters the water treatment system. Do not allow water to enter natural watercourses which may permanently establish ToBRFV in alternative host weed species nearby.
- Monitor weeds species on site and control as necessary.
- When using contractors ensure their equipment is clean and sterilised before allowing entry on-site.
- Clean and disinfect all site architecture using products known to eliminate ToBRFV. Ensure all products are used at their recommended rates, pressures and temperatures and applied to maximise their effectiveness e.g. foam.

<u>After an infection occurs</u>

- Isolate any suspicious plants and test for ToBRFV. When outbreaks are confirmed carefully double bag infected plants and remove them from the glasshouse. Remove additional plants along the row and plants from the rows opposite the infected plant (exact number to be removed to be taken on a case by case basis).
- When suspicious plants are quarantined, shut down bee hives in these areas, until a result is returned. If a positive result is returned these hives should be destroyed.
- When an outbreak does occur, quarantine the affected house and immediately notify Defra, or appropriate plant health authority, and comply with their recommendations.
- Removed infected plants must be transported off-site using a covered truck and destroyed e.g. incineration or composting.

6. SCIENCE SECTION

Introduction

Tomato brown rugose fruit virus (ToBRFV) is a highly infectious emerging tobamovirus of tomato and pepper. Emerging in Israel in 2014, ToBRFV has the potential to become one of the major factors limiting current global tomato production; the virus became widespread in Israel before it was classified as ToBRFV in Jordan in 2015. Though the geographical origin of the virus is unclear, it is postulated to have originated in North Africa and is an older virus than the similar tobamoviruses, Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV) (Aviv Dombrovsky, personal comment). Initial spread of ToBRFV is believed to be due to a series of events enabling the virus to transfer from an unknown host (e.g. solanaceous weed species) into production plants. Since 2014, the virus has spread rapidly across many of the world's largest production regions. As of December 2019, ToBRFV presence has been confirmed in twelve countries (Table 1), though it has now been eradicated from the UK, Germany and the USA. Initial spread was sporadic, and the virus is most likely present in other countries, but has not yet been identified or reported. Potentially infected countries include Chile, Peru, Ethiopia, Sudan, Saudi Arabia and Thailand.

Table 1. Confirmed worldwide geographical outbreaks of ToBRFV (December 2019).

Country ((region(s))	**Date first reported /identified**	**EPPO reference (where applicable)**
Israel (widespread)	October 2014	N/A
Jordan (widespread)	April 2015	N/A
Germany (North Rhine-Westphalia)	July 2018	EPPO 2019/012
Mexico (Yurecuaro, Guanajuato)	September 2018	EPPO 2019/014
USA (Arizona, California)	September 2018	N/A
Italy (Sicilia, Piemonte)	October 2018	EPPO 2019/013
Turkey (Antalya)	January 2019	EPPO 2019/123
China (Shandong province)	April 2019	EPPO 2019/143
UK (Kent)	July 2019	EPPO 2019/163
Greece (Crete)	August 2019	EPPO 2019/210
Netherlands (Westlands region)	October 2019	EPPO 2019/209
Spain (Almeria)	October 2019	EPPO 2019/238

Unlike most other viruses of tomato and pepper, ToBRFV has no natural insect vector. The main route of transmission is via mechanical contact, including hands, clothing, crates, equipment, etc.. ToBRFV became widespread in Israel and Jordan via movement of people and equipment in 2014.

Historically, both ToMV and TMV have infected tomato crops in the UK. The presence of these viruses in commercial production decreased with the move towards protected cropping and improved phytosanitary measures. In addition, the incorporation of resistance genes including *Tm-2*^{2}*,* provided protection to almost all commercial tomato varieties grown in Europe. Sweet pepper varieties do not contain the *Tm-2*^{2} gene, instead carrying the *L*^{4} gene to confer resistance to these tobamoviruses.

Before resistance was developed, cross-protection via artificially infecting plants with a mild strain was commonly used to control ToMV in the UK. Mild strains do not prevent infection occurring, but reduce symptoms. They provide an insurance policy, increasing marketable yield in situations where a viral outbreak does occur. Many UK growers choose to inoculate with a mild strain(s) of Pepino Mosaic Virus (PepMV)—another serious viral issue in the UK—immediately after arrival of plants on site. Mild strains of ToBRFV are in development, however if a mild strain was available to market today, it would take four to five years to gain registration.

The impact on plants of dual viral infection—with ToBRFV and PepMV, —is unknown. A long-term laboratory-based experiment has shown that there are no additional negative impacts on plants infected with mild strain PepMV and ToBRFV, compared with ToBRFV alone. It should be noted that this work was commissioned by the producer of a PepMV mild strain and the full experimental conditions of this work were not provided. No scientific literature is available on plants infected with ToBRFV and full-strength strains of PepMV, though anecdotal observations have reported that fruit is smaller and harder, with the vigour of infected plants greatly reduced. Pants infected with ToBRFV and Paprika mild mottle virus isolate from Japan (PaMMV-J) have been shown to exhibit more severe symptoms (Luria, *et al.* 2018).

Unlike ToMV and TMV, ToBRFV breaks the *Tm-2*^{2} resistance in tomato. To date, no commercially available variety has demonstrated resistance to ToBRFV, although resistance is in development. The presence of the *L*^{4} resistance gene in pepper maintains resistance for almost all European production varieties, but some heritage varieties will not carry this gene and will be susceptible to ToBRFV infection. No such infections have been officially reported.

In regions where most of the pepper varieties grown lack the *L*^{4} gene—e.g. Mexico—the impact of ToBRFV on production has been catastrophic, with 100% of plants infected at some

large sites. Currently, there is very little research on ToBRFV is being undertaken in Mexico, but there is some joint collaboration with the USA, who import a large quantity of fresh produce from Mexico. Breeders can incorporate the L^4 gene into their commercial varieties, but this process takes time. Genetically modified (GM) produce is grown and sold in Mexico/USA, and this may provide a faster route to the incorporation of resistance genes into popular varieties.

Thankfully, ToBRFV is not present within the seed endosperm, but it may be present on the seed coat. If seeds are cleaned and processed effectively, there will be negligible risk of young plants becoming infected. However, if a viable inoculum source is present on the seed coat, and the seeds are not processed completely, the developing seedlings could become infected. Seed infection in this manner is believed to be a rare occurrence and is extremely unlikely to happen on commercially processed seed.

The fact-finding trip

As a response to the threat ToBRFV poses to the UK tomato and pepper industries, AHDB Horticulture funded Dave Kaye (ADAS Plant Pathologist) to undertake a fact-finding study tour of protected edible production sites and specific crop research institutes in the Netherlands, Germany and Israel, where ToBRFV has been found, reported and acted upon. Seed producers, propagators, growers, consultants, researchers and diagnostic laboratories were contacted and visited during October (Europe) and November (Israel) 2019.

It was anticipated that direct face-to-face contact would facilitate honest, open discussions between the project lead and the individuals/sites visited. Not only would these discussions provide insights into ToBRFV management and avoidance strategies, it would also establish a platform for continued information exchange.

As a consequence of the high infectivity rate of ToBRFV, most industry visits in the Netherlands and Germany have been stopped, and communication within the industry has become restricted. This has also happened in the UK, where only essential visits are now allowed.

Just before the visit to the Netherlands, the first official outbreak of ToBRFV in the Westland region was reported, and several more have been reported since. Seven outbreaks occurred in Germany in 2018, all at sites in a grower cooperative. These infections were successfully eradicated, and no new outbreaks have been reported. Israel and Jordan grow in the soil and these countries continue to be plagued by ToBRFV. This will remain the case until resistant varieties are developed. Only one UK outbreak has occurred (Kent, July 2019), which has

also been successfully eradicated. At the time of writing, no further ToBRFV infections in the UK have been reported.

Over the course of the two trips, more than twenty people at seed producers, propagators, grower sites, and research and diagnostic laboratories were met with. In addition, communication via email and telephone was made with other businesses/institutes which could not be visited during the two study tours. This information has been collated and reported under three headings, representing the different stages of tomato/pepper production; Seed Production, Propagation and Main Crop Production.

During the trips, a considerable amount of information was gathered, some of it anecdotal in nature. In situations where the reliability of this information was questionable, it has not been included in this report. Where information has been included, but has no direct robust scientific backing, this is noted in the text. This information has been included if it is standard practice used by the industries, or it may identify future research needs.

7. Results and Discussion

Seed Production

ToBRFV is not present in the seed endosperm, but is found on the seed coat (Dombrovsky A, 2017). Seed which is comprehensively cleaned and treated will pose no risk of spreading ToBRFV. Though not technically seed borne, if the developing seedling does come into direct contact with an infected seed coat, this seedling could become infected. The rate of viral transmission to seed in this manner is currently unknown, but is expected to be low. At the time of writing, this is being established at the Volcani Centre (Agricultural Research Organisation, Israel). Over 1100 plants from untreated seeds collected from infected plants have been tested (as of 11/11/19), and grown in isolation to prevent inadvertent infection occurring. No plant has tested positive for ToBRFV yet, but testing will continue until it is found.

A major European—and the two largest Israeli—seed producers were visited, and contact made with two others via telephone/email. All seed-transmissible pathogens, including Clavibacter, Tobamoviruses, PepMV, and *Pseudomonas* and *Xanthomonas* spp. pose significant risks to seed producers. Commercial seed is typically produced outside of Europe, normally at isolated locations, and is subject to high phytosanitary measures, including GSPP . In places where seed production is carried out in areas of dense tomato/pepper production, this may be moved to a more geographically isolated area. One seed producer in Israel considered moving production to Turkey, but chose to remain, despite being surrounded by ToBRFV infected sites. They have so far managed ToBRFV effectively, avoiding infection by

implementing and following high phytosanitary procedures and limiting access to sites, and believe that they can continue to do so.

In Turkey, ToBRFV has spread much slower. This is attributed to cooler conditions where the majority of tomato is grown. Unlike Israel, where symptoms are more severe during the hot summers, symptoms in Turkey are worse during the winter where the crops can reach temperatures as low as 4°C. Almost all discussions over the course of the fact-finding trips have suggested that plant stress is a trigger for enhanced symptom expression.

Most—if not all—seed production is now grown under protection, with few crops grown in the open field, mainly due to pest issues. Seed production sites have developed dedicated, risk-based emergency ToBRFV protocols. Production from parental material will only occur if this has been fully tested and confirmed to be disease free. This parental material is kept isolated, with access to it limited. Frequent crop monitoring is carried out by phytopathologists, and suspicious plants are tested in-situ using generic tobamovirus lateral flow devices (LFDs), e.g. Agdia Immunostrips. Any plants which test positive for tobamovirus will be removed and further tested to confirm ToBRFV infection.

Dependent on the company/site, if a plant is suspected of being infected with ToBRFV, this—and a number of additional plants—will be removed. The recommended number of plants to remove was discussed at several meetings, with no consensus reached. Removal may simply be ten plants either side of the infected plants along the row, with additional plants taken from the neighbouring rows. However, if an infection is confirmed, one Israeli seed producer stated that they may remove and destroy the entire crop, preventing anything from entering the market. They believe it is better to have a zero-tolerance approach and take no chance of spreading ToBRFV. This seed producer has divided their seed production houses into distinct isolated blocks, limiting staff access to different parts of the structure. Should an outbreak occur here, areas can be quarantined whilst the rest of the crop processed. Emergency protocols are in place, but the actions taken will be judged on a case by case basis, assessed on the extent and location of infection. Thus far, no seed producer met has implemented any of their emergency procedures, suggesting their hygiene standards are sufficient.

Seed is processed and shipped to a central packaging and distribution centre, often in the Netherlands. This route represents the first high-risk entry point for ToBRFV and appropriate measures need to be identified and put in place to mitigate against any infected seed entering commercial production. Until the point at which treated seed has been confirmed to be pathogen free, it is restricted from use.

Seed houses already have procedures confirming their product is free from seed transmissible disease before dispatch, including seed treatments. This typically involves a

series of processes to clean seed, followed by treatment to deactivate any residual viral inoculum. Information was received that products hydrochloric acid, sodium hypochlorite and trisodium phosphate can be used in these situations. All seed lots are tested for pathogens, including tobamoviruses, and certificates of cleanliness are made available to customers. Propagators (and growers) should always request that these certificates are provided before seeds/young plants are allowed on-site, including the number of seeds which have been tested.

Seed testing

Most seed producers follow ISTA practices for determining the viability of seed transmissible viruses. Currently no ISTA seed testing protocols are available for ToBRFV, however validated seed health testing methods are available for the detection of the other tobamoviruses in tomato, including ToMV and TMV (ISTA, 2014). In addition, the ISTA Seed Health Committee recommends diagnostic laboratories follow ISHI best practices for seed health.

Most of the large seed houses will also have or follow other seed quality certifications, including Naktuinbouw Authorized Laboratories (NAL), GSPP and SKAL (control authority responsible for the inspection and certification of organic companies in the Netherlands). Membership of these includes auditing, which ensures all procedures are correctly followed.

Seed testing is only as accurate as the test procedure itself, and the limit of detection will vary with the number of seeds sampled (Table 2). No test will give 100% certainty of seed cleanliness.

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

Seed Volume	**Detection limits (95%) confidence**
3000	0.1% infection
1000	0.3% infection

From discussions with seed producers, propagators and diagnostic labs, at least 3000 seeds should be tested, but one Israeli propagator stated that they would test as low as 2000 seeds if necessary. One propagator and a member of the phytosanitary controls on imports, exports and business registrations team at the Landwirtschaftskammer (LWS, German Chamber of Agriculture) said they would rather more seeds were tested (up to 5000).

Although seed is expensive to purchase, it is mass produced and cheap from the perspective of the producers (around €0.02/seed). It is not possible to process 3000 seeds at once and

before testing, the seed sample needs to be ground up and several batches need to be prepared. Sampling 5000 seeds would add significant time to this process, likely delaying reporting. It would also add additional costs for consumables, as most lab items are single-use to prevent cross-contamination of samples and false positive results. The general consensus was that testing 3000 seeds was sufficient.

The most commonly used test for ToBRFV is ELISA (enzyme linked immunosorbent assay). This test uses specific antisera (antibodies) which target and bind to the target virus. A second antisera containing a marker is then added and the presence of the virus confirmed by the marker changing colour when an appropriate substrate is added. This test is rapid, and normally done in a 96-well plate enabling a large quantity of samples to be run simultaneously.

Initially, there was no specific ToBRFV antisera available, and testing of a ToBRFV positive sample would provide a positive tobamovirus result, not distinguishable from ToMV or TMV (cross-reactivity). ToBRFV specific antisera is in development, with varying degrees of specificity achieved. To date, no antisera completely specific to ToBRFV is available.

ToBRFV infection can be accurately determined by other diagnostic tests and the absence of highly specific antisera is not a major concern. Seed producers cannot allow any tobamovirus to be present on their seed, regardless of if it is ToMV, TMV or ToBRFV. The Senior Seed Pathology Researcher at the major Dutch seed producer visited during this work stated that apart from testing specifically for ToBRFV, they had not changed their treatment practices as a response to ToBRFV and they believe their processes are already sufficiently robust.

The seed treatment process aims to remove/inactivate any residual virus present on the surface of the seed. Viruses are not considered to be ‘living’ entities and it is not possible to determine whether a virus is either infectious, or inactive, from ELISA alone. In circumstances where ELISA tests are positive, a bioassay is performed to check that seed treatment has been 100% effective.

The bioassay involves artificially inoculating leaves of indicator plants, *Nicotiana tabacum* cv. Xanthi NN or *Nicotiana glutinosa,* with a tomato or pepper seed extract. Typically, local lesions will rapidly develop on these species when they are inoculated with infectious virus particles from infected seed, demonstrating the viability of the virus. This process is much slower than ELISA, taking one to two weeks based on the conditions the plants are grown under, and tests can require the use of large amounts of growing space.

Seed extract TaqMan Real-Time (RT) qPCR can also be used as an alternative to ELISA for exact identification of ToBRFV. Naktuinbouw (Netherlands Inspection Service for Horticulture) developed a RT-PCR method which will detect a single infected seed in sub-samples of 1000 seeds. This test is very selective, only detecting ToBRFV, so will not identify

other tobamoviruses which may also be present in the sample. Similar to ELISA, RT-PCR will not distinguish between infectious and non-infectious ToBRFV and a bioassay is required to confirm infectivity. The ability of the Naktuinbouw RT-PCR method to positively detect the virus using only 1000 seeds reduces the amount of seed wasted and is beneficial for testing trial variety seed where limited numbers are often available for testing.

Care must be taken when running RT-PCR tests, any damage to the sample material can result in a false negative result. For this reason, samples should only be sent to an accredited diagnostic lab, e.g. Fera in the UK. While ELISA remains the most commonly used diagnostic test, RT-PCR is used to complement ELISA, definitively identifying the tobamovirus as ToBRFV.

Due to growing practices and the lack of the L^4 resistance gene in pepper, ToBRFV infection has seriously impacted tomato and pepper production in Mexico. Some samples sent from Mexico—where they tested positive for ToBRFV—to a diagnostic laboratory in the Netherlands tested negative in their second test. This difference is likely down to the sensitivity of the ELISA tests used in Mexico, including cross-reactivity with other tobamoviruses which may be present on-site, providing a false positive result. This result further underlines the importance of sending samples to certified diagnostic laboratories.

Currently, different antisera are available for use with ELISA, all varying in their degree of specificity. Research at many organisations is ongoing, and it is likely that specific antisera to ToBRFV will be developed which will allow confident identification of ToBRFV via ELISA. All seed companies, propagators and diagnostic labs visited expressed a desire for a definitive ‘gold-standard’ protocol to be developed for all diagnostic tests. ISHI protocols offering guidance do exist, but these are supported by seed producers and may not represent real best practices. Fera—alongside the Julius Kühn-Institut (J.K.I., Federal Research Centre for Cultivated Plants in Germany)—are working together to achieve this goal. The development of this protocol will ensure the best detection methods are consistently used, allowing complete confidence in the results. As the tests continue to be refined, or their sensitivities improved, amended protocols can be developed and distributed.

Many diagnostic laboratories are NAL accredited, following the same procedures/standards as Naktuinbouw. These laboratories are audited to ensure results are consistent. Similarly, Naktuinbouw receives samples from NAL accredited labs to ensure their own procedures are robust. Since 2018, no Dutch samples were received at Naktuinbouw from growers which tested positive for ToBRFV, but it is likely these samples do not reflect the ToBRFV situation on nurseries in the Netherlands, particularly since the notified outbreak in 2019. From November 1st 2019, ToBRFV gained a quarantine status. Since this date, all outbreaks have

become subject to a European emergency regulation, requiring all cases of the virus be made public. All diagnostic laboratories in the Netherlands were required to submit any records of positive results for ToBRFV infection and are now legally obliged to report any new outbreaks which might occur in the future. Although these measures have been put in place to protect the industry, they do not necessarily mean the virus will actually be eliminated.

In Israel, several laboratories test for ToBRFV and two of these were visited. The Volcani Centre receives around 500 samples per week, whilst at the other laboratory, around 30-40 are tested. The large number of samples received in Israel is in part a consequence of their intensive cropping system, and also the fact that ToBRFV is present at most commercial production sites.

In December 2018, a positive sample for ToBRFV was detected in aubergine (*Solanum melongena*) in Mexico (*Senasica*, 2019). However, an earlier inoculation study showed that *S. melongena* was not a host of ToBRFV (Luria et al., 2017). Aubergine root stock has been considered an option for protecting soil-grown crops from developing infections from ToBRFV in infected soil. However, the use of these rootstocks will not protect against mechanical transmission between plants. Ungrafted aubergine varieties in Europe root poorly, and commercial growers graft their varieties onto tomato rootstocks, e.g. Beaufort. This is done as a response to increased incidence of Pythium and Fusarium in the soil. Therefore, the use of aubergine rootstocks in Europe is unlikely to be used as a ToBRFV management strategy. Work to investigate these contradictory results is planned by research organisations including the J.K.I. and the Volcani Centre.

Resistance breeding

No commercial variety has been demonstrated to be fully resistant to ToBRFV, however some varieties in Israel are asymptomatic. Growing these varieties may increase marketable yields, but asymptomatic ToBRFV infections will likely impose a yield penalty. Despite being symptomless, infected plants will contain infectious ToBRFV particles, and these plants will act as inoculum sources for mechanical transmission to other susceptible varieties.

Historically, breeding for resistance involved screening to identify plants with the desired characteristics, including inoculating plants with ToBRFV. Modern day breeding incorporates genotyping into this process, increasing the rate of resistance development. It is believed that resistant commercial varieties may be available in as little as four years' time, but some breeders have suggested this will take considerably longer (>10 years).

Many of the largest seed producers and research institutes are screening for full, or partial, ToBRFV resistance. Two organisations visited during the study-tour to Israel, have developed ToBRFV resistance to some extent.

The Volcani Centre have developed a rootstock resistant to ToBRFV which will be made available in 2020. Intensive repeat cropping in the same soil has led to a significant ToBRFV presence within the soil. A resistant rootstock would prevent the infection route from the soil to plants, but will not prevent mechanical transmission. The development of a resistant rootstock will be unlikely to directly impact the ability of European growers to control ToBRFV infections, as most production is in artificial substrates, but this demonstrates that resistance is being developed. Were outbreaks to develop in organic crops in the UK, the use of resistant rootstock could be useful, but it is unclear if this would be possible with the Q-status of ToBRFV in Europe and this may be too risky to other production.

The second source of resistance is being produced by an Israeli seed producer and is entering the final rounds of testing. This company believes that they may have identified a full resistance gene, but whether it differs from the resistance gene developed by the Volcani Centre is unclear. If true resistance has been developed it will be trialled over the next couple of years on grower sites. If successful, resistance will begin to be bred into commercial varieties. Israel has been dealing with ToBRFV since 2014, and their breeding programmes are ahead of most other countries, especially as they have the ability to grow in infected soil. It is positive to see that resistance is already being developed and it is reasonable to assume that other sources of resistance may have also been identified.

Unlike other viruses such as PepMV, the genome of ToBRFV has been found to be remarkably conserved across all sequenced samples (>99%). This is positive news, and it is unlikely that resistance breaking strains will develop quickly once resistance has been bred in.

Propagation

A large Dutch propagator and the two largest propagators in Israel were visited. Propagators are required to provide vigorous disease-free plants to their customers. Any infection which develops on plants at this stage—including ToBRFV—could negatively impact several production sites. The high infectivity of ToBRFV means that delivery of infected plants could rapidly spread the virus further.

The Netherlands

Tomato propagation is high-risk and there is currently no compensation available to UK or Dutch propagators/growers who suffer from ToBRFV infections. The Dutch propagator visited has a responsibility for over 600 employees, and a confirmed ToBRFV infection could be financially disastrous, leading to job loses—or even site closure—and they take the threat of ToBRFV very seriously.

The Dutch propagator was visited shortly after the first official outbreak of ToBRFV was reported in the Netherlands (1st October 2019). They correctly stated that the actual ToBRFV situation in the Netherlands was likely greater than had been reported so far.

Propagators have no option but to trust that the seed lots provided by seed producers are free of seed transmissible diseases. Seed certificates are always sought, providing information on the nature and robustness of any diagnostic tests carried out on the seed, including the quantity of seed that was tested. Seed producers are required to keep in-depth records and samples of each seed batch are always kept back, allowing these seed lots to be revisited, and re-tested if necessary.

Due to the epidemiology of ToBRFV, there is a latent period from infection to visible symptom expression. Young plants are unlikely to become symptomatic before dispatch from the propagator in the Netherlands, unless they are stressed, in poor health or suffering a considerable ToBRFV viral load. In Israel, propagators only grow to the plug stage, meaning even shorter propagation times. ToBRFV symptoms will never develop on Israeli plants until after they are dispatched.

The Dutch propagator visited considers their site hygiene practices as comprehensive. To date, they have had no issue with ToBRFV, PepMV or Cucumber Mild Mosaic Virus (CMMV). This propagator does not think they will suffer from a ToBRFV infection and believes that, given time, the virus will be eradicated in Europe (though did highlight that the industry is only as strong as its weakest link). Changes in practice, as a result of ToBRFV, have been introduced to improve their current systems and they will continue to make changes as new information emerges. All entry points onto their sites, whether plant production or administrative, contain a mechanised foot and hand cleaning device (hygiene lock, Figure 2). All staff and visitors, without exception, are required to pass through these when entering or exiting. The detergent used in the foot brushes is unknown, but the hand sanitiser was alcohol based, which has not shown efficacy against ToBRFV. When used correctly, these machines are an effective tool in reducing ToBRFV risk, and also provide a visible reminder of the seriousness of excellent on-site hygiene.

The machines are only as effective as the disinfectants used, and all products must be used at the correct rate to ensure continual effectiveness. Machines must be well maintained, and routinely cleaned down with a disinfectant such as Virkon S.

Figure 2. Hygiene lock at the entrance/exit of a propagator in the Netherlands – image with permission of the propagator.

As with seed producers, only essential on-site visits are permitted. Visitors are considered high risk, especially NVWA extension workers (Netherlands Food and Consumer Product Safety Authority, formerly the National Plant Protection Organisation (NPPO)). Site visits are only permitted first thing in the morning, ensuring visitors have not been to other tomato production facilities that day. All visitors to the administration centre are escorted directly to meeting rooms and back to the exit after the meeting concludes. Customer visits are not advised, with the propagator quoting ToBRFV as the reason for this. Where customers insist on visiting the site, plants are brought to them, rather than the customer brought to the plants.

Crop workers are requested to arrive in clean clothing, and are then provided with fresh clothing to change into. This clothing is laundered on site, using normal detergent, and never leaves the propagator. Should this Dutch propagator develop any issues with ToBRFV in the future, they will investigate the alternative of using medical grade viricidal detergents for their clothing. Shoes are kept on site and are routinely disinfected, and vinyl gloves provided for use.

Any subcontractors who enter production sites are requested to change directly into new disposable oversuits, gloves, hats and shoe coverings before leaving their vehicles, and are

not to get changed until after they have returned to their vehicles. Trucks must be cleaned before use with a suitable disinfectant, e.g. Virkon S.

Returned trays have been identified as a significant risk and all trays used during plant delivery are now single-use, never returning from growers. This propagator uses a cart cleaning machine, rather than relying on individual staff to clean carts, saving time and ensuring that the carts are cleaned consistently.

Almost everything which leaves the site is not allowed back on site. All areas are assessed based on their relative risks and designated as Green Zones (low risk) or Red Zones (higher risk). It should be noted that Red Zones are areas which are considered at a greater risk of developing ToBRFV, but this risk will still be very low with the current procedures in place. Any equipment from off site, or equipment which is to be moved from a Red Zone to a Green Zone, is addressed and controlled. Only once all risks have been mitigated will anything be moved to a Green Zone. Staff and equipment can move freely between Green Zones, or from a Green to Red Zone as long as their hands are disinfected. Movement and work between Red Zones is more managed, with requirements for hands to be disinfected frequently, and more regular changes of disposable gloves.

Changes at the grafting stage have also been introduced, with blades replaced at the start of each tray, and hands disinfected with Enno Rapid (benzoic acid). Some customers have requested a more frequent change of blades/hand disinfection and this is accommodated, at an additional cost.

Irrigation is performed at the request of the customer e.g. overhead, ebb and flood etc., but as is now standard for the Netherlands, the irrigation system is closed, with water recirculated. This site uses sodium hypochlorite and high-power UV bulbs to sterilise their water.

Historically, trial varieties have represented one of the greatest risks for propagators. Growers are keen to ‘try before they buy’, trialling new varieties before committing to growing them in large quantities. Often, the seed available for some varieties is limited, restricting the amount which is used in seed testing, often too far below the recommended 3000. Since the outbreaks of ToBRFV in Europe, the number of trial varieties propagated for the 2020 season has decreased considerably. Growers who plan on continuing to grow trial varieties in the future should ensure that sufficient seeds are tested and reconsider varieties if the number of seeds tested is lower than 3000.

Some propagators carry out their own testing for other seed transmissible pathogens—including PepMV—which provides additional certificates to clients as an extra guarantee of seed/plant cleanliness. These tests will be performed in laboratories which are accredited

with certifications such as GSPP, NAL, etc. Propagators are unable to test directly for ToBRFV on site, and all samples are tested by the NVWA.

The ToBRFV diagnostic tests performed by the NVWA include ELISA, and the follow-on bioassay if necessary. These have a turnaround time of six weeks (two for testing, four for reporting). Six weeks may seem excessive, but is likely a response of the quantity of samples which require testing.

The Q-status of the virus means that any suspected outbreaks of ToBRFV need to be quarantined until the test results are released. During quarantine, access to suspicious plants is strictly prohibited. Propagation of plants for the European market takes around six weeks and theoretically, should several young plants appear suspicious, entire propagation sites could be quarantined. Under these circumstances, regardless of the whether plants test positive for ToBRFV or not, they are effectively removed from production.

As a response to the restrictions Q-status places on production, tomato growing areas at some Dutch propagators have been condensed to a few sites. Each production area has been sited to enable certain sections to be isolated. This allows main production to continue should an area of tomato be quarantined. Moving production to a few sites also isolates tomato production away from other crops which may be impacted if areas were quarantined. Most pepper varieties are grown alongside tomato, but heritage pepper varieties—which do not contain the L^4 gene—are grown separately. Should more than one suspected infection occur, all production could stop. The complete loss of tomato production at some propagators could shut these businesses down, having significant repercussions for their customers.

Israel

It was only possible to visit one of the two Israeli propagation sites due to escalating political tensions within the region, but the phytopathologist for the largest tomato propagator was met at the Volcani Centre. Both of these propagators supply the West Negev region, an arid desert region in the South of Israel, where 70% of tomato production is located. Unlike Europe, almost all of tomato production in Israel is in soil, but growers still require healthy, disease-free plants.

Until recently, almost all young plant production in Israel was ungrafted. Plants were grown with a single head, as the choice of varieties grown and environmental conditions meant taking additional heads was inadvisable. Recent restrictions in soil sterilants has led to a resurgence in soil-borne pathogens, including Fusarium and Pythium, and root stocks are increasingly being used to help manage these diseases. Another benefit of using a rootstock is that two heads can be taken. As a response to ToBRFV infection, Israeli growers switched to two crop cycles, rather than one. Two heads enable growers to maximise yields in these

shorter cycles. The switch to two crop cycles as a response to ToBRFV, and an increase in the number of plants being grafted, has benefitted both seed producers and propagators, effectively doubling their sales/work. It is likely that once resistance to ToBRFV has been developed, most plants in Israel will continue to be grafted.

Site hygiene at this propagator was similar, but not as stringent as the Dutch propagator. The disease management strategy in Israel is delay-based, rather than avoidance-based. The development of symptoms is seen as inevitable to most growers, but plants which are well established before infection occurs will produce the most marketable fruit. Most growers have ToBRFV infested soil, with other infected production sites often located as little as 10m away. Delivery of clean plants is therefore essential to delay symptom onset, maximising marketable yields.

Similar to Europe, certificates are sought from seed producers to ensure any seed sourced is free of disease. As an additional service, this propagator sends additional samples to Israeli diagnostic labs, e.g. Volcani Centre/Microlabs, to confirm the results of the seed certificates. This is an expensive and time-consuming process, but provides customers with additional peace of mind. The Israeli propagators want to see a standard test developed, with additional work on improving the sensitivities of current testing procedures. As ToBRFV symptoms will not develop until young plants have been planted seed certificates offer the only guarantee to growers. Everything is recorded, with full traceability of plants back to seed lots, including locations where the plants were grown. If an issue is raised, the entire process can be reviewed and any entry points of the virus addressed.

Hygiene protocols are developed and reviewed as information becomes available. All visitors are required to wear disposable shoe coverings, oversuits and gloves before entering any production areas and hand sanitiser is present throughout. All staff must ensure they have clean clothing, and gloves are worn and frequently changed, especially during grafting. At this time, blades are also frequently replaced.

A consequence of supplying only plug plants for direct planting into the soil—rather than the larger plants typically produced by European propagators—the total area required for tomato production is much smaller in Israel. Two to three million plants will be propagated in one production house over the course on one year, with 70% of these grafted on Beaufort rootstock. Should an outbreak occur, this could put a large amount of production at risk.

Plants are dispatched to grower sites in plastic plug trays, which are returned for re-use at one of the two propagators. It is known that many of these trays will return contaminated with infectious ToBRFV, and without treatment the virus could be transmitted to an uninfected crop. In AHDB project PE 033, work by Fera on the survivability of ToBRFV on different

surfaces demonstrated the virus's prolonged persistence on plastic surfaces. This site uses a specialist tray cleaner which washes and disinfects trays before they enter onto the main site. Each tray is washed separately at high pressure with sodium hypochlorite, before rinsing with clean water. This process has not been tested against ToBRFV, but is likely to be effective as no early ToBRFV infections have been reported at this propagator. Histil Nurseries, the largest tomato propagator in Israel, will not risk re-using their plastic trays, and all trays are now single use. This method is expensive, and environmentally questionable, but they are not willing to take any chances.

Plant wounding is thought to be the major route of infection into plants—especially within the soil, where ToBRFV inoculum is present at planting. Shorashim nurseries have developed a biodegradable organic film which surrounds the plug. Transplanting invariably results in some degree of root wounding, and this film prevents direct contact between the roots and the soil during this high-risk period. This film will eventually dissolve, by which time any root wounds from planting will have healed. However, the use of this organic film will not benefit protected cropping in Europe, where much larger plants are propagated and grown in artificial media, but could potentially be useful in organic production.

Due to the close proximity of most of Israeli tomato production to their sites, some propagators continue to monitor their plants during cropping. Early development of symptoms could indicate plants were infected before planting. Both propagators believe that the plants they supply are free of ToBRFV infection and that their hygiene practices are sufficient. The number of large tomato propagators in Israel is limited, and early ToBRFV from infections occurring at just one propagator could seriously impact the yields of a large portion of the industry in Israel.

Main crop production

At the time of writing, ToBRFV outbreaks have been officially confirmed in seven European countries, including Turkey (Table 1). There are frequent rumours of outbreaks in other European countries, but until these are officially confirmed, they remain speculation and will not be commented on further in this report.

The Netherlands produces over 90,000 tonnes of tomatoes a year, with an export value of 1.6 billion euros in 2018 (Statistics Netherlands, CBS). During the European study tour, only one official outbreak had been confirmed in the Netherlands. The identity of this site was unknown, and it was not visited during the trip. Shortly after the tour, several other Dutch production sites were also confirmed to be infected. As of 6th December 2019, ToBRFV infections have been found on the nurseries of at least ten tomato growers, with several other

growers having plants considered seriously suspicious of infection. Despite best efforts, no site in the Netherlands were willing to discuss ToBRFV and contribute to this work.

Germany

The first official European outbreak was seen on a production site in July 2018, in the North Rhine-Westphalia region of Germany by an extension worker (Plant Protection advisor) for the LWS. This was later confirmed to be ToBRFV via high throughput sequencing and electron microscopy, with the assistance of the J.K.I. who are responsible for training LWS extension workers. The Germans were the first to offer reasonably specific antisera for ToBRFV detection, but cross reactivity with other tobamoviruses still occurs. Antisera with a greater specificity to ToBRFV is in development by the J.K.I. and other laboratories, and similar to Naktuinbouw, the J.K.I is responsible for auditing the accuracy of ToBRFV tests used by other German diagnostic laboratories.

The infected site where symptoms were first seen is part of a collective of seven growers, and all members' sites had developed ToBRFV infections by the end of 2018. Despite suffering infection for some time, clean-down procedures were effective, and after months of extensive sampling and testing, Germany was declared ToBRFV free in July 2019 (EPPO 2019/145).

The original ToBRFV infection in Germany occurred earlier in the year, at a different site within the grower group to the one visited. Unknowingly infected, this grower continued production. Dependent on the variety, the initial health of the plant pre-infection, and the time of the year, it is possible to continue to produce marketable fruit despite infection. However, the volume of marketable fruit may be so low that crop inputs exceed profits, making continued production uneconomical.

The German crops were grown in coir, in boxes, rather than disposable slabs, and this coir was reused over multiple crops. Due to the absence of ToBRFV in Europe, the first German grower was unaware that they were infected with ToBRFV. There is general consensus that plant stress is a driver of increased symptom expression. The summer of 2018 was consistently warm and sunny—excellent growing conditions—and this may have reduced symptom expression.

It is important to note that marketable fruit harvested from infected plants is still infected, just asymptomatic of viral symptoms. Continuing to grow infected plants on sites will lead to a build-up of ToBRFV inoculum. This will increase the likelihood of mechanical transmission to uninfected areas and make eradication much more difficult. The single UK outbreak occurred on young plants, which had not been de-leafed or harvested. The limited interaction with this crop will have increased the likelihood of successfully achieving complete eradication.

Most tomato producers have banned the consumption of tomatoes on their sites—it has been reported that fruit infected with ToBRFV have been found in supermarkets in Florida on fruit imported from Mexico (Florida Department of Agriculture and Consumer Services, 2019). Infected fruit poses a real risk, especially when imported from countries where the virus has been confirmed, e.g. Spain. Some sites provide their own fruit for staff consumption, but this is not advised. If fruit is supplied to workers from a house which is infected, but not yet symptomatic of ToBRFV, the virus could be rapidly spread across the entire site. To date, no testing of imported supermarket fruit for ToBRFV has been performed in the UK.

Distribution of infected fruit will also contaminate the supply chain, including pack house facilities and supermarket crates. Contaminated crates represent one of the most significant risks to the grower community. This was demonstrated in an experiment by the LWS, where an infected leaf was placed directly onto a plastic tray for one second. Later swabbing revealed that sufficient levels of the virus had been transmitted to the tray for detection during this brief contact.

The Q-status of the virus in Europe, means that all outbreaks must to be made public. It is up to each EU member state to determine what measures need to be taken upon discovery of the virus in production. As no outbreak has occurred in the UK since the legislation came into place, it is not known what these measures will be.

A visit was made to the German site where the first outbreak of ToBRFV was identified, and the extension worker who originally identified the disease was met, alongside the grower. There was some disagreement as to whether the original infection was PepMV or ToBRFV, as symptoms for both diseases are similar at an early stage. In addition, the plants had been treated with a PepMV mild strain. ToBRFV had not yet been found in Europe—despite rumour of its presence—but fortuitously, the extension worker had recently attended some ToBRFV training. They noted patterns in the symptoms, with infected plants appearing to move down rows. This is unusual for PepMV, which often appears sporadically around infected houses. These symptoms were consistent for a disease spread via mechanical transmission, likely from people or trolleys. Samples were collected and sent away for testing at the LWS.

Results confirmed that the crop was infected with PepMV, but also ToMV and TMV. This was surprising as all the varieties tested were resistant to these tobamoviruses. No ToBRFV specific ELISA test was available at this time, and the TMV and ToMV positive result was a consequence of cross-reactivity. ToBRFV was later confirmed via high throughput sequencing.

Although symptoms were present in all varieties, on all sites, two were severely affected, including var. Juanita. These varieties had performed poorly the entire season, were stressed

and generally less vigorous than others grown on this site. In 30 years, the extension worker had never seen a disease with such rapid spread of symptoms—one third of the Juanita crop was dead within six weeks of visible symptom development. It was not possible to say that these varieties are more susceptible to ToBRFV infection than others, or if the symptoms seen were a result of the poor health/vigour of the plants. More information may be available from growers in the Netherlands who have recently suffered infections. Future trial work could also look at the relative susceptibilities of varieties grown in the UK to ToBRFV infection.

Varietal differences were clear, some plants only showed symptoms on leaves, but fruits failed to ripen; other varieties exhibited symptoms on the fruit, but not on leaves. Generally, there was more drooping on ToBRFV infected young leaves than you would expect from a PepMV infection alone. Leaves were smaller, and when looking up at the canopy, “a lot more sky” was visible than normal.

At this site, sterilised knives are now made available for each row (Figure 3). These knives are soaked overnight in Enno Rapid, and placed out on hooks at the end of the rows before work starts each morning. Workers must change knife before beginning on each new row. Enno Rapid hand sanitiser is available for use, and staff are requested to sterilise their hands frequently. Any knives which are damaged/rusty are removed and replaced. The process of soaking knives for long periods of time (≥12 hours) is likely an effective one, and the one knife-one row approach sensible.

Some sites will treat their trolleys by spraying them with a knapsack containing Menno Florades after each row. However, the effectiveness of this brief surface disinfection is unclear, and contact times may be insufficient to inactivate the virus. Also, any hard to reach areas where the virus may persist, are unlikely to be treated. The degree of management practices undertaken by any aspect of the industry is risk-based and will always need to factor in the costs associated with each practice.

Figure 3. Using a dedicated knife for each row helps to prevent infection of neighbouring rows. Photo: Heike Scholz-Döbelin.

The initial source of ToBRFV in Germany is unknown, but local spread between the seven grower sites is likely due to site visits, or the sharing of crates/boxes. Despite infection, marketable fruit continued to be sold until the crops were quarantined at the end of the season. For six of the seven sites, symptom development did not occur until late in the season. This is likely due to the timing of the initial infection, and/or plant stress from shorter day lengths.

The method of disposal of infected material differs between countries; no international standard has been enforced. The Netherlands compost their waste at facilities which are certified to meet a high temperature requirement and maintain this for sufficient duration to deactivate the virus. They do not recommend incineration, due to the high cost. In Germany, crop waste from ToBRFV infections must be incinerated, as here it is viewed as the only guaranteed method for complete elimination.

Many German growers grow in plastic boxes, only replacing the coir every few years. They believe reusing growing media gives a better yield in subsequent years. As part of their clean-up process in response to ToBRFV, the growers disposed of their coir and the boxes. The coir will be replaced each year, with boxes replaced where an outbreak occurs in the previous

season. The drip pegs and laces were also removed and replaced on the seven sites as an additional part of their clean-down process

Although it is unlikely that infection arose from propagation, as a precaution, plants delivered to the group this year were tested for ToBRFV before delivery, and these plants were not allowed on site until they were confirmed to be ToBRFV free. Plants continued to be routinely re-tested throughout the season, to ensure that clean-down was effective at eliminating the virus. Crop monitoring continues, with any suspicious plants sampled and tested.

There is concern that the measures put in place to limit ToBRFV infection will not be followed as comprehensively after a few years. However, the biosecurity and hygiene approaches put in place to manage ToBRFV will reduce the risk posed by other plant pathogens, and best practice should always be maintained where possible.

Harvested fruit from the German group of growers is sent to a central packing facility. Since the outbreaks, fruit is carefully transferred from grower crates to packhouse crates, allowing crates to be returned to their original sites and not shared between sites. The efficacy of this practice is dubious, as it is unknown if these crates are disinfected, or if any disinfection practices are sufficient to eliminate ToBRFV. Fruit quality issues have also arisen as a result of this additional handling.

At the time of the German outbreak, very limited information was available on the best practices for site clean-down. Little literature was available for disinfectant products which have been proven to effectively inactivate ToBRFV. Based on work on other tobamoviruses, the LWS have examined what was likely to be effective, with the assistance of the Julius Kühn Institute and Volcani Centre, amongst others.

Since the first outbreak, the clean-down procedure recommended in Germany for response to an on-site ToBRFV infections has been updated. The requirements for the disinfection and decontamination of ToBRFV in an empty greenhouse are located in Table 3. It is important to note that some chemical disinfectants recommended for use in Germany may not be authorised for use in the UK, and products must only be applied in an empty greenhouse, free of plant material. Appropriate personal protective equipment (PPE), should always be worn. Please note before using any product or protocol you must check legality and compliance with current UK Health and Safety law and legislation.

Table 3. German recommended procedure for the disinfection and decontamination of ToBRFV in an empty greenhouse.

Step	Product	Concentration	Approach	Comments
Clean up all plant debris and glasshouse structure.	Brooms, etc.	N/A	Remove all organic material from the site.	The cleaner, the better; residual organic material may impact the efficacy of the disinfectants used.
Clear sap residues, and everything which has had contact with plant material.	Venno 'Hortisept Clean Plus'(or equivalent)	Up to 2%	Foaming (warm water, 30 °C) for 5 minutes (product must not dry). Rinse thoroughly with water, allow to dry.	Application with spray lance or spray nozzle, e.g. injector nozzles. ○ or Skumix facility ○ or high-pressure cleaner with foam nozzle ○ or Menno dosing foam syringe
OR, in organic production	Fadex H+ (formic acid)	Up to 2%	Foaming (warm water, 30 °C) for 5 minutes (product must not dry). Rinse thoroughly with water, allow to dry.	Application with spray lance or spray nozzle, e.g. injector nozzles. ○ or Skumix facility ○ or high-pressure cleaner with foam nozzle - or Menno dosing foam syringe
Disinfection after thorough cleaning	Menno Florades	4%	Reaction time: - 4 hrs as a foam, otherwise 8-16 hrs Effective also after drying	Foam application offers higher effectiveness. Attempts by the University of Wageningen in 2019 gave good results against ToBRFV.

Step	Product	Concentration	Approach	Comments
	Sodium hypochlorite (content: NaOCl, 12.5%) or stabilised NaOCl Note: storage reduces effect.	3% dilution of 0.1-0.4% NaOCl in spray mixture.	Apply with spray boom with usual spray nozzle; leave to absorb for 2 hours (wet).	Monitor for corrosion and ensure correct personal protective equipment (PPE) is worn. - Research by the University of Wageningen in 2019 at 1% concentration gave no kill after 8 hours. - NaOCl gave good control against Tobamo viruses in Israel.

This table was sourced and translated from the Tomato Brown Rugose Fruit Virus (ToBRFV) – “Jordan Virus”, Practical information and experiences webpage.

https://www.landwirtschaftskammer.de/landwirtschaft/pflanzenschutz/gemuesebau/tobrfv.htm

German production sites are generally not as advanced as those in the UK or the Netherlands. Very little growing is done using lit crops and German growers will usually have two full months between plant removal and replanting. In situations where turn-around times are very short, and where other crops are present in other houses, the ability to eradicate ToBRFV will be constrained, underlying the need for common sense reporting and prompt, careful plant removal. Elimination will also be difficult where the virus has been present on sites for a long period of time, due to the build-up of high viral loads. The German disinfection process described in Table 3, combined with the long period of time the houses were left empty, will have aided eradication there.

The German approach to the ToBRFV outbreaks was excellent. Once identified as a problem, the virus was identified quickly and processes put in place to restrict spread. Despite not knowing which disinfectants were effective against the virus, LWS established contact with relevant institutes/individuals and developed a hygiene and disinfection protocol which was effective in eradicating the virus, even on a site where it had been present for most of 2018. This reinforces the importance of effective, proactive sharing of knowledge for the benefit of all.

The Netherlands

The Dutch outbreak was originally identified as a part of an EU sampling survey, with 8% of the crop reported as infected. Once the virus was reported in the Netherlands, diagnostic labs—including Naktuinbouw—who offer ToBRFV testing, began to develop ToBRFV protocols.

Once inside the plant, ToBRFV moves within the vasculature tissue. As a consequence of this movement, high levels of ToBRFV develop in the young leaves. Sampling these leaves requires the use of trolleys, and sampling takes time. Naktuinbouw are investigating the suitability of other parts of infected plants to be used as sample for virus detection, including older leaves, calyces, side shoots and roots, etc. to develop the most efficient sampling protocols for in-crop infections. Other laboratories in the Netherlands, including Groen Agro Control, also offer ToBRFV testing, including real time PCR assay which can be performed on many types of samples, including crops, water, or swab samples from tools or surfaces.

In recent years, many Dutch growers have come together to form larger groups. Consequently, many staff work across multiple production sites each day, which represents an additional risk. Similar to the UK, the risk from contact with staff clothing is now being considered. Research institutes such as Groen Agro Control are investigating materials which survive industrial cleaning temperatures (95°C wash), including using viricidal products which are currently used in the medical sector. Where disinfected clothing is used, all individuals

will need to follow a ‘dressing protocol’ to minimise risk of transferring ToBRFV from their personal clothes onto work clothing. Requiring staff to shower before and after work is also being considered.

Despite reluctance from the Dutch growing community to meet and discuss ToBRFV, contact was made with an extension worker who provides advice to many large Dutch growers. Since the obligation to report came into force on 1st November 2019, many Dutch sites have reported ToBRFV infections and it stands to reason that many of these sites were infected before this time. This will mean that marketable, but infected fruit has entered the supply chain, and there is currently no guidance on what sites are meant to do with this marketable fruit.

It is not known when the original Dutch infection occurred, or what the source of this infection was. Now that the virus has been reported, the seed houses and propagators which supplied the infected sites will be investigated by Naktuinbouw to try to identify the cause. The first rumours of ToBRFV infections in the Netherlands started in February/March 2019, though these decreased as the season progressed. This could be due to improved growing conditions (lower levels of plant stress)—which may have reduced symptom expression—or be simply that infection was not present at all. Unofficially, symptoms have been reported to have increased shortly before the first reported case in October. At this time, plants are becoming stressed (due to shorter day lengths, etc.), and visible PepMV symptoms often begin to develop. This timescale also mirrors what happened in Germany on the infected sites in 2018.

Similar to Germany, site visits have all but stopped, unless strictly necessary. As a consequence of this, communication between growers is now poor, but this may improve now that ToBRFV has been reported. Consultants continue to visit sites, but have made some small adaptations to reduce the risk of spreading ToBRFV. Shoe coverings tear easily and are often not suitable for long days, needing to be replaced. Instead of this, shoes are provided and stored on all sites, and never removed from sites. As an additional biosecurity check, one consultant will put on one glove, puncture a hole at the end of the sleeve of the oversuit, and place their thumb through it. A second glove is then worn on top. The benefit of this is that the protective clothing is now continuous where a wrist gap would normally be left exposed. It is debateable that this would make much difference, especially as consultants would disinfect their hands before entry into production areas, but it shows that they are taking ToBRFV seriously.

Other adaptations to growing practices have been implemented as a response to ToBRFV on sites. Unlike the one knife-one row policy used by the German growers, some Dutch

growers have started using a two-knife policy. Each trolley contains two knives, one is used for working up the row whilst the other is soaking in disinfectant (Virkon S). At the end of the row, the knives are switched and the pre-disinfected knife is used down the opposite row. The length of time it takes to process up one row is considered to be sufficient for the second knife to be fully disinfected. Knives are also left to soak overnight in Virkon S, and any rusty, damaged, or excessively dirty knives are replaced. In the UK, Virkon S is not authorised for use during cropping as it can cause phytotoxic effects on fruit. It is not considered to be crop safe and may leave detectable levels of residue.

Due to the concern of infected but asymptomatic plants, more growers are collecting de-leafed material, rather than allowing this to drop to the floor. In the event of an infection, this would reduce the viral load present on sites and make eradication of the virus easier. If material is asymptomatic, it should be treated as potentially infected and disposed of in a safe way.

The ease at which ToBRFV is spread mechanically—and its high infectivity—has made growers concerned about the cleanliness of any equipment which moves within crops. Similar to the German growers, some sites require staff to spray disinfectant (Virkon S) over trolleys after each row is worked. At least one grower uses time management systems to monitor staff performance, and has their workers log the time when they have finished working a row to ensure it is done efficiently. This worker is then required to clean the trolley/equipment, including wiping it down with a cloth left to soak in Virkon S solution for five minutes. This action is then logged before work starts on the next row. This process allows supervisors to monitor that the equipment is being cleaned correctly, and that sufficient time is being spent on this. The knowledge that they are being monitored will also mean that staff are more likely to clean/disinfect equipment correctly.

Dutch growers are concerned about their crates being returned infected with ToBRFV. Similar to the UK, effective tray washing is being investigated and experimental work on the efficacy of disinfectants is underway. Trays disinfection needs to be carried out at high temperature and pressure, and any damaged or excessively dirty trays are discarded. If dirty trays emerge from the washer, the entire cleaning process needs to be reassessed as it is not effective. Similar to some of the Dutch and Israeli propagators, some growers have switched to using disposable, single use crates (cardboard or plastic) to mitigate this risk.

Gloves are to be worn at all times by staff, and replaced often. Hand sanitation stations containing Enno Rapid are made available at several points in the glasshouses, and bins are routinely emptied and disinfected. Some growers provide laundered clothing for staff and are considering compulsory showers for staff and visitors, before and after shifts.

Growers in the Netherlands are required to recirculate their water and there is concern over the effectiveness of water treatments. If ToBRFV does survive the water treatment process, it could be rapidly spread across entire sites. Due to the small size of the ToBRFV virus (300nm), the effectiveness of slow sand filters is uncertain, although the biologicals present within the filters may be sufficient to remove the virus. One option is water treatment with hydrogen peroxide; if followed by a UV treatment (at a sufficient dose), this will lead to ozone formation, which removes any residual hydrogen peroxide and further sterilises the water. The likely effectiveness of on-site water treatment facilities should be assessed—is the disinfectant dose sufficient? Are more effective formulations of disinfectants available, e.g. hydrogen peroxide vs. stabilised hydrogen peroxide (e.g. Huwa-San)? UV is effective at sufficient doses, but UV efficacy is reduced where greater organic loads are present in the system.

It is recommended that any remaining 'clean' water from production sites at the end of the season be disposed of down the drain to enter into the water treatment system. Waste water should never be disposed of in a manner in which it can enter the natural aquatic system, releasing the virus into the local environment. New builds could also consider running separate irrigation systems for each house to limit potential spread through the irrigation system. This would be expensive, and funds may be better placed in improved water treatment facilities.

As the first official outbreak of ToBRFV was reported shortly before the study tour to Europe, it is unknown what additional disinfection practices are being used in the Netherlands to eliminate ToBRFV. It is expected that this will include a similar approach to that used in Germany, which effectively eliminated the virus in 2018, and included high pH products. Fera are currently carrying out AHDB Horticulture-funded testing of disinfection products, and Dutch laboratories are also working on disinfectants, including Groen Agro Control, who have developed and released a ToBRFV hygiene protocol, including recommended disinfectant products, which has been updated as new information became available. Groen Agro Control recommend that their protocols are reviewed carefully and a bespoke plan be made for each site. Time needs to be taken to train all staff on ToBRFV, not just growers.

Similar to the UK, external companies are contracted in to help with site clean-down. This process normally involves the rolling of reusable fabric matting down each row, collecting waste plant material for removal. The fabric, machinery used and staff, etc. all pose a significant biosecurity risk. Sites should ensure that a contractor's equipment is completely cleaned and sterilised before entering onto sites, including their clothes, as multiple sites can be visited by contractors each day. Ensuring contractors and their equipment is clean will add to costs and slow this process. Some sites supply their own fabric rolls, or choose single-use

plastic over the rolls normally provided by contractors. Sites which supply their own reusable rolls must ensure the rolls are disinfected or replaced before reuse the following year, especially after an outbreak has occurred near to the site.

Although incineration is the recommended method for destroying infected plant material in Germany, this is difficult due to the high water content of stems. This, and deep burial (≥2m), have been considered as options in the Netherlands, but both are expensive. In the Netherlands it is recommended that plant material be composted, along with organic substrates. Composting must be done at an accredited site, which can guarantee that the duration of the process and the temperatures achieved are sufficient to deactivate ToBRFV. Most composting facilities will not accept plant waste material which includes non-biodegradable string, so this will need to be removed before it can be sent for processing. Biodegradable string is available, and its use is increasingly common by growers who compost plant waste.

Under normal conditions, growing media is often piled up outside the entrance to sites following removal, to be collected at a later date. This practice is not advised, and media could act as a source of ToBRFV inoculum, potentially infecting future crops. Similarly, any residual water containing ToBRFV within this media could leach into the local environment, infecting alternative host weed species, especially solanaceous weeds, and permanently establishing the virus on site.

Removal of infected plant material/media should be done with care, and with thorough assessment of the associated risks. Transportation off site should be done in a covered truck to prevent infected material escaping on route to the composting facility. Ideally, removal should be completed before new plants arrive on site.

Rockwool does not need to be composted, if sent to re-processing this process produces more than enough heat to destroy the virus. Where rockwool is not sent for recycling, it will need to be disposed of away from site.

On a larger scale, some growers who are investing in infrastructure changes are considering moving new pack house facilities away from their main production sites, especially where fruit is imported from countries suffering ToBRFV infections. There are also growers who are considering stopping importing fruit from abroad altogether.

Israel

Before ToBRFV was identified in 2015 in Jordan, it spread rapidly across most of Israeli and Jordanian tomato production. This was exacerbated by the fact that 70% of tomato is intensively grown, in densely packed sites in the West Negev region of Israel. Communication

between growers is very poor and no grower co-operatives exist, with all sites independent. The lack of any formal grower groups restricts communication, general improvements and relevant knowledge transfer which would benefit the entire region. Growers are disappointed by the slow response to ToBRFV taken by the Ministry of Agriculture (MoA).

Initial infections were catastrophic, but differences in varietal susceptibilities were quickly seen, as was observed during the outbreaks in Germany. The varieties which exhibited significant fruit symptoms are no longer widely grown. With changes in varieties and cultivation practices, the average yield loss in Israel is now around 20%, whereas it had been closer to 100% in some instances. With the virus endemic in Israel, and the avoidance of new infections unlikely, the focus now centres on delaying infection.

The rapid spread of ToBRFV has meant that most growers are on a level footing with each other, and generally businesses have not suffered in the extreme. In fact, yield reductions of 20% have led to wholesale price increases of between 30-40%. Despite the costs associated with growing two crops per year, many growers are profiting and over 500 ha of new net houses were built in the West Negev region in 2018. When resistance is bred into commercial varieties, growers will face the prospect of mass overproduction of tomato in Israel. Growing two crops per year has also had a beneficial impact on both propagators and seed producers.

Research carried out in Israel revealed that bumblebees are able to mechanically spread ToBRFV (Levitzky, 2019), though the virus must already be present on plants on site for this to occur. By the time the virus has developed to sufficient quantities within plants for it to be transmitted by bumblebees, it is likely that it will have already been transmitted by staff and equipment. In situations in Europe or Israel, where a low number of plants are under suspicion of ToBRFV infection, it may be prudent to shut down hives until confirmation of the presence/absence of ToBRFV. Where one house is infected with ToBRFV on one site, but other areas remain clean, it may be necessary to remove hives containing ‘infected’ bees to prevent potential transmission between houses.

Most crop workers are Thai and technical communication between growers and workers about managing ToBRFV is difficult. Some growers hire translators to come in twice a year, to better communicate with their staff. Staff typically work on several sites and equipment is often shared between growers. Generally, sanitation is extremely poor, and these practices further increased the rate of disease spread between sites. In the West Negev, crops are grown in net houses (50 gauge). Glasshouses would be too costly for this region and temperatures too hot for production for much of the year. Net houses provided protection from pests, whilst allowing light to reach crops. Plants are irrigated by drip pegs and in almost all cases, crops are planted directly into the soil.

Almost all Israeli growers follow a two crop growing cycle as a response to ToBRFV. Symptoms are exacerbated during the high summer temperatures where crops are under high heat stress, and summer yields are typically 10-20% down on winter yields, though the summer season is shorter. Two crop cycles are more economical, with most growers taking around eight trusses per cycle, though some will take as few as six.

Different varieties have been shown to exhibit different symptoms, and in Israel, cherry tomato varieties are less susceptible than beef tomato varieties. No information is currently available on the differences in susceptibilities of tomato types in European varieties. Some varieties are only symptomatic on foliage and/or the stems/trusses (Figure 4), and others just on fruit (Figure 5), similar to what was reported in Germany in 2018. In most cases, varieties will show both fruit and foliar symptoms. The degree of fruit mottling varies; some fruit symptoms are severe, others are mild, and some may show no characteristic symptoms of the disease at all, but may never ripen.

Figure 4. Truss marking and discolouration in a ToBRFV infected plant, var. Ikram – image with the permission of the grower .

Figure 5. Marbling symptoms on ToBRFV infected fruit, var. Ikram - image with the permission of the grower.

There are some varieties which are completely asymptomatic of ToBRFV. This characteristic should not be mistaken for resistance and these varieties will still contain infectious viral particles. Despite showing no visible symptoms, these varieties will likely have a yield penalty from hosting ToBRFV, which may be reduced by cropping for shorter periods of time. Issues with fruit set have been reported by some growers, but not all and it is unclear why this is the case.

One variety is grown more than any other—var. Ikram. This variety is the most popular for supermarkets/consumers in Israel as it has good post-harvest characteristics. Ikram shows a greater severity of symptoms compared with other varieties. Its popularity is such that most growers will continue to grow this variety, despite knowing they will get better yields with others.

The presence of *Tomato Yellow Leaf Curl Virus* (TYLCV) is believed to significantly reduce the fitness of plants infected with ToBRFV, leading to stunting (Figure 6), although to date there is no evidence in the literature supporting this. Plants infected with ToBRFV and Paprika mild mottle virus isolate from Japan (PaMMV-J) have been shown to exhibit more severe symptoms (Luria, *et al.* 2018)

Similar issues are expected in plants with combination treatments of ToBRFV and PepMV. PepMV has not yet been officially reported in Israel and hence, no work is currently being carried out to look into these interactions. No literature is available on plants infected with ToBRFV and full-strength strains of PepMV, though anecdotal observations have reported that fruit is smaller and harder, with the vigour of infected plants greatly reduced.

Figure 6. Severely stunted tomato plant with combined ToBRFV and TYLCV infection – image with the permission of the grower.

The introduction of PepMV across Israel would be very negative, especially if mixed infections of PepMV and ToBRFV are serious. In hot countries, PepMV symptoms actually develop during cooler times of the year, when ToBRFV symptoms are worse. Israeli growers are accustomed to dealing with ToBRFV. PepMV can kill plants and may pose an even greater threat to the industry than ToBRFV. Mild PepMV strains could offer a solution, however these have not been developed for use in hot climates and their impacts are unknown.

Some growers may still grow one crop, choosing to diversify into other crops during the hottest periods when symptom expression is greatest and yields are restricted. Alternative crops include watermelon, melon, cabbage and cauliflower. ToBRFV can survive for several months

in plant debris in the soil. Growing these alternative crops may provide an additional income during high risk periods, but will not be sufficient for the inactivation of the virus in the soil. Also, it is not known whether these alternative crops are also asymptomatic hosts of the virus.

Many growers solarise empty sites for one month, during the hottest period of the year by covering soil in polythene. The duration of this process and the high temperatures achieved are said to be sufficient to eradicate ToBRFV. It is likely that a large reduction in soil viral inoculum load would be possible, but it is unlikely that the virus would be completely eradicated in this manner, with some level persisting to the subsequent crops.

Israel: Site visits

Three houses—belonging to two growers—were visited, each suffering from ToBRFV infection. Normally, phytosanitary practices are poor in Israel, but the first grower visited followed excellent sanitation practices. It was requested that the visit to this house take place early, ensuring we had not visited other infected sites that day.

Whereas most growers in the West Negev region have decided to expand their production to cope with increased loses, this grower focussed on improving site hygiene, increasing yields this way. Unusually, this grower plants out their crops in coir slabs, rather than in the soil, and these are replaced before each new crop cycle. The movement from soil to coir was actually a response to Fusarium/Pythium in the soil, but will benefit ToBRFV management, avoiding the need for careful planting. The grower also covers the floor with woven ground cover matting (Figure 7).

Figure 7. An Israeli tomato production site with high hygiene standards – image with the permission of the grower.

At this site laundered work suits are supplied to workers before work begins each day, with corresponding colours and numbers 1-6 (Sunday to Friday) to ensure suits are never worn twice before being washed. Shoes (which have been disinfected overnight), disposable gloves and hats must be worn. Shoe dips (sodium hypochlorite) are present at all entrances and exits. All tools and equipment are disinfected overnight in sodium hypochlorite (concentration unknown). All equipment is owned by this grower and nothing is shared. If staff do work in other houses they always move from younger to older crops, never the other way around.

As little contact is made with the plants as possible, with nothing touched for the first two to three weeks after planting, and minimal contact made for the first two months. A consequence of short cycle crops is that plants do not grow tall. Plants are strung, but only lowered once

or twice a cycle to avoid stressing the plants and inducing symptom expression. The string is replaced each year (after two cycles).

Due to the relatively young age of the crop, symptoms were visible, but not severe. These careful management practises work well for this grower, and he manages to get eight to ten trusses—normally ten—per cycle. After cropping, the site architecture is cleaned with a stabilised chlorine product at 1000 ppm. The soil is treated at 2000 ppm with stabilised chlorine through the drip irrigation system. Trisodium orthophosphate (TSOP) and Kemphos (a new chlorinated TSP product) can be run through the drip pegs for one hour the day before planting to disinfect the soil. This is flushed with clean water to remove any residual product. Additional products used in Israel are Huwa-San (stabilised hydrogen peroxide), which is authorised for use in organic cropping, and Green Up, a high pH product (pH 13.5, citrus extract) which can reportedly eliminate the virus within 30 seconds and is being investigated by the Volcani Centre.

Under Israeli conditions, ToBRFV is unable to survive in its naked form, free of any plant material, for longer than three months (Aviv Dombrovsky, personal comment). However, it can remain viable in plant debris for much longer. Healthier soils have an enhanced breakdown of plant material due to diverse levels of microbial communities, increasing the rate of viral inactivation over time. The longevity of ToBRFV in high quality UK organic soils is unknown and should be investigated. Organically certified soils are a precious commodity, taking many years to gain full accreditation.

For organic production in the UK soil sterilisation via solarisation is not a viable option and steaming is expensive, and unlikely to deactivate 100% of the virus due to issues with soil penetration and treatment difficulties associated with site architecture. These soil treatment options would destroy the biological nature of the soils. Bio-fumigation was discussed, but the effectiveness of this against viral pathogens is unknown, and unlikely to be effective. No effective means of managing ToBRFV in organic systems were identified in this work.

In addition to the practises listed above, the grower has a barcode system for every row which workers must scan on mobile devices (a system called ‘Pickup’, Figure 8). Similar to the Dutch system, this enables the crop to be closely monitored by phone or computer. As most crop workers are Thai, language barriers make communication between staff difficult. Understanding that they are being constantly monitored, workers are more likely to comply with instructions, reducing the high risk actions. Any issues which do arise can also be traced back to individuals and further training needs be addressed.

Figure 8. ‘Pickup’ barcode system used to monitor crop worker movements – image with the permission of the grower.

Water efficiency is important in the West Negev. Waste water is not recirculated, but used on other crops, including spinach. Infectious ToBRFV particles have been found in recirculated water in protected tomato in Europe, where water treatment is insufficient to destroy it. Waste water from Israeli tomato production is not treated in any way. It is likely that this water is entering into watercourses, potentially infecting wild plant hosts e.g. solanaceous weeds, which will help spread the virus further.

In periods of drought, water produced by desalination may be used. Desalinated water is low in certain key minerals, including magnesium, which can lead to deficiencies developing in plants if this is not carefully monitored. Stress related to mineral deficiencies or reduced plant health can exacerbate ToBRFV symptoms, impacting yields. Israeli growers are unfortunate in that they are largely unaware of when desalinated water will be used, so struggle to mitigate against this. Deficiencies are not an issue for fertigated protected crops, but this demonstrates the importance of optimal feed regimes in promoting high plant health and reducing viral symptom expression. Trials have been run by the Israeli Ministry of Agriculture, where plant nutrition was increased in an attempt to delay symptom onset, but these were unsuccessful. In this work, it is likely that infection occurred early from inoculum present in the soil and the root system. As a consequence, the roots never developed sufficiently to efficiently utilise this

additional resource. If infection had developed later on in the season, differences in ToBRFV symptoms may have been seen.

Despite best efforts and the high hygiene standards of the first grower, the site continues to develop infections in each crop cycle. This grower uses new virus-free coir slabs, and infection will not develop from the soil, but from mechanical transmission. Therefore, the use of the resistant rootstock will not help this grower.

After cropping, the spent coir is left outside the site (Figure 9). In Israel, there is no regulation for the composting of infected materials. These slabs will be collected once a year and reused in pineapple production. It is not known whether these slabs continue to be a source of infection—this is due to be investigated by the MoA—or if pineapple is a host of ToBRFV.

Figure 9. Spent coir left outside the tomato house, awaiting reuse in pineapple production – image with the permission of the grower.

Two sites were visited at a second grower who follows a phytosanitary approach more typical for Israel, and the differences between the first house visited and these two was stark. These crops were the same variety (var. Ikram), planted one week earlier. Symptoms were significantly advanced, but the extra week of production alone would not account for the extent of the differences seen.

It was abundantly clear on walking into these two houses that the plants were infected. It was possible to see “a lot more sky” when looking at the canopy, as had been noted during the German infections of 2018. No foot dips, hand sanitiser or other hygiene measures were

present. When growing on bare soil, most growers would use a mulch to keep weeds and volunteer seedlings down, but these growers did not. There were volunteer tomato plants and weed species present which were visibly infected (Figure 10), acting as another source of inoculum. Research at the Volcani Centre is currently establishing which of the main weed species in Israel are wild hosts of ToBRFV. Weed species in Israel differ from those found in the UK and Europe, and these should be investigated. Weed species can host numerous plant pathogens and sites should be frequently monitored and treated for them.

Figure 10. A symptomatic weed host of ToBRFV present in an Israeli tomato crop – image with the permission of the grower.

Given the normal hygiene standards, and different cropping styles of the Israeli growers, there is little that can be learned for improving UK practice. The grower following high sanitation

standards is an excellent example of why hygiene matters, and that the industry cannot afford to cut corners. Although solarisation is not practical in the UK, the ability of the virus to persist for only three months outside of plant tissue suggests a fallow season, perhaps with a black plastic covering, could recover organic soils which have been infected to some extent. This might be impractical with sites growing both organic and conventional crops, but could be worth investigating.

No treatment is effective in treating ToBRFV, or available to prevent infection. The MoA tested over 30 biostimulants—products designed to increase plant vigour—attempting enhancing disease resistance. No differences were seen in infection levels in treated plants compared with the untreated control. Again, these products were tested in plants which likely became infected shortly after planting. Differences in symptom expression may be seen in European crops, which may be infected later in the season.

8. Best practice

The following lists the 'best practice' procedures identified from the two study tours. Implementation of these practices may not be practically, or economically viable for all sites, but demonstrates what can be done to effectively mitigate against ToBRFV infections developing on site, and how to address infections which do occur on sites.

The visit to Israel provided invaluable insights into ToBRFV infections, including its epidemiology, but was unable to provide many suggestions to improve current ToBRFV management in Europe. However, the differences between symptoms at a site which followed excellent hygiene standards, compared to others with very poor standards was blatant and highlights how critical hygiene is in controlling any pathogen.

Pre-infection

- Review and update site hygiene protocols to include new ToBRFV management practices, and train staff on ToBRFV symptoms and what to do if an outbreak occurs.
- Become a member of, or follow GSPP, ISTA/ISHI guidelines etc.
- Install foot and hand cleaning machines at the entrances to each site. These must be maintained, ensuring that the active ingredients used are at recommended rates and have activity against ToBRFV. Alcohol hand gel has no activity against ToBRFV.
- Maintain strict biosecurity practices and stop all site visits unless essential. Where visits are required request they take place first thing in the morning. No tomato products should be brought onto, or consumed on-site.
- Ensure visitors read and understand site hygiene protocols, including wearing protective clothing at all times, ideally changing into this before leaving their vehicles. Where sites

are visited by individuals frequently, shoes can be purchased to remain on-site and be routinely disinfected.

- Consider supplying fresh laundered clothing for staff to wear during shifts, which is replaced each day. Compliance could be ensured by providing different coloured/numbered clothing each day. Staff should wear disposable gloves at all times, replacing them often and frequently sterilise their gloved hands with an appropriate disinfectant.
- Limit staff movement between production areas on each site, do not move equipment or machinery between areas. Identify and designate high and low risk areas on site, and control movement between these. Consisted investing in staff performance monitoring systems to ensure compliance.
- Ensure seed health certificates are sourced from producers for each variety grown, especially trial varieties. Check the diagnostic tests which were performed by an accredited laboratory, including that a sufficient number of seeds is tested (3000).
- During grafting change blades after each new tray, or more frequently if necessary.
- Avoid growing varieties which are extremely sensitive to ToBRFV (currently unknown).
- Ensure trucks are disinfected with a suitable disinfectant e.g. Virkon S, or Menno Florades, before arrival on-site and again before use.
- Monitor crops for disease symptoms every few days.
- Focus on maximising plant health to suppress symptom onset should infections occur.
- Use the German one knife per row, or Dutch two knife method, in which knives are continually swapped after a period in sterilant, to ensure disinfected knives are used at all times.
- Clean and disinfect all equipment with an effective, crop-safe disinfectant between each row, or use a trolley cleaning machine.
- Avoid excessively stressing plants and take care to avoid unnecessary wounding which may act as an entry point for infection to occur.
- Ensure crates are cleaned using a crop-safe disinfectant, or alternative method e.g. low pressure steam treatment, before they are used.
- Where water is recirculated, the sterilisation process should be checked to ensure it is sufficient to eliminate infectious ToBRFV.
- Ensure all waste water enters the water treatment system. Do not allow water to enter natural watercourses which may establish ToBRFV in alternative host weed species nearby.
- Monitor weeds species on site and control as necessary.

- When using contractors ensure their equipment is clean and sterilised before allowing entry on-site.
- Clean and disinfect all site architecture using products known to eliminate ToBRFV.

Post infection

- Isolate any suspicious plants and test for ToBRFV. When outbreaks are confirmed carefully double bag infected plants and remove them from the glasshouse. Remove additional plants along the row and plants from the rows opposite the infected plant (exact number to be removed to be taken on a case by case basis). Restrict access to this area to essential workers and consider a changing area between this area and the rest of the crop.
- When suspicious plants are quarantined, shut down bee hives in these areas, until a result is returned. If a positive result is returned these hives should be destroyed.
- Removed infected plants must be transported off-site using a covered truck and destroyed by e.g. incineration or composting.
- When an outbreak does occur, quarantine the affected house and immediately notify Defra, or appropriate plant health authority, and comply with their recommendations.

9. Conclusions

The industry is only as strong as its weakest link and clean seed, clean plants and clean houses are needed to effectively manage ToBRFV. In Israel, and other countries where plants are grown intensively in soil, or where phytosanitary practices are poor, ToBRFV is here to stay and will continue to be an issue until resistant varieties are developed.

Successful eradication of the virus in Germany and the UK shows it is possible to eliminate ToBRFV from infected sites, as long as ToBRFV protocols are developed and biosecurity practices are strictly adhered to. If all sites follow best practices the impact of ToBRFV on European production can be minimised.

Overall findings from this investigation

The virus

- ToBRFV originated in Israel and spread rapidly before being positively identified in Jordan in 2015. It is a serious, emerging tobamovirus threat to UK and European tomato production.
- ToBRFV breaks the *Tm-2*^{2} resistance gene which confers resistance to TMV and ToMV.
- Mexican varieties lack the *L*^{4} resistance gene and crop losses of up to 100% have been reported. Most commercial UK pepper varieties are resistant, containing the *L*^{4} gene.

- ToBRFV has no insect vector. Mechanical transmission is the main route of ToBRFV spread e.g. hands, clothing, equipment, machinery and crates.
- Existing hygiene protocols, or emergency ToBRFV protocols have been developed and/or updated by seed producers, propagators and growers as a response to ToBRFV and all sectors of the industry.
- ELISA, (RT) and qPCR can be used to identify ToBRFV infection in seed and plant material, but bioassays are required to confirm infectivity of ToBRFV.
- Cross-reactivity with other tobamoviruses is common with current ELISA tests, however ToBRFV specific antisera is in development by several research institutes/laboratories.
- Multiple ELISA protocols are available. Identification of the 'gold standard' ToBRFV protocol, recommended for use by all laboratories is currently in development.

  Virus survival and spread in plants and hygiene measures within the tomato crop
- Plant stress has been implicated as a major trigger of symptom expression. ToBRFV symptoms developed in the Netherlands in autumn 2019, at a similar time to when they developed in Germany in 2018, when plant stress increased as a consequence of shorter day lengths.
- Almost all sites have limited access with essential visits permitted only, preferably first thing in the morning.
- Sites are considering supplying fresh, clean clothing for staff. Clothing capable of being laundered at ~ 95°C is being researched alongside viricidal detergents.
- Returning trays are considered one of the greatest risks of spreading ToBRFV between sites. Some sites now using automated machines to disinfect trays, whilst many have switched to using single use trays.
- Water treatment must be sufficient to eliminate the virus from recirculated water e.g. high level UV.
- Many European and Israeli sites used Virkon S to clean down equipment/machinery e.g. knives and trolleys. Virkon S is not considered food safe in the UK and can cause phytotoxic effects on fruit and leave detectable residues.
- All propagators and main crop growers should request certificates confirming all seeds are confirmed free of ToBRFV and sufficient seeds (3000) were tested.
- Trial varieties pose a risk to propagators (and growers). Trial varieties must not be grown unless suitable seed certificates are available, ensuring sufficient seeds (3000) were tested.
- From November 1st 2019, ToBRFV gained a Q-status and all ToBRFV outbreaks are legally required to be reported to relevant plant health organisations.
- It is currently unclear if aubergine is a host of ToBRFV, research in this topic is underway.

- The ToBRFV Q-status means that all outbreaks must be reported to the relevant plant health authorities.
- Weed host species yet to be determined, so all weeds should be taken as potential hosts until shown otherwise

Seed production

- ToBRFV is not present in the endosperm of the seed, but infections may develop from inoculum sources on the seed coat and seed transmission is predicted to be very rare.
- Seed is processed and treated with disinfectants including hydrochloric acid, sodium hypochlorite and trisodium phosphate to eliminate seed transmissible pathogens, known viricidal products.
- Seed is already tested for similar tobamoviruses including TMV and ToMV, and confirmed free of all tobamoviruses (and other seed transmitted viruses), before dispatch.
- Many seed producers are members and follow ISTA, ISHI, NAL, GSPP or SKAL guidelines.
- Some producers have moved production to lower risk areas regions keeping parental material isolated.
- Seed production areas on some sites are structured to allow areas of production to be isolated, allowing production to continue if suspicious plants are found.
- Resistance breeding is underway in Israel with potentially two source of resistance identified, including a ToBRFV resistant rootstock.
- In-depth records, and samples of all seed batches, are kept for re-testing if required.
- Gloves must be replaced often, and hands sterilised with Enno Rapid. Blades can replaced at the start of each tray if requested.

Propagation

- All propagators should request certificates confirming all seeds are confirmed free of ToBRFV, including that sufficient seeds were tested (3000).
- Visible ToBRFV symptoms are unlikely to develop until after plants have been dispatched and planted out on grower sites.
- Hygiene standards may already be adequate to prevent ToBRFV infections occurring on site, but changes to protocols have been made as a response to new information on ToBRFV.
- Customer visits are not advised. Where customers do wish to see plants, the plants should be brought to the customer.

- Increased use of grafted plants allows splitting of plants into two heads, maximising marketable yields in the two crop cycles in Israel.
- ToBRFV management in Israel is to delay the onset of symptoms, rather than avoidance, and disease free plants are necessary to maximise the number of trusses produced per cycle.
- Plant wounding is considered a key entry point for early infection. Israel follows careful planting practices to delay the date of infection, including the use of organic, biodegradable films surrounding the plug preventing direct contact with wounded roots and infected soil at planting.

Main crop production

- ToBRFV has been officially confirmed in 12 countries, seven in Europe, including the Netherlands and the UK, but has been eliminated from Germany, the UK and USA.
- The first European outbreak occurred in Germany in 2018, affecting all seven members of a grower collective. Early crop losses in Israel were 100%, however movement to less susceptible varieties, careful planting, grafting and a two crop-cycle has reduced losses to 20%.
- Varietal differences in the severity of ToBRFV have been seen in Europe and Israel, with some varieties asymptomatic of ToBRFV infection. Some varieties show symptoms on leaves alone, but fruits failed to ripen; other varieties exhibited symptoms on the fruit, but not on leaves.
- The true susceptibilities of the varieties infected during the German outbreak is unclear. The severity of symptoms may have been confounded by poor plant vigour in certain varieties.
- Nutrient deficient plants, as a consequence of using desalinated water in Israel, have enhanced symptom expression. Excellent plant health/nutrition will likely reduce symptom expression.
- Asymptomatic varieties will still contain viable inoculum and provide an inoculum source to spread ToBRFV to other varieties.
- Dependent on the variety, initial health of the plant pre-infection, and the time of the year, it is possible to continue to produce a degree of marketable fruit despite infection.
- Good growing conditions and high levels of plant health may reduce symptom expression.
- Sampling of suspicious plants during cropping can identify if ToBRFV infections are present. The most efficient sampling practices are currently being investigated.

- Heat stress will exacerbate symptoms. In Israel symptoms are most severe at the height of midsummer, whilst symptoms are greatest in Turkey when crops are exposed to very cold temperatures (~4°C).
- Some Israeli growers grow different crops during the summer, including watermelon and cauliflowers, but this is not an option for UK growers, although one Dutch grower now grows cucumber instead of tomato.
- Mixed infections of ToBRFV and mild strain PepMV do not appear to be more severe than plants infected with ToBRFV alone.
- The impact of mixed infections with ToBRFV and wild strains of PepMV is unknown.
- Mild strain 'vaccines' are in development, and may provide cross-protection to ToBRFV, but these are several years away from being registered for use.
- Plants infected with multiple viruses, in addition to ToBRFV will likely suffer more than plants infected with just ToBRFV.
- Some German and Dutch growers sterilise their knives overnight in Virkon S or Menno Florades. One Dutch grower use a two knife practice, re-sterilising each blade whilst the other is in use whilst a German grower provides a pre-sterilised knife for use down each row.
- Some sites choose to spray down trolleys using a knapsack containing Virkon S between rows, but this is unlikely to be fully effective. Other production sites monitor staff electronically through recording software, ensuring that sufficient time is spent disinfecting equipment.
- Some growers use a trolley cleaning machine, which is likely to be the most effective method of disinfection, so long as the disinfectant has activity against ToBRFV and is used at its recommended rate.
- Bumblebees have been demonstrated to spread ToBRFV, but will only spread in houses where ToBRFV is already present, and likely already widespread. In cases where a suspicious plant is found, hives should be closed until the plants has been tested and results returned.
- ToBRFV infected material must be incinerated in Germany, but composted in the Netherlands.
- The infected German growers now replace their coir each year, rather than reused year-on-year. Boxes and drip pegs were also replaced.
- Effective disease management and comprehensive site clean-down, using disinfectant products identified to be effective against tobamoviruses, led to Germany being officially declared ToBRFV free in July 2019.

- Solarisation for one month can be used to reduce inoculum levels in Israel, but this is not suitable for protected crops in the UK. No effective means of managing ToBRFV have been identified for organic producers in the UK.
- Fruit infected with ToBRFV is likely being sold in UK supermarkets, especially with the recent confirmation of the disease in Spain. Some sites are now reconsidering importing fruit into their business.
- Slabs/plant material should be disposed of in a covered truck and not stored outside the site for long periods of time.
- Waste water which may contain infectious ToBRFV should be flushed into the sewage system, rather than released where it may enter watercourses and establish in weed species nearby.
- During clean-down all contractor equipment should be disinfected before entry on-site, including any fabric rolls used to remove plant material. Contractors should ideally be wearing clean clothing, not worn to other production sites.
- New build projects should consider ToBRFV virus when situating and designing houses/packing facilities.
- Weed species can act as alternative hosts of ToBRFV and these need to be identified in the UK.

10. Future work

1. What are the different susceptibilities of the main varieties of tomato grown in the UK to ToBRFV? Screening of other varieties and/or breeding for ToBRFV tolerance/resistance
2. What impact do nutritional deficiencies have on ToBRFV expression in infected plants?
3. What effect does enhanced nutrition/plant health have on reducing ToBRFV symptoms, (including the use of biostimulants)?
4. Is symptom expression reduced under lit crops? Might there be a difference between the use of high pressure sodium (HPS) vs. Light emitting diode (LED)?
5. Effective disinfectants for use at crop clean down (in the absence of plants) and during cropping.
6. Effective crop safe disinfectants for use on trays/crates.
7. The development of a ‘gold standard’ protocol for the detection of ToBRFV.
8. The development of highly specific antisera to ToBRFV to prevent cross-reactivity with other Tobamoviruses, development of a ToBRFV lateral flow device for in-crop confirmation.
9. Confirm if aubergine is a host of ToBRFV.

10. Determination of which UK weed species can become hosts, including asymptomatic hosts, of ToBRFV.
11. Has the virus been found in tobacco production? Does smoking pose a biosecurity risk?
12. Establish impact of dual infections with full strength PepMV and ToBRFV on symptoms.
13. Establish the persistence of infectious ToBRFV in 'standard' organic soils, as naked particles or within plant debris.
14. Investigate methods of recovering ToBRFV infected organic soils without undue harm to soil health, including biological diversity of the soil.
15. Confirm whether ToBRFV infected tomato fruit is being sold in UK supermarkets.
16. Establish if slow sand filtration is sufficient to remove infectious ToBRFV particles.

11. Knowledge and Technology Transfer

1. Results will be shared as part of an AHDB ToBRFV webinar in February 2020
2. To be confirmed

Results were also shared with the UK Tomato Grower Association Technical Committee on December 4th 2019.

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