



Department for Environment Food & Rural Affairs

Rapid Pest Risk Analysis (PRA) for: *Tomato torrado virus*

March 2015

Stage 1: Initiation

1. What is the name of the pest?

Tomato torrado virus (ToTV), family *Secoviridae*.

A disease affecting tomatoes in Spain, initially called torrao or cribado disease, was first seen in 2001 according to Alfaro-Fernández *et al.* (2006), though Verbeek *et al.* (2007) consider the first observations to date from only 2004. The virus was formally identified and characterised by Verbeek *et al.* (2007). ToTV was assigned as the type species of the new genus *Torradovirus*, and this genus was included in the new family *Secoviridae* by Sanfaçon *et al.* (2009).

2. What initiated this rapid PRA?

A review of pests absent from the UK but present in other parts of the EU identified several pests and pathways of potential concern to the UK. During discussions of this review, the Plant Health Risk Group considered the risks posed to the UK by ToTV. As a result of these discussions, this PRA was requested to further investigate potential risks, especially with regard to identifying pathways of relevance to the UK.

3. What is the PRA area?

The PRA area is the United Kingdom of Great Britain and Northern Ireland.

Stage 2: Risk Assessment

4. What is the pest's status in the EC Plant Health Directive (Council Directive 2000/29/EC¹) and in the lists of EPPO²?

This virus is not directly listed in the EC Plant Health Directive, though legally it would fall under the part of the legislation covering viruses transmitted by one of its vectors, *Bemisia tabaci*. ToTV is not recommended for regulation as a quarantine pest by EPPO, though it was on the EPPO alert list between 2009 and 2013.

5. What is the pest's current geographical distribution?

Table 1: Distribution of *Tomato torrado virus*

North America:	No reports
Central America:	Panama (Herrera-Vásquez <i>et al.</i> , 2009)
South America:	Colombia (Verbeek & Dullemans, 2012)
Europe:	Hungary Csongrád, Öcsöd and Szeged regions (Alfaro-Fernandez <i>et al.</i> , 2009) Poland Wielkopolska and Mazowsze regions (Pospieszny <i>et al.</i> , 2007; Pospieszny <i>et al.</i> , 2010) Spain Mainland provinces Alicante, Almería, Barcelona and Murcia, as well as the islands Gran Canaria, Mallorca and Tenerife (Alfaro-Fernandez <i>et al.</i> , 2010b)
Africa:	No reports
Asia:	No reports
Oceania:	Australia (South Australia only) (Gambley <i>et al.</i> , 2010)

ToTV is also present in Italy (Davino *et al.*, 2010), where the official status is transient, under eradication (EPPO, 2014). Additionally, there have been reports from Belgium (EPPO, 2014) and France (Verdin *et al.*, 2009), but ToTV has now been declared eradicated from both countries by their respective NPPOs (EPPO, 2014).

¹ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2000L0029:20100113:EN:PDF>

² <https://www.eppo.int/QUARANTINE/quarantine.htm>

6. Is the pest established or transient, or suspected to be established/transient in the UK/PRA Area?

There have been no interceptions or outbreaks of ToTV in the UK.

7. What are the pest's natural and experimental host plants; of these, which are of economic and/or environmental importance in the UK/PRA area?

Table 2. Recorded hosts of *Tomato torrado virus*

Family	Host	Situation	Source
Amaranthaceae	<i>Amaranthus</i> sp.	Weed	Alfaro-Fernandez <i>et al.</i> (2008)
Caryophyllaceae	<i>Spergularia</i> sp.	Weed	
Chenopodiaceae	<i>Atriplex</i> sp.	Weed	
	<i>Chenopodium</i> spp.	Weed	
	<i>Halogetum sativus</i>	Weed	
Cruciferae	<i>Senebiera didyma</i>	Weed	
Malvaceae	<i>Malva</i> sp.	Weed	
Polygonaceae	<i>Polygonum</i> sp.	Weed	
Solanaceae	<i>Capsicum annum</i>	Experimental	Amari <i>et al.</i> (2008)
	<i>Datura inoxia</i>	Experimental	Pospieszny <i>et al.</i> (2010)
	<i>Datura stramonium</i>	Experimental	Alfaro-Fernandez <i>et al.</i> (2010b)
	<i>Nicandra physaloides</i>	Experimental	Pospieszny <i>et al.</i> (2007)
	<i>Nicotiana glauca</i>	Weed	Alfaro-Fernandez <i>et al.</i> (2008)
	<i>Nicotiana</i> spp.	Experimental	Amari <i>et al.</i> (2008), Pospieszny <i>et al.</i> (2007)
	<i>Petunia hybrida</i>	Experimental	Pospieszny <i>et al.</i> (2007)
	<i>Physalis floridana</i>	Experimental	Amari <i>et al.</i> (2008)
	<i>Solanum lycopersicon</i>	Main economic host	Many authors, e.g. Verbeek <i>et al.</i> (2007)
	<i>Solanum melongena</i>	Experimental	Amari <i>et al.</i> (2008)
	<i>Solanum nigrum</i>	Weed	Alfaro-Fernandez <i>et al.</i> (2008)
<i>Solanum tuberosum</i>	Experimental	Pospieszny <i>et al.</i> (2007)	

The main economic host is tomato (*Solanum lycopersicon*), and it is this host on which the majority of damage has been seen. In studies with experimental inoculation of different tomato cultivars, the impact of the virus is very variable, with some cultivars showing severe damage, others with mild symptoms, and even no detected infection in a few cultivars (Pospieszny *et al.*, 2010). Alfaro-Fernandez *et al.* (2010b) used RT-PCR on leaf samples to detect ToTV in four tomato cultivars at 15 and 45 days following the release of viruliferous whitefly. The cultivar Cedrico was most susceptible, followed by Boludo, while no infection was detected in the cultivars Marmande and Marglobe (Alfaro-Fernandez *et al.*, 2010b).

ToTV is also capable of infecting many other hosts, both naturally and experimentally. Table 2 lists hosts that have tested positive for ToTV, including both asymptomatic and symptomatic hosts. Of these, tomatoes and the experimentally infected host *S. tuberosum* (potatoes) are the hosts of most economic importance in the UK.

8. What pathways provide opportunities for the pest to enter and transfer to a suitable host and what is the likelihood of entering the UK/PRA area?

ToTV has been detected in five European countries other than its putative origin of Spain, has been introduced to Australia, and detected in two Central/South American countries. This indicates that the virus can be moved in trade, but no pathways have been definitively identified for any of these incursions/introductions.

ToTV is distributed in several tomato growing areas of Spain (see Table 1 for a regional list). In the mainland provinces, 50 out of 52 of the greenhouses tested in Murcia between 2001 and 2008 contained at least one ToTV positive plant, though it isn't clear how many fields were repeatedly tested over the eight years of sampling and how many were unique sites (Alfaro-Fernandez *et al.*, 2010b). Other mainland provinces in Spain had relatively few glasshouses tested during the survey period (Alfaro-Fernandez *et al.*, 2010b), and, with these low numbers of sites, the prevalence within these regions is unclear. In 2008, Alfaro-Fernandez *et al.* (2010b) studied four greenhouses in Murcia. A total of 1527 randomly selected plants were examined (about 10% of the total), of which 8.4 to 37% showed symptoms of ToTV (Alfaro-Fernandez *et al.*, 2010b).

Growing plants, seeds, infected tools and packaging and infected whiteflies may all be pathways for the introduction of ToTV to the UK, and are discussed separately below. Tomato fruit, however, is not considered to be a pathway for entry, though there is a substantial trade between Spain and the UK. The UK imports a large amount of fruit from Spain – between 2004 and 2013, 131,000 to 186,000 tonnes were imported each year (EUROSTAT, 2015). Many UK growers have links with Spanish sites, so during the period when their UK crops are unproductive, they can import fruit and ensure a year-round supply. Data on trade in tomato, other than fruit, are not collected.

Direct pathways

Seeds: There is some evidence that seed transmission is possible, at least under experimental conditions. Pospieszny *et al.* (2012) studied tomato plants of a variety highly susceptible to ToTV (Beta Lux). Seeds were collected from infected plants, washed and dried before storage, and finally surface disinfected with 10% phosphoric acid before sowing. Very low levels of ToTV were found in the young plants after 4-5 weeks, detected using a specialised assay (immunocapture real-time reverse transcription-polymerase chain reaction, or IC real-time RT-PCR) (Pospieszny *et al.*, 2012). The transmission rate in this preliminary study was considered to be 0.315%, using 5400 seedlings (Pospieszny *et al.*, 2013). Similar experiments on peppers showed seed transmission rates of 0–0.81%,

depending on the pepper variety (with the number of seedlings tested per variety ranging from 1468 to 2110) (Pospieszny & Borodynko, 2014). However, Gambley *et al.* (2010) did not detect ToTV in seedlings grown from infected plants, for any of the three cultivars tested (Beatrice, Ediez and Loretto), though a total of only 751 seedlings across all three varieties were tested, reducing the chances of detection. As the details of any seed transmission of ToTV are still unknown, it is unclear if acid extraction or equivalent measures (as required in EC Directive 2000/29/EC, Annex IVA, for movement of all tomato seed, including that from inside the EU) would be effective at limiting the spread of this virus. Seed transmission has been suggested as a possible (though unproven) pathway for the findings in Australia (Gambley *et al.*, 2010), and infected seed or seedlings are thought to have been responsible for the introduction of ToTV into Poland (Pospieszny *et al.*, 2010). However, seed producers are careful about quality and, if ToTV was to occur in a glasshouse, the use of pest control would help to reduce the whitefly-mediated spread, and hence incidence, of ToTV within a production site. Tomato seed for commercial production in the UK is imported, as the UK does not have a tomato seed production industry (Sansford & Morris, 2009). While much of the imported seed is traded through Dutch seed houses, the origins of the seed is unclear, as seed can be bulked up in third countries (Plant Health and Seeds Inspectorate (PHSI), pers. comm., April 2015). Overall, this pathway is considered to be **unlikely** with **medium confidence**.

Growing plants for planting: Infected seedlings or seeds are thought to have been the pathway by which ToTV was introduced to glasshouse tomatoes in Poland (Pospieszny *et al.*, 2010), who found that the genetic similarity of Polish and Spanish isolates of ToTV was very high. The UK imports a significant volume of tomato seedlings from Dutch growers. Producers source plants from varying locations, with some using only UK-grown seedlings, and others sourcing between 25 and 60% of their seedlings from the Netherlands (PHSI, pers. comm., April 2015), though no official data on the trade is collected. ToTV is absent from the Netherlands, as confirmed by repeated surveys (EPPO, 2014). However, it is not known where the Dutch growers source their seed (see the earlier discussion under the seed transmission pathway), and as very young plants are moved in trade, they are unlikely to be showing symptoms at this stage, and thus there is still considered to be a potential risk associated with Dutch seedlings. Another factor is, given the wide host range of ToTV, it is possible that ornamental plants, especially Solanaceae, could also be infected with the virus. As some hosts of ToTV can be asymptomatic (Alfaro-Fernandez *et al.*, 2008), there is a possibility that these plants may present a higher risk than tomato plants, as they could not be detected as infected by visual inspection. Overall, this pathway is considered to be **moderately likely** with **medium confidence**.

Mechanical transmission: Transmission methods of ToTV, other than whitefly vectors, are not well understood. Mechanical transmission has been tested experimentally (by applying infected sap extract to leaves which have been damaged), and appears to be less effective than whitefly transmission (Alfaro-Fernandez *et al.*, 2010b). However, it is unclear if mechanical transmission occurs in the field, and if there is the potential for the virus to survive transportation and enter the UK on contaminated boxes, tools or other sources of equipment. No data were found on the length of time the virus can survive on its own. A number of UK tomato growers have links with tomato growers in continental

Europe, with the European tomatoes being packed in the UK during the period that UK glasshouse crops are unproductive, Boxes used to transport European fruit to the UK are often stored and re-used in UK production sites, though it isn't clear if any tools or other equipment is moved between sites. However, precautions against the spread of *Pepino mosaic virus*, where mechanical transmission is known to be a high-risk pathway, are also judged to reduce the chance of ToTV moving into the UK via this pathway. Overall, the assessment of **unlikely** is made with **medium confidence** as there are many uncertainties and a lack of data.

Vector mediated pathways

Whiteflies are likely to be only a semi-persistent vector of ToTV. Experimental work on another tomato torrado virus, *Tomato marchitez virus* (ToMarV), by Verbeek *et al.* (2014) demonstrated that *T. vaporariorum* could transmit ToMarV efficiently to a new host for at least 8 hours after becoming infected, but the whitefly usually died after periods longer than 8 hours if they had not been able to feed. If the whitefly fed on an intermediate (non-virus host) plant, their longevity increased, but a much lower percentage of final hosts were infected, and after 24 hours on the intermediate host, the whiteflies were not able to infect any of the final hosts (Verbeek *et al.*, 2014). In order to introduce ToTV to the UK, infected whitefly vectors of any species must arrive alive and still infectious in the UK, and find a host susceptible to ToTV within the (relatively short) period the individual whitefly is assumed to be capable of transmitting the virus. An additional assumption is that the three whitefly species considered below are attracted to all hosts (including the weed species) of ToTV. The degree of host susceptibility to ToTV outside the Solanaceae is also unknown.

Infected glasshouse whitefly (*Trialeurodes vaporariorum*): This whitefly species is a known vector of ToTV. Experimentally, a colony of glasshouse whitefly from a ToTV-infested glasshouse was introduced into a clean glasshouse. The infection rate varied by tomato cultivar, and ranged from two thirds of plants tested for 'Cedrico', down to zero for 'Marmande' and 'Marglobe' (Alfaro-Fernandez *et al.*, 2010b). In another study, Pospieszny *et al.* (2007) initially found a glasshouse whitefly transmission efficiency of 100%, which was confirmed by later work (Pospieszny *et al.*, 2010), though Verbeek *et al.* (2014) found only half of the *Nicotiana hesperis* plants tested were infected with ToTV by *T. vaporariorum*. In Australia and Poland, ToTV infections were noted to co-occur with high populations of glasshouse whitefly (Gambley *et al.*, 2010; Pospieszny *et al.*, 2007). Glasshouse whitefly is found in much of UK protected cultivation, and even if it was detected on imported plants or produce, no action would be taken against the consignment on the basis that this whitefly is present. Overall, entry of ToTV on the pathway of infected glasshouse whitefly is considered **unlikely**, but confidence in this assessment is **medium** as specific tests on the retention period of ToTV in *T. vaporariorum* have not been done, and the data used are based on a related virus.

Infected tobacco whitefly (*Bemisia tabaci*): Most research on vectors has concentrated on glasshouse whitefly, but *B. tabaci* is also a known vector of ToTV. First reported as a vector of ToTV by Amari *et al.* (2008), Verbeek *et al.* (2014) confirmed this ability. In addition to the general whitefly vector limitations listed earlier, the UK has protected zone

status for *B. tabaci*. Therefore, statutory action is taken against this whitefly when it is detected on imports of plant material, whether plants for planting or produce. Overall, infected *B. tabaci* as a pathway for ToTV is considered to be **very unlikely**. This judgement is made with **medium confidence**, as detailed research into the vector capability of *B. tabaci* for ToTV has not been carried out to date.

Infected banded-winged whitefly (*Trialeurodes abutiloneus*): This whitefly is only distributed in the Americas and has only rarely been intercepted in the UK. It has been shown experimentally to transmit ToTV (Verbeek *et al.*, 2014), but no instances of this species doing so under field conditions are known. In the Americas, ToTV is only present in Panama and Colombia, and, of these two countries, *T. abutiloneus* has only formally been recorded from Colombia (Calvert *et al.*, 2001), and not from Panama. Statutory action would be taken in the UK against findings of *T. abutiloneus*, though there is a potential for confusion of this species with glasshouse whitefly at ports of entry. Additionally, all the general vector limitations considered earlier will also apply to this species. Overall, therefore, it is considered **very unlikely** that *T. abutiloneus* could arrive in the UK while infective for ToTV, and this judgement is made with **high confidence**.

Seeds Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Growing plants for planting Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Mechanical Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Infected glasshouse whitefly Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Infected tobacco whitefly Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Infected banded-winged whitefly Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

9. How likely is the pest to establish outdoors or under protection in the UK/PRA area?

Given that the host range of ToTV includes a number of weed species, the availability of hosts is unlikely to be limiting. Establishment outdoors in the UK may, however, be limited by the lack of a suitable vector. The whitefly *T. vaporariorum* is not often found in the wider environment in the UK, and thus any ToTV infections outdoors may only be localised and transient, as the virus may be unable to spread from infected plants without an insect vector and many of the recorded hosts are annuals that die in winter. However, given the wide host range, it is possible that some perennial plants could be hosts, such as the solanaceous *Atropa* species (nightshades), allowing ToTV to overwinter in the wider environment.

Establishment under protection is much more likely. Tomatoes are widely grown under protection in the UK, and *T. vaporariorum* is commonly found in glasshouses (Hayman *et al.*, 2009). ToTV is known to be present in glasshouses in Hungary and Poland (e.g. Alfaro-Fernandez *et al.*, 2009; Pospieszny *et al.*, 2010). An early report on “torrao” suggests that, despite growing cucumbers in the glasshouse as a break between tomato crops, or leaving the glasshouse empty for three months, the disease reappeared in tomato crops when they were re-planted (Jordá *et al.*, 2003). However, this could have been due to re-infection of the new crop from outside, rather than persistence of the virus in the glasshouse.

Overall, establishment outdoors is considered **unlikely**, with **high confidence**. Establishment in protected cultivation is considered **very likely** with **high confidence**.

Outdoors Very unlikely Unlikely Moderately likely Likely Very likely

Confidence High Confidence Medium Confidence Low Confidence

Under Protection Very unlikely Unlikely Moderately likely Likely Very likely
Confidence High Confidence Medium Confidence Low Confidence

10. If the pest needs a vector, is it present in the UK/PRA area?

ToTV is known to be vectored by three species of whiteflies (Verbeek *et al.*, 2014), but the aphid *Myzus persicae* was not found to be a vector in transmission studies by Pospieszny *et al.* (2010).

Trialeurodes vaporariorum (glasshouse whitefly) is common and widespread in protected cultivation in the UK. However, this whitefly is only found in transient populations outdoors, even in the warmer months. It is not clear what factors prevent it establishing in the wider environment, as temperature should not be a limiting factor, based on published thermal requirements (e.g., Greenberg *et al.*, 2000; Soto *et al.*, 2000).

Bemisia tabaci (tobacco whitefly) is a species against which the UK has Protected Zone status. Statutory control measures are taken against findings of this species, aimed at eradicating outbreaks on growing plants, and preventing the entry of imported plant material where this whitefly is detected.

Trialeurodes abutiloneus (banded winged whitefly) is absent from the UK (and Europe).

11. How quickly could the pest spread in the UK/PRA area?

Natural spread is considered to be at a **moderate pace**, with **medium confidence**. No detailed studies have been done, but ToTV in glasshouses has been associated with the presence of *T. vaporariorum* (Pospieszny *et al.*, 2007), suggesting that vector transmission could be the main route for local dispersal. Alfaro-Fernandez *et al.* (2008) suggested that whitefly-assisted transmission might explain the infection of weeds present in glasshouses infected with ToTV in Spain. Jordá *et al.* (2003) noted that ToTV (as an undescribed tomato disease) spread rapidly through the first glasshouse, and also infected two neighbouring greenhouses despite precautions, though it's not clear what those measures were.

Spread in trade could be **very quick**, this judgement made with **high confidence**. ToTV has already spread to a number of new countries internationally, though the exact pathways are unclear. Studies on the genetic similarity of Spanish, Hungarian and Polish ToTV isolates showed a very low level of genetic diversity between samples, and only

samples from Grand Canaria showed any evidence of geographical differentiation compared to the other samples tested (Alfaro-Fernandez *et al.*, 2010a). If it is capable of international spread, it would also be capable of rapid spread within the UK, including movement of infected seedlings from UK propagators into places of production. Though propagators will produce plants of a high quality, tomato seedlings are traded while very small, probably before any symptoms develop. On a local level, mechanical transmission through routine crop work may also aid spread within a glasshouse, though there are no data on whether this has occurred in the current range of ToTV. In experimental studies on mechanical transmission, Alfaro-Fernández *et al.* (2006) reported a transmission rate of 1.8% for mechanical and grafting inoculation experiments. Pospieszny *et al.* (2007) regarded mechanical transmission of ToTV as poor, reporting rates of 50-70%. In later work, the transmission rate was found to vary with the plant species used as a source of virus, and was 70-90% (Pospieszny *et al.*, 2010). Spread of ToTV through grafting is of greater concern. Tomato plants in UK cultivation are commonly grafted, with one rootstock having two lateral stems grafted onto it (Adrian Fox, pers. com., 4 Feb 2015), and thus if any one of the three plants is infected, there is the potential for the virus to spread through the graft to the whole plant.

<i>Natural Spread</i>	Very slowly <input type="checkbox"/>	Slowly <input type="checkbox"/>	Moderate pace <input checked="" type="checkbox"/>	Quickly <input type="checkbox"/>	Very quickly <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input type="checkbox"/>	Medium Confidence <input checked="" type="checkbox"/>	Low Confidence <input type="checkbox"/>		
<i>With trade</i>	Very slowly <input type="checkbox"/>	Slowly <input type="checkbox"/>	Moderate pace <input type="checkbox"/>	Quickly <input type="checkbox"/>	Very quickly <input checked="" type="checkbox"/>
<i>Confidence</i>	High Confidence <input checked="" type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input type="checkbox"/>		

12. What is the pest’s economic, environmental and social impact within its existing distribution?

ToTV infection in tomatoes starts with necrotic spots at the bases of young leaves, which are surrounded by paler areas (Verbeek *et al.*, 2007). The spots can produce shot holes in the leaves, and long necrotic streaks can appear on stems (Alfaro-Fernandez *et al.*, 2010c). Later, the necrosis continues to develop, leading to overall growth reduction in the plant and a burnt appearance, from which the virus gets its name (Verbeek *et al.*, 2007). As well as leaves, fruit can show symptoms. Most commonly seen on green fruit, damage typically starts with brown or black spots or streaks, and as the fruit grows, it can split at the lesions (Alfaro-Fernández *et al.*, 2006). Such fruit is clearly unmarketable. Overall stunting of plants can lead to substantial yield loss (Navas-Castillo *et al.*, 2011), even if fruit is not directly affected. The production of tomatoes has been reported to be severely affected by ToTV infection, for example in the Canary Islands (Alfaro-Fernández *et al.*, 2007), Italy (Davino *et al.*, 2010) and Panama (Herrera-Vásquez *et al.*, 2009), and serious economic losses in Spain were reported by Amari *et al.* (2008). Leaf and fruit damage have been reported from commercial glasshouses in Hungary (Alfaro-Fernandez *et al.*,

2009), while in Polish glasshouses leaf damage and stunting have been reported (e.g. Pospieszny *et al.*, 2007; Pospieszny *et al.*, 2010) There are some reports that symptoms in experimental crops are less severe than those seen in commercial glasshouses (Navas-Castillo *et al.*, 2014), and it should be noted that some cultivars are apparently symptomless, at least following experimental inoculation (Pospieszny *et al.*, 2010). Pepper plants have shown stunting and viral mosaics, but no necrosis (Amari *et al.*, 2008), though no reports of natural infections of this host have been found. Overall, though no quantitative data were found, the impact of ToTV was assessed as **large**, and this judgement is made with **high confidence**.

<i>Impacts</i>	Very small <input type="checkbox"/>	Small <input type="checkbox"/>	Medium <input type="checkbox"/>	Large <input checked="" type="checkbox"/>	Very large <input type="checkbox"/>
<i>Confidence</i>	High Confidence <input checked="" type="checkbox"/>	Medium Confidence <input type="checkbox"/>	Low Confidence <input type="checkbox"/>		

In addition to impacts due to ToTV alone, mixed infections with other viruses can occur. Alfaro-Fernandez *et al.* (2010b) found that, of plants tested for mixed infections, almost half were also infected with *Pepino mosaic virus* (PepMV), and lower proportions with *Tomato chlorosis virus* (ToCV) and *Tomato spotted wilt virus* (TSWV), though not all plants in the study were tested for the last two viruses. The damage to tomato leaves at the cellular level was greater in plants with co-infections due to synergistic effects, and the most damage was seen in a triple infection with PepMV and TSWV (Alfaro-Fernandez *et al.*, 2010c). In contrast, Gomez *et al.* (2010) found that plants infected with ToTV alone had symptoms just as severe as those with mixed ToTV and PepMV infections, though the levels of viral RNA over time showed a complex interaction in mixed infections. In the UK, PepMV is present only with a limited distribution, TSWV is found in pepper crops, again with a limited distribution, and ToCV is absent from this country. There has been one report of a mixed infection with *Cucumber mosaic virus* (CMV) and ToTV in Panama, but the symptoms were the same whether the plants were infected with both viruses, or only ToTV (Herrera-Vásquez *et al.*, 2009). CMV is widespread in the UK.

13. What is the pest’s potential to cause economic, environmental and social impacts in the UK/PRA area?

Tomato is an important glasshouse crop in the UK, with a total area of over 200 ha and an annual value of around £100 million per year (Defra Basic Horticultural Statistics, 2008-2012). Economic impacts in the UK would probably only be evident in glasshouse crops, but commercial tomato production in the UK is all under glass. Additionally, the UK grows high-quality tomatoes, where even minor fruit damage is unacceptable and will result in substantial losses for the grower. As ToTV can produce symptoms on fruit (Alfaro-Fernández *et al.*, 2006) as well as more general yield losses, the potential impact on UK production could be greater than the potential impact some of other tomato viruses, such

as ToCV and *Tomato infectious chlorosis virus* (TICV) which do not directly impact fruit, though yield losses can be considerable. Glasshouse environments have been suitable for ToTV elsewhere, for example Poland (Pospieszny *et al.*, 2010). Glasshouse whitefly is very common within UK tomato crops, and while it is usually kept at an acceptable level with biocontrol, and occasionally IPM-compatible insecticides, high populations can build up on occasions (Rob Jacobson, pers. comm. April 2015). Infestations of glasshouse whitefly are more common with particular tomato cultivars (Hayman *et al.*, 2009). It should, however, be noted that production systems do differ within Europe. Tomatoes in Spain are often grown under cover, but not under full protection. Glasshouse whitefly is found outdoors in Spain, and can easily enter the tomato production sites. In the UK, glasshouse whitefly is not found outdoors, and so the vector population will probably be lower, with much less chance of movement of vectors between weed hosts outdoors and tomato crops indoors. However, due to the fruit damage, the potential impact of ToTV in the UK is assessed as **large**, but with **medium confidence**, as limitations on the vector populations may mean that the rate of spread through a glasshouse would be more limited.

Environmental impacts are considered to be **very small**, with **high confidence**. While ToTV does infect solanaceous and other weed hosts naturally, no reports of environmental impacts from the existing range of ToTV could be found.

Social impacts were also considered to be **very small** with **high confidence**. While tomatoes are commonly grown in gardens and allotments, local spread appears to be largely reliant on whitefly vectors, which are not widespread in the wider environment in the UK. Therefore, though localised impacts may occur in individual sites, on a wider scale these are not considered significant.

<i>Economic Impacts</i>	Very small	<input type="checkbox"/>	Small	<input type="checkbox"/>	Medium	<input type="checkbox"/>	Large	<input checked="" type="checkbox"/>	Very large	<input type="checkbox"/>
<i>Confidence</i>	High Confidence	<input type="checkbox"/>	Medium Confidence	<input checked="" type="checkbox"/>	Low Confidence	<input type="checkbox"/>				
<i>Environmental Impacts</i>	Very small	<input checked="" type="checkbox"/>	Small	<input type="checkbox"/>	Medium	<input type="checkbox"/>	Large	<input type="checkbox"/>	Very large	<input type="checkbox"/>
<i>Confidence</i>	High Confidence	<input checked="" type="checkbox"/>	Medium Confidence	<input type="checkbox"/>	Low Confidence	<input type="checkbox"/>				
<i>Social Impacts</i>	Very small	<input checked="" type="checkbox"/>	Small	<input type="checkbox"/>	Medium	<input type="checkbox"/>	Large	<input type="checkbox"/>	Very large	<input type="checkbox"/>
<i>Confidence</i>	High Confidence	<input checked="" type="checkbox"/>	Medium Confidence	<input type="checkbox"/>	Low Confidence	<input type="checkbox"/>				

14. What is the pest's potential as a vector of plant pathogens?

N/A: this is a pathogen.

15. What is the area endangered by the pest?

All tomatoes in protected cultivation could be at risk from ToTV. Theoretically, other solanaceous crops (e.g., pepper) may also be at risk, though no reports of natural infections of this crop were found.

Stage 3: Pest Risk Management

16. What are the risk management options for the UK/PRA area?

Exclusion: Trade in Solanaceae plants from third countries, other than European and Mediterranean countries, is prohibited, though seeds can be imported from outside the EU. However, as ToTV is present within the EU, exclusion could be problematic, as intra-EU trade is covered by plant passports and monitoring arrangements, thus it is not a priority for official inspection. Detection of symptomatic plants for planting would theoretically be possible, though visual inspection would be impractical. Tomatoes are traded as very young plants, probably before symptoms are expressed, and, given the trade in imported seedlings, the numbers of plants that would require testing would be prohibitive. Additionally, not all infected tomato plants show necrotic symptoms (Alfaro-Fernandez *et al.*, 2010b) and, when external symptoms are visible, they are similar to those caused by other species of torradovirus (though it should be noted that no tomato infecting torradoviruses are present in the UK). Molecular tests are available, which allow the detection of ToTV from plants that are suspected of being infected. Examples of such tests include those detailed in Verbeek *et al.* (2012) (two generic *Torradovirus* primer pairs, which allow RT-PCR detection of the known and tentative species in the genus *Torradovirus*), Panno *et al.* (2012) (simultaneous RT-PCR screening method for 7 tomato RNA viruses including ToTV), and Wieczorek and Obrepalska-Steplowska (2013) (a protocol involving multiplex RT-PCR for simultaneous detection of ToTV and *Pepino mosaic virus* in tomato). However, no tests are currently available for detecting ToTV in seed.

Due to the difficulties associated with testing, the best means of exclusion of ToTV for the UK would be requirements that tomato plants (and possibly other hosts) have been produced at a place of production which is known to be free from ToTV. However, as there is a possibility of seed transmission, and plants are traded before they are likely to express symptoms, there remains a risk of infected plants being traded.

Eradication and containment: These options would seem to be possible, at least in theory. Both France and Belgium have reported that they have eradicated ToTV following outbreaks (EPPO, 2014), suggesting that isolated findings of the virus can be successfully eliminated. It is not known what measures were used in France and Belgium to eradicate ToTV. However, early reports from Spain suggest that a three month crop break in one glasshouse did not prevent a newly planted crop showing symptoms of ToTV some weeks

after planting (Jordá *et al.*, 2003), though this may have been due to re-infection from outside rather than persistence of ToTV in the glasshouse. ToTV can infect a number of weed species (Alfaro-Fernandez *et al.*, 2008), and so there is a possibility of these acting as reservoirs for the virus, making eradication more difficult. However, the glasshouse whitefly, the only vector in the UK, is rarely found outside glasshouses. Therefore, reinfection from weeds outside the glasshouse is less likely, and containment or eradication is more likely to be successful.

Non-statutory controls: There has been some work on developing tomato cultivars that are resistant to ToTV, with patents on a gene for resistance filed by Maris *et al.* (2007), though these resistant plants are not apparently commercially available at the time of writing. Different cultivars of tomato that are currently sold do show varying susceptibility to this virus (Alfaro-Fernandez *et al.*, 2010b; Navas-Castillo *et al.*, 2014) and if less susceptible varieties were planted, this would seem likely to reduce the impacts of the virus. However, only a very few tomato varieties have been formally tested for susceptibility to ToTV, and, in reality, identifying such cultivars could be challenging for growers due to this lack of knowledge. *Trialeurodes vaporariorum* appears to be important at the local level for spread of ToTV (e.g. Alfaro-Fernandez *et al.*, 2010b; or Pospieszny *et al.*, 2010) and thus control of whitefly populations may help to reduce impacts of ToTV. Though there are currently no data on this, if mechanical spread was found to be responsible for local spread, then good hygiene would help to slow the spread within and between glasshouses.

17. Summary and conclusions of the rapid PRA

This rapid PRA shows:

Symptoms of *Tomato torrado virus* (ToTV) were first seen in Spain in the early 2000s, and the virus was formally described in 2007. The main economic host is tomatoes, on which most impacts have been seen, and the host upon which most research has been carried out. However, ToTV can infect a number of other hosts within the Solanaceae, as well as non-solanaceous weed hosts. ToTV is now present in limited parts of Europe, Panama, Colombia and southern Australia. This virus is vectored by three species of whitefly, including glasshouse whitefly (*Trialeurodes vaporariorum*), which is widespread in protected cultivation in the UK and Europe. Other (potential) means of ToTV transmission currently have less evidence to support them, and there is considerable uncertainty about which pathways have allowed ToTV to spread to new countries.

Risk of entry

Entry on seeds was considered unlikely with medium confidence. The available evidence suggests that ToTV is transmissible by seeds. The UK imports seed, which may have been bulked up in third countries, meaning the exact origin of a batch is unclear. Seeds have also been suggested – but not proven – as the pathway of introduction to Poland and Australia.

The pathway of plants for planting was assessed as moderately likely. The UK imports a significant volume of tomato seedlings from the Netherlands, but ToTV is not present in the Netherlands. Infected seedlings have been suggested as a possible pathway for ToTV being introduced to Poland. The import of solanaceous ornamental plants may pose another risk, as not all hosts are symptomatic, and this factor contributes to the rating of medium confidence for this pathway.

Mechanical transmission was considered unlikely with medium confidence. Data are lacking on how long the virus can persist on boxes, tools, etc., and it is also unknown how much movement of potentially infected equipment there is between sites in Europe where ToTV is present and the UK. However, precautions against the introduction of another virus, PepMV, are considered to reduce the risk of ToTV moving on this pathway.

Whiteflies are semipersistent vectors of ToTV, with the period during which they are infective likely to be less than 24 hours (however, these are assumptions based on research on whitefly transmission of a different species of tobravirus). Entry of ToTV with infected glasshouse whitefly (*T. vaporariorum*) was thought to be unlikely with medium confidence due to the whitefly needing to find a susceptible host within the period it is still infective. Entry with infected tobacco whitefly (*Bemisia tabaci*) was considered very unlikely with medium confidence, as *B. tabaci* is a regulated pest in the UK. The third known vector whitefly, banded winged whitefly (*T. abutiloneus*), is only present in the Americas, and has rarely been intercepted in the UK. If detected, statutory action would also be taken against this species. Overall, banded winged whitefly is judged very unlikely to introduce ToTV to the UK, with high confidence.

Risk of establishment

Outdoors, this was judged to be unlikely with high confidence. The lack of a suitable vector outdoors in the UK meant that the virus was considered less likely to be able to persist due to lack of transmission and it may be that only transient populations are possible. Under protection, establishment was considered very likely with high confidence, as ToTV is a known pest of protected cultivation, and its vector *T. vaporariorum* is widespread in UK glasshouses.

Economic, environmental and social impact

In the current area, impacts are considered large, with high confidence. Though quantitative data are lacking, ToTV causes reduction in plant growth with consequent decline in yields. Unlike several other tomato viruses of concern, fruit on plants infected with ToTV may show necrotic symptoms, rendering it unmarketable. The potential economic impacts in the UK are also considered to be large with medium confidence, as fruit is grown for a high-quality market, ToTV is known to be in glasshouses and a whitefly vector is common in UK protected cultivation. However, it should be noted that there are potentially more factors limiting whitefly populations in UK glasshouses, compared to countries in the current range of ToTV, and lower vector populations may reduce potential

impacts. Potential environmental and social impacts are considered small, with high confidence.

Endangered area

All protected tomato cultivation is at risk.

Risk management options

Exclusion would be difficult. ToTV is present in the EU, and the movement of tomato plants from other EU MS into the UK is covered under plant passporting and monitoring arrangements and thus is not a priority for official inspection. No official data is collected on the volume of this trade. While visual examination would not reliably detect all infected plants, molecular tests for ToTV in plants are available, but testing sufficient plants to be confident of detecting infection would be impractical. There are currently no tests that can detect ToTV in seed.

Eradication has been successful in France and Belgium, though there are complicating factors such as possible reservoirs of infected weed hosts. Containment, given the UK vector of ToTV is seldom found in the wider environment, may also be an option if there was an outbreak.

Non-statutory controls could include the use of tomato cultivars which are less susceptible to ToTV, though there has been very limited research into which varieties show fewer symptoms and, in reality, identifying these less susceptible varieties would be challenging given the current state of knowledge. Controlling glasshouse whitefly populations and other aspects of good glasshouse hygiene also seem likely to reduce the rate of spread of ToTV, and hence the impacts.

Key uncertainties and topics that would benefit from further investigation

As ToTV is a recently-identified virus, research is still actively being carried out on many basic aspects of ToTV biology. Much of the available information is often in the form of preliminary studies, which can contain contradictory or partial data. For example, details of many of the modes (or potential modes) of transmission are not known, which affects all the judgements made on pathways. There is a lack of epidemiological information at a large scale, which affects areas such as the impacts and importance of weed reservoirs.

18. Is there a need for a detailed PRA or for a more detailed analysis of particular sections of the PRA? If yes, select the PRA area (UK or EU) and the PRA scheme (UK or EPPO) to be used.

(For completion by the Plant Health Risk Group) ✓ (put a tick in the box)

No	<input checked="" type="checkbox"/>		
Yes		PRA area: UK or EU	PRA scheme: UK or EPPO

This rapid PRA has summarised all the currently available information. The uncertainties described within it are due to the limited knowledge about this recently described virus. Therefore, a more detailed PRA will not help to clarify these uncertainties, until such time as more research into ToTV has been conducted. A major review of torradoviruses written by van der Vlugt, Verbeek, Dulleman, Wintermantel, Cuellar, Fox and Thompson is due out soon, and will be a valuable summary of current information.

19. Images of the pest

Images of symptoms on leaves and fruit are available in Alfaro-Fernández *et al.* (2006). The electronic article is freely available via this link:
<http://www.magrama.gob.es/en/ministerio/servicios/publicaciones/RevistaPlagas.aspx>

20. Given the information assembled within the time scale required, is statutory action considered appropriate / justified?

[For completion by the Plant Health Risk Group] (put a tick in the box)

Yes No
 Statutory action Statutory action

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Name of Pest Risk Analyst(s):

Anastasia Korycinska, Adrian Fox, Sharon Matthews-Berry, Richard Baker, Helen Anderson, Peter Reed and Melanie Tuffen



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Any enquiries regarding this publication should be sent to us at

The Office of the Chief Plant Health Officer,
Department for Food, Environment and Rural Affairs,
Room 11G35,
Sand Hutton,
York, YO41 1LZ

Tel: 01904 465635

Email: plantpestrisks@defra.gsi.gov.uk