APPROVED: 20 December 2018

doi:10.2903/sp.efsa.2019.EN-1573

Pest survey card on *Toxoptera citricida*

European Food Safety Authority (EFSA),
Gritta Schrader, Melanie Camilleri, Ramona Mihaela Ciubotaru, Makrina Diakaki, Sybren Vos

Abstract

This pest survey card was prepared in the context of the mandate on plant pest surveillance (EFSA-Q-2017-00831), upon request by the European Commission. The purpose of this document is to assist the Member States in planning annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with the current international standards. The data requirements for such activity include the pest distribution, its host range, its biology, risk factors as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards and generic documents relevant for all pests to be surveyed, including, the general survey guidelines and statistical software such as RiBESS+.

Keywords: plant pest, survey, risk-based surveillance, *Toxoptera citricida*, black citrus aphid

Requestor: European Commission

Question number: EFSA-Q-2018-00355

Correspondence: ALPHA@efsa.europa.eu

Acknowledgements: EFSA wishes to acknowledge the Netherlands Food and Consumer Product Safety Authority (NVWA) and the Julius Kuehn Institute (JKI), in Germany, for the support provided to this scientific output in the context of the tasking grants GP/EFSA/ALPHA/2017/02. EFSA also wishes to thank EFSA Plant Health Panel member Josep Anton Jaques Miret, for reviewing this document as well as the working group members that contributed to the preparation of this output.

Suggested citation: EFSA (European Food Safety Authority), Schrader G, Camilleri M, Ciubotaru RM, Diakaki M and Vos S, 2019. Pest survey card on *Toxoptera citricida*. EFSA supporting publication 2019: EN-1573. 16 pp. doi:10.2903/sp.efsa.2019.EN-1573

ISSN: 2397–8325

Table of contents

Introduction

The information presented in this pest survey card was summarised from the EFSA pest categorisation of *Toxoptera citricida* (EFSA PLH Panel, 2018), European and Mediterranean Plant Protection Organization (EPPO) diagnostic protocol (EPPO, 2006), the CABI datasheet on *Toxoptera citricida* (CABI, 2018) and the EPPO datasheet on *Toxoptera citricida* (EPPO and CABI, 1997).

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *Toxoptera citricida* in EU Member States (EFSA, 2018). This document is part of a toolkit that is being developed to assist and support Member States plan a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention (IPPC) guidelines for surveillance (FAO, 2016). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:

   a. The pest survey card on *Toxoptera citricida.* [1]

ii. General documents:

   a. The general survey guidelines (to be finalised in 2019)

   b. The RiBESS+ manual available online[2]

   c. The statistical tools RiBESS+ and SAMPELATOR which are available online[3] with open access after registration.

T. citricida is known as the most efficient vector of Citrus tristeza virus (CTV) that is considered as a threat to citrus cultivation in the EU. For this reason, surveillance of this insect is particularly relevant for the EU territory.

1. The pest and its biology

1.1. Taxonomy

Scientific name: *Toxoptera citricida* (Kirkaldy)

Class: Insecta, **Order:** Hemiptera, **Family:** *Aphididae*, **Genus:** *Toxoptera*, **Species:** *Toxoptera citricida*

Synonyms: *Aphis aeglis*, *A. citricidus*, *A. nigricans*, *A. tavaresi*, *Myzus citricidus*, *Paratoxoptera argentinensis*, *Toxoptera aphoides*, *T. citricidus*, *T. tavaresi*

Common name in English: black citrus aphid (preferred), brown citrus aphid, oriental black citrus aphid and tropical citrus aphid

Although the genus name, *Toxoptera* Koch, is feminine, the feminine/masculine genus/species combination *Toxoptera citricidus* is widely used in the literature (CABI, 2018).

T. citricida is a well-defined and clearly identifiable aphid species that can be distinguished using available taxonomic keys for adult aphids (EFSA PLH Panel, 2018).

[1] The content of this EFSA Supporting Publication is reproduced as a live document available at https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489 where it will be updated whenever new relevant information becomes available.
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shiny-efsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

1.2. EU pest regulatory status

T. citricida, is listed in Annex II/AI of Council Directive 2000/29/EC[4], on plants of *Citrus*, *Fortunella*, *Poncirus* and their hybrids, other than fruit and seeds. Moreover, importation from non-member countries of *Citrus* plants other than fruit and seeds for planting is prohibited (Annex III Part A). In addition, Annex IV/AI lays down that fruits shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark. *T. citricida* is known as the most efficient vector of CTV, which is considered as a threat to citrus cultivation in the EU. Citrus tristeza virus is a quarantine virus regulated in the Council Directive 2000/29/EC specifically for European isolates and for non-European isolates.

1.3. Pest distribution

T. citricida is believed to be native to Asia where citrus plants originated. Since the first half of the 20th century, the aphid has been known to be widely distributed on citrus in Asia, India, New Zealand, Australia, Pacific Islands (including Hawaii), Africa south of the Sahara, Madagascar, Indian Ocean Islands and South America (CABI, 2018).

The pest is present in the EU (Figure 1) in Madeira and in continental EU in the coastal area of the northwest quadrant of the Iberian Peninsula, extending from the north of the Portuguese province of Beira Litoral (Regiao Norte) to the Basque province of Bizkaia (EFSA PLH Panel, 2018).

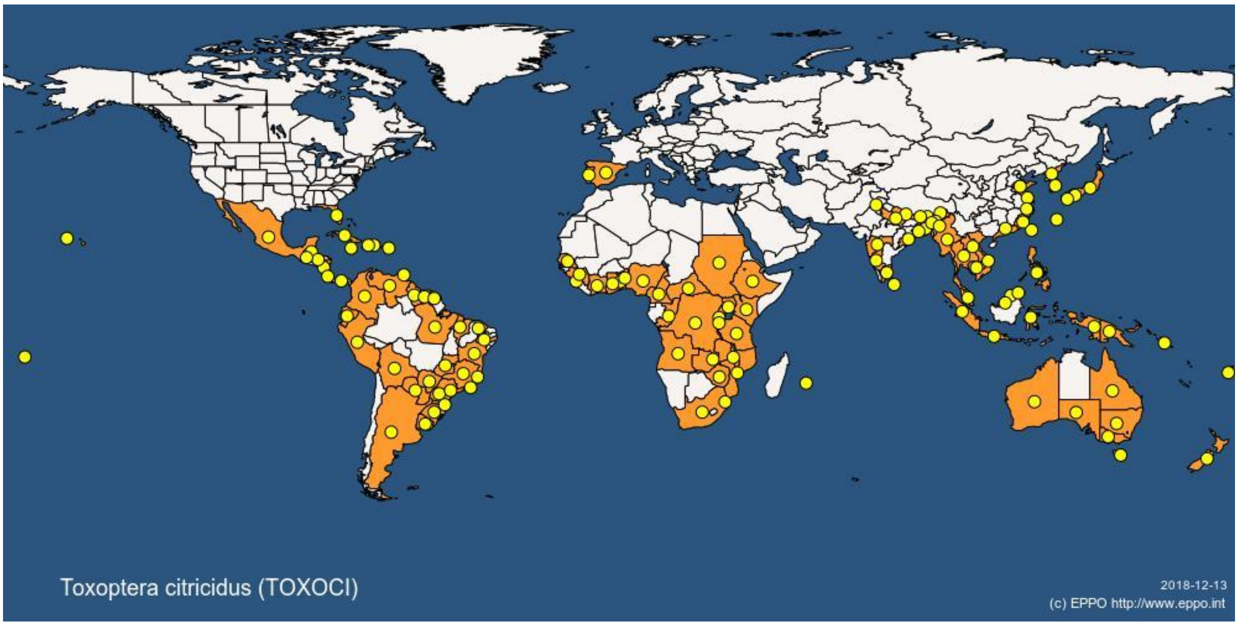

Figure 1: Global distribution of *Toxoptera citricida*. Yellow dots indicate the pest status in countries or states as present (Source: EPPO global database, www.eppo.int)

1.4. Life cycle and biology

For the insect itself, the life cycle of *T. citricida* is linked to the phenology of plants belonging to the genus *Citrus* (Michaud, 1998; Uygun et al., 2012) (EFSA PLH Panel, 2018).

T. citricida is anholocyclic (males are absent, females reproduce parthenogenetically, viviparous) (Figure 2), and thelytokous (females are produced from unfertilised eggs) over most of its range (Yokomi, 2009).

[4] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–112. Consolidated version of 01/04/2018

For the insect as a vector of viruses:

- *T. citricida* is the most efficient vector of CTV (Michaud, 1998; Moreno et al., 2008; Gottwald, 2010), a phloem-limited closterovirus (Bar-Joseph et al., 1989). CTV is semi-persistently transmitted by citrus aphids (Raccah et al., 1976). Aphids acquire the virus from an infected tree with feeding times as short as 5–10 min, but transmission efficiency increases with feeding times up to 24 h. There is no latent period and the virus does not multiply or circulate in the aphid. The time required to inoculate a plant is the same as for acquisition. The aphid is capable of spreading the virus for 24–48 h without reacquisition (Meneghini, 1948). Two types of CTV strains are economically important: (1) those that cause decline of citrus budded onto sour orange (*Citrus aurantium*) rootstock; and (2) those that cause stem pitting of grapefruit and sweet orange regardless of rootstock. Both are readily transmissible by *T. citricida*.
- Several other viruses are reported to be transmitted by *T. citricida*, namely Citrus vein enation virus, stem-pitting virus, Eureka-seedling virus and bud union decline of citrus. Furthermore, the aphid is reported to transmit mosaic viruses of abaca, pea, yam and zucchini and the potyvirus chili veinal mottle virus (EPPO, 2006).

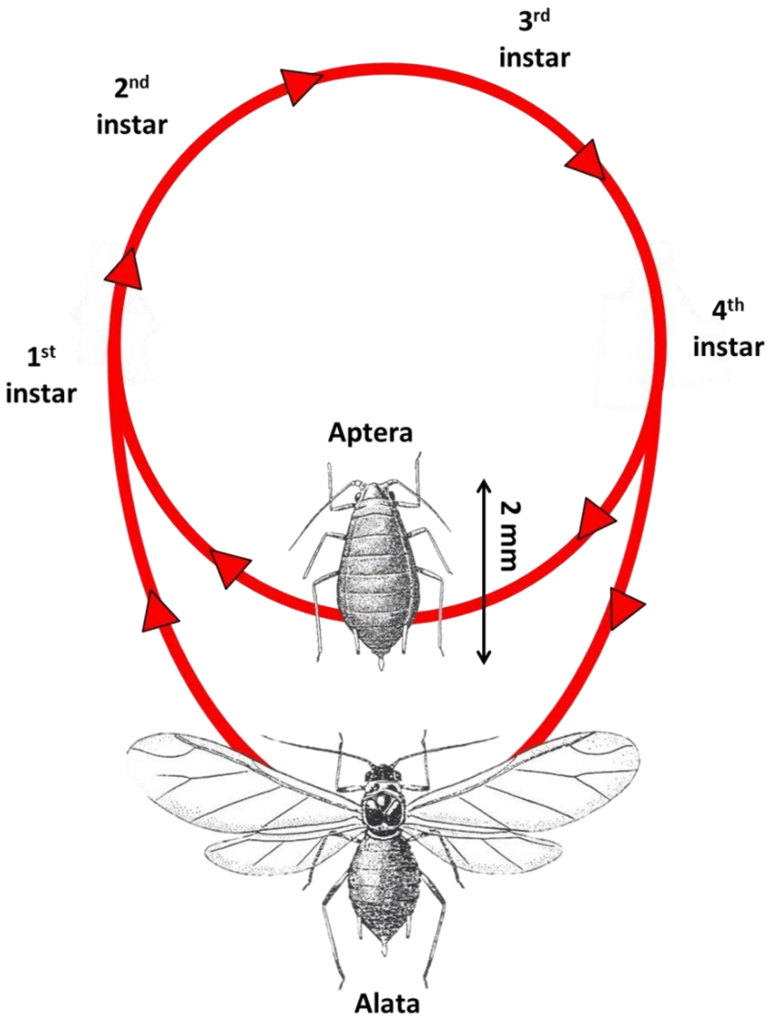

Figure 2: General life cycle of a facultative anholocyclic aphid. Wingless females (apterae) reproduce parthenogenetically until the host (the leaf flush) is exhausted. They then produce winged females (alatae) which will disperse and colonize new unexploited leaf flushes, where they will reproduce parthenogenetically to produce more wingless females. Both wingless and winged females are present on the host(s) and can be found throughout the whole year. (Source: Josep Anton Jaques Miret)

1.5. Host range and main hosts

Rutaceae are the preferred hosts of *T. citricida*. However, in non-member countries it has also been observed that relatives such as calamondin (× *Citrofortunella microcarpa*) and orange jessamine (*Murraya paniculata*), rough lemon (*Citrus jambhiri*), sour orange (*C. aurantium*), box orange (*Severiana buxifolia*) and lime berry (*Triphasia trifolia*) can support the development of *T. citricida*.

There are many other additional host records that may offer a temporarily host when no new citrus leaf or flower flush is available. For a list, see EFSA PLH Panel (2018; citing Michaud, 1998). Nevertheless, there is high uncertainty about the suitability of these plants to support the complete development and reproduction of *T. citricida*. No records of interception of *T. citricida* in the EUROPHYT database (EFSA PLH Panel, 2018).

For the host plants grown in the EU, the EPPO Global Database lists *Citrus limon*, *C. paradisi*, *C. reticulata* and *C. sinensis* as major hosts for *T. citricida*. Host plants are present in citrus-growing areas in the EU and are found in Cyprus, Spain, France, Greece, Croatia, Italy, Malta, and Portugal (Figure 3). These citrus species are retained as the target hosts for surveillance activity.

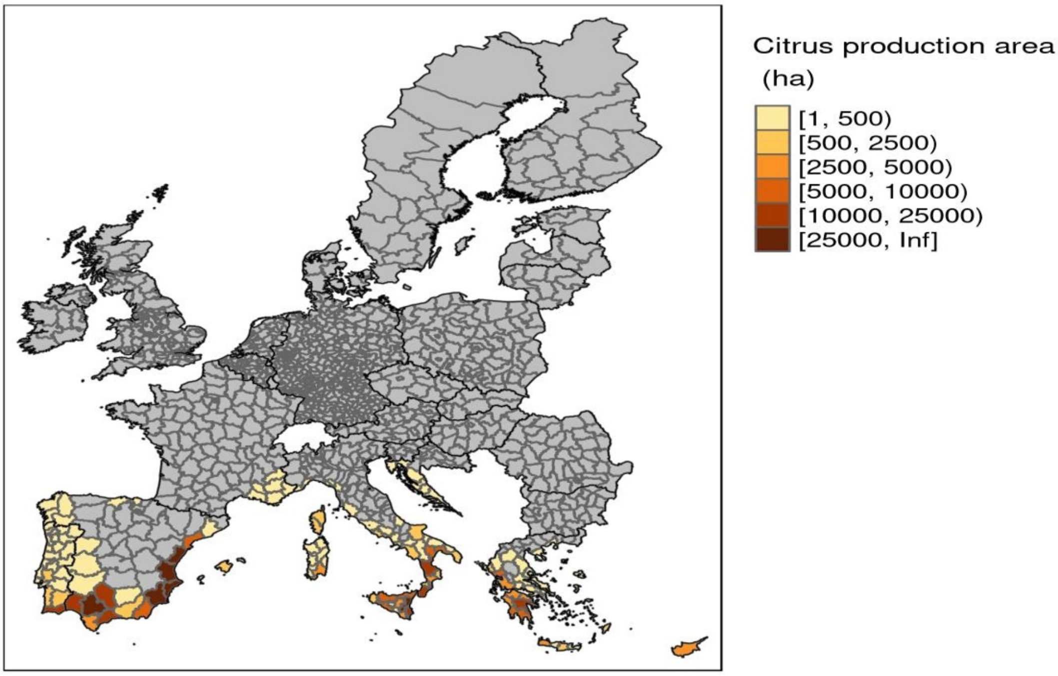

Figure 3: Citrus-growing areas in the EU (Cyprus, Spain, France, Greece, Croatia, Italy, Malta, and Portugal) at NUTS3 level, extracted from EFSA PLH Panel (2014, Appendix F)

1.6. Environmental suitability

Although *T. citricida* prefers warm climates, it can tolerate colder areas such as southern Japan by developing a holocyclic stage and overwintering as eggs (Komazaki, 1993). Development time is temperature dependent. At 20°C, the nymph development time is 6–8 days with an average pre-reproductive period of 8.1 days, longevity is 28.4 days. Fecundity is 58.5 offspring/female with an intrinsic rate of natural increase (r_c) of 0.36, net reproductive rate of 56.2 and mean generation time of 11.2 days. Its thermal threshold is 8.4°C and it requires 125 degree days for development (Komazaki, 1982). Takanashi (1989) reported slightly longer generation times under similar conditions and differentiated between alata and aptera development time. Winged aphids develop either when populations get too large or when food sources lack quality. They disperse then search for new hosts to start new colonies (Yokomi, 2009).

In principle, one single female can start a new colony if it finds a host plant. *T. citricida* can be found in almost all citrus-growing regions world-wide except for California and the Mediterranean (except for a restricted distribution in Portugal and Spain).

Climate conditions in the Mediterranean basin are likely to be favourable for the establishment of this aphid (EFSA PLH Panel, 2018). In particular, the citrus-growing areas in the EU are considered to be suitable for the pest and are therefore the target area for surveillance of the aphid.

1.7. Spread capacity

Human-assisted dispersal via plants for planting of citrus and other host plants is most likely to be the main dispersal mechanism of *T. citricida*. In addition, agricultural equipment used in the infested areas and not properly cleaned (e.g. clothing, tools, empty fruit boxes, etc.) could contribute to the spread.

Winged adult females can naturally disperse by themselves and both winged and wingless forms can be passively dispersed by thermal updraft currents and storms for passive long distance (EFSA PLH Panel, 2018).

1.8. Risk factor identification

A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest. The risk factors that are relevant for surveillance are those that result in different effects on different parts of the target population depending on its structure and its variability.

Identification of the risk factors and their relative risk estimation is essential for performing a risk-based survey. It needs to be tailored to the situation of each Member State. The proportion of the target population for each risk factor needs to be known or estimated by each Member State. This section presents examples of risk factors. Different Member States may have different risk factors.

Plants for planting of different genera being potential pathways for the pest into the EU, higher risk areas can be delimited in the citrus-growing areas as:

- the citrus fields contiguous to nurseries and other locations where plants for planting are being produced or traded such as garden centres;
- citrus trees in the backyards and garden in the areas where the pest occurs in Spain and Portugal.

2. Detection and identification

2.1. Visual examination

2.1.1 Pest

Adults of *T. citricida* are 1.5–2.8 mm long, shiny, and dark brown to black (Figure 4). When disturbed they may stridulate with the hind legs without audible sounds. By this, the aphid can also be differentiated from *T. aurantii*, which produces a sound that can be heard over a distance of 45 cm. Squeezing adults of *T. citricida* onto a white surface produces a red colour. Alcohol is coloured deeply red when adults are immersed in it. Other *Toxoptera* species do not show this effect.

To be able to fully identify this species, a permanent slide mount is needed (see Appendix 1 of EPPO, 2006). Keys to identify adult apterous (wingless) and alate (winged) aphids associated with citrus are provided by Blackman and Eastop (2000) and by Martin (1991). To identify first instar nymphs of *Toxoptera* spp., the key of Martin (1991) can be used, see also the EPPO Diagnostic Protocol (EPPO, 2006), Appendix 4. Other instars and the species level can only be identified if reared to the adult stage.

These keys can also be found in Appendices 2 and 3 of the EPPO Diagnostic Protocol (EPPO, 2006) and are summarised in Table 1.

Table 1: Comparison of *Toxoptera* species (extracted from EPPO, 2006)

Character	*T. citricidus*	*T. aurantii*	*T. odinae*
Size of apterae and alatae	1.5–2.8 mm	1.1–2.0 mm	1.3–2.4 mm
Colour – apterae	Shiny, very dark brown to black	Shiny, reddish brown, brown–black to black brownish	Grey-brown to reddish brown
Colour – immatures	Brown	Brownish	Brown
Colour – alatae	Shiny black abdomen	Dark brown to black abdomen	Reddish brown to dark brown abdomen
Antennae – apterae	Black-and-white banded	Black-and-white banded	Pale
Antennae – alatae	Segment III – black with a pale base; segment IV – pale	Segments III and IV – white with a dark tip	Segments III and IV – pale
Antennal setae – segment III	Longer than the basal diameter of the segment	Shorter than the basal diameter of the segment	Twice as long as the basal diameter of the segment
Forewing – media	Normally twice branched	Normally once branched	Normally twice branched
Forewing – pterostigma	Pale	Black	Pale
Siphunculi	Black – longer than cauda	Black – longer than cauda	Dusky – three-quarters length of cauda
Cauda	Black	Black	Black
Cauda setae – apterae	25–54	9–20	15–18
Caudal setae – alatae	25–40	8–19	15–18
Stridulation	No audible sound to the human ear	An audible sound to the human ear	Not known
Preserved species	Colour fluid deep red (also squashed aphids colour a white surface red)	Do not colour fluid or a white surface red	Do not colour fluid or a white surface red
Host plants	Rutaceae almost exclusively – only occasionally on members of other plant families	120+ species in more than 10 plant families including Rutaceae	25+ species in more than 15 plant families including the Rutaceae, although not common on *Citrus* spp.

Risk of misidentification:

According to Blackman and Eastop (2000) there are 19 species of aphids that feed on citrus, of which six can be confused with *T. citricida*. These are: *Aphis craccivora*, *A. gossypii*, *A. nerii*, *A. spiraecola*, *T. aurantii*, and *T. odinae*. Except for the latter, which occurs in Asia and sub-Saharan Africa (Favret and Miller, 2014), the remaining aphid species occur in citrus in the EU. They can be distinguished using available taxonomic keys for adult aphids both wingless (apterae) and winged forms (alatae) (Michaud, 1998; Ilharco et al., 2005; Uygun et al., 2012).

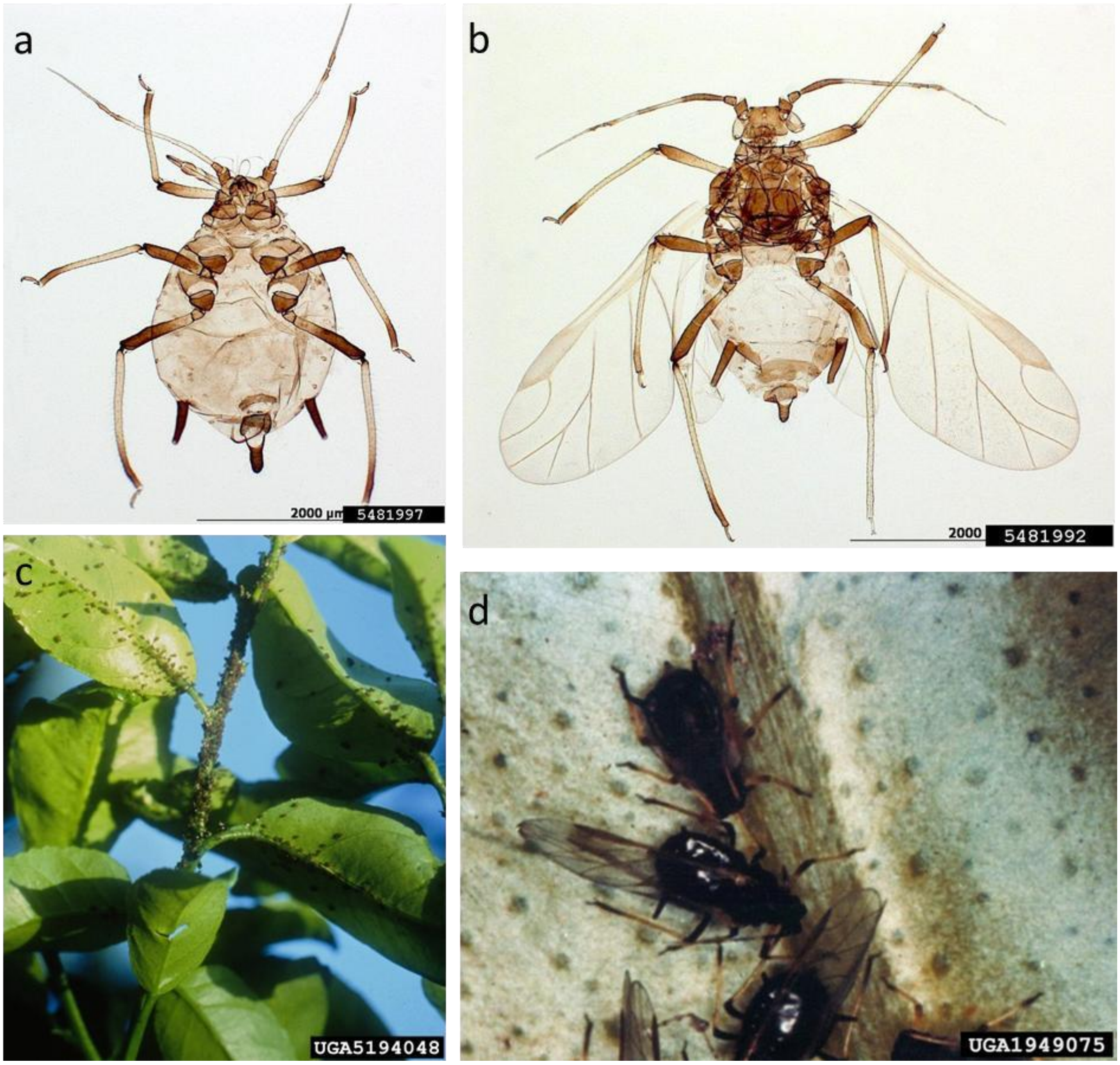

Figure 4: Adults of *Toxoptera citricida* (a) wingless female vivipara (Source: Brendan Wray, AphID, USDA APHIS PPQ, Bugwood.org), (b) winged female vivipara (Source: Brendan Wray, AphID, USDA APHIS PPQ, Bugwood.org), (c) adults aggregated on infested citrus shoot (Source: Jeffrey W. Lotz, Florida Department of Agriculture and Consumer Services, Bugwood.org) and (d) adults on leaf (Source: Bayer Pflanzenschutz, Bayer Pflanzenschutz, Bugwood.org)

2.1.2. Symptoms

Growth of shoots is significantly reduced and leaves become distorted, brittle, wrinkled and curled. Branches may become deformed. A few aphids on a young shoot can stop blossom bud development and induce bud dropping. Infested flowers do not open completely, or open only abortively, due to deformed ovaries.

A general sign of an infestation with aphids is honeydew on leaves, on which black, sooty mould develops, as well as the presence of ants that collect the honeydew from the aphids.

As other aphid species on citrus also cause leaf distortion and because mixed colonies of two or more species are common (Halbert and Brown, 1998), species needs to be confirmed by examination of slide-mounted specimens in the laboratory. Specimens can be stored in 70–80% alcohol before slide mounting.

2.2. Traps

With yellow sticky or water traps or with suction traps, populations of alatae (winged aphids) can be monitored, but this technique is not species-specific and can only indicate that adult aphids are present in that area. Such traps are not suitable for early detection. Sensitivity is very low.

2.3. Laboratory testing

Molecular methods are under development (e.g. Wang and Qiao, 2009) and may be available in the near future. So far, these methods, combined with morphometric methods have shown that the genus *Toxoptera* is paraphyletic, with *T. aurantii*, *T. citricida* and *T. odinae* not being closely related (Kim and Lee, 2008; Wang and Qiao, 2009; Kim et al., 2010). These results may lead to *Toxoptera* soon becoming a subgenus of *Aphis*.

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation of each Member State. The size of the defined target population and its structure in terms of the number of epidemiological units need to be known. When several pests have to be surveyed in the same crop, it is recommended to use the same epidemiological and inspection units for each pest in order to optimise the survey programme as much as possible. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for a survey of *Toxoptera citritida* in citrus

	Definition	Unit
Target Population	Citrus growing area including backyard gardens in each Member State	total Ha
Epidemiological Units	Citrus orchards and backyard gardens	Half Ha*
Inspection Units	Young shoots of plants for planting	

*In Spain, half a hectare of citrus orchard is assumed to represent the average size of a farm area in which the cultivar (citrus species and variety), the cultural practices and the ownership are similar or the same.

The general guidelines for the risk-based, statistically sound surveillance are presented in a separate document and describe step- by-step the process of the survey design and include:

1/ the choice of the type of survey to develop depending on the objectives of the survey

2/ manual for guiding the user through the statistical tools for sample size calculations

3/ essential considerations when:

- choosing the sampling sites and taking the samples

- collecting the data

- reporting the data and the survey results

References

Bar-Joseph M, Marcus R and Lee RF, 1989. The continuous challenge of Citrus tristeza virus control. Annual Review of Phytopathology, 27, 291–316.

Blackman RL and Eastop VF, 2000. Aphids on the world's crops: an identification and information guide. 2nd Edition. pp. i–x, 1–466. Wiley, Chichester, UK.

CABI (Centre for Agriculture and Bioscience International), 2018. *Toxoptera citricida*. In: Invasive Species Compendium. Wallingford, UK: CAB International. Available online: https://www.cabi.org/ISC/datasheet/54271 [accessed 10 December 2018].

EFSA (European Food Safety Authority), Ciubotaru RM, Cortiñas Abrahantes J, Oyedele J, Parnell S, Schrader G, Zancanaro G and Vos S, 2018. Technical report of the methodology and work-plan for developing plant pest survey guidelines. EFSA supporting publication 2018:EN-1399. 36 pp. doi:10.2903/sp.efsa.2018.EN-1399

EFSA PLH Panel (EFSA Panel on Plant Health), 2014. Appendix F: Data supplement Available online at: https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903%2Fj.efsa.2014.3557&file=3557ax1-sup-0001.pdf. Accessed 13 December 2018.

EFSA PLH Panel (EFSA Panel on Plant Health), 2018. Scientific opinion on the pest categorisation of *Toxoptera citricida*. EFSA Journal, 16(1): 5103, 22 pp. https://doi.org/10.2903/j.efsa.2018.5103

EPPO and CABI, 1997. Data sheets on quarantine pests: *Toxoptera citricidus*. Contract 90/399003.

EPPO (European and Mediterranean Plant Protection Organization), 2006. PM 7/75 (1). Diagnostics *Toxoptera citricidus*. EPPO Bulletin, 36, 451–456.

FAO (Food and Agriculture Organization of the United Nations), 2016. Plant pest surveillance: a guide to understand the principal requirements of surveillance programmes for national plant protection organizations. Version 1.1 published March 2016. FAO, Rome, Italy. Available online: https://www.ippc.int

Favret C and Miller G, 2014. *Toxoptera odinae*. AphID. Identification Technology Program, CPHST, PPQ, APHIS, USDA; Fort Collins, CO, USA. http://aphid.aphidnet.org/*Toxoptera*_odinae.php. Accessed 12 November 2018.

Gottwald TR, 2010. Concepts in the epidemiology of Citrus tristeza virus. In: Karasev AV and Hilf ME (eds). Citrus tristeza virus complex and tristeza diseases. APS Press, St Paul, MN, USA. pp. 119–131.

Halbert SE and Brown LG, 1998. *Toxoptera citricida* (Kirkaldy), brown citrus aphid, identification, biology and management strategies. Entomology circular no. 374. Division of Plant Industry Florida Department of Agriculture & Consumer Services, USA.

Ilharco FA, Sousa-Silva CR and Alvarez A, 2005. First report on *Toxoptera citricidus* (Kirkaldy) in Spain and continental Portugal (Homoptera, Aphidoidea). Agronomia Lusitanica, 51, 19–21.

Kim H and Lee S, 2008. A molecular phylogeny of the tribe Aphidini (Insecta: Hemiptera: Aphididae) based on the mitochondrial tRNA/COII, 12S/16S and the nuclear *EF1α* genes. Systematic Entomology, 33, 711–721.

Kim H, Lee W and Lee S, 2010. Morphometric relationship, phylogenetic correlation, and character evolution in the species-rich genus *Aphis* (Hemiptera: Aphididae). PLoS One, 5, e11608.

Komazaki S, 1993. Biology and virus transmission of citrus aphids. Technical Bulletin – Food and Fertilizer Technology Center Taipei, Taiwan. Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC, ASPAC), No. 136, ii + 9 pp. cpc/abstract/19951101474. Available at www.fftc.agnet.org/library.php?func=view&id=20110712190621

Komazaki S, 1982. Effects of constant temperatures on population growth of three aphid species, *Toxoptera citricidus* (Kirkaldy), *Aphis citricola* van der Goot and *Aphis gossypii* Glover (Homoptera: Aphididae) on *Citrus*. Applied Entomology and Zoology, 17, 75–81.

Meneghini M, 1948. Experiencias de transmissao doc doenea 'tristeza' dos citrus pelo pulgo da laranjera. Biologico, 14, 115–118. cpc/abstract/19480302893

Michaud JP, 1998. A review of the literature on *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). The Florida Entomologist, 81, 37–61.

Martin JH, 1991. A new *Toxoptera* species from Rutaceae in Hong Kong (Homoptera: Aphididae). Bulletin of Entomological Research, 81, 277–281.

Moreno P, Ambrós S, Albiach-Martí MR, Guerri J and Pena L, 2008. Citrus tristeza virus: a pathogen that changed the course of the citrus industry. Molecular Plant Pathology, 9, 251–268. https://doi.org/10.1111/J.1364–3703. 2007.00455.X

Raccah B, Loebenstein G and Bar-Joseph M, 1976. Transmission of citrus tristeza virus by melon aphid. Phytopathology, 66, 1102–1104.

Takanashi M, 1989. The reproductive ability of apterous and alate viviparous morphs of the citrus brown aphid, *Toxoptera citricidus* (Kirkaldy) (Homoptera: Aphididae). Japanese Journal of Applied Entomology and Zoology, 33, 266–269.

Uygun N, Hermoso de Mendoza A and Başpınar, H, 2012. Chapter 9. Aphididae. In: Vacante V and Gerson U (eds). Integrated Control of Citrus Pests in the Mediterranean Region. Bentham Books, Dubai, UAE. pp. 126–136.

Wang JF and Qiao GX, 2009. DNA barcoding of genus *Toxoptera* Koch (Hemiptera: Aphididae): identification and molecular phylogeny inferred from mitochondrial COI sequences. Insect Science, 16(6), 475–484.

Yokomi RK, 2009. The brown citrus aphid, *Toxoptera citricida*. In: D'Onghia AM, Djelouah K and Roistacher CN (eds). Citrus tristeza virus and *Toxoptera citricidus*: a serious threat to the Mediterranean citrus industry. Bari: CIHEAM, 2009. pp. 35–46 (Options Méditerranéennes: Série B. Etudes et Recherches; n. 65) http://ressources.ciheam.org/om/pdf/b65/00801385.pdf. Accessed 13 December 2018.

Glossary

Term	Definition*
Component (of a survey)	In the general framework of surveillance, with the goal of demonstrating pest freedom, a component is an activity characterised by a given sensitivity of the method of detection and identification. The overall confidence of the survey for pest freedom will result from the combination of the different components. Two components of the same survey could have different target populations. E.g. Survey on an insect performed by trapping of the pest (component 1) and sampling the host plants for visual examination of signs or symptoms (component 2).
Confidence	Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).
Design prevalence	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence. (EFSA, 2018)
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).
Epidemiological unit	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2018).
Inspection unit	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place. (EFSA, 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2018).
Method sensitivity	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and

	confirmed as positive.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2018).
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2018).
Population size	The estimation of the number of plants in the region to be surveyed (EFSA, 2018).
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (Cameron et al., 2014).
RiBESS+	An online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at: https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2018).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2018).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: • Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)
Test	Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2018).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (DSp) is the probability that a truly negative epidemiological unit will test negative and is related to the analytical specificity. In freedom from disease it is assumed to be

	100%.
Visual examination	The physical examination of plants, plant products or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2018).

*References
Cameron A, Njeumi F, Chibeu D, Martin T, 2014. Risk-based disease surveillance. FAO (Food and Agriculture Organization of the United Nations), Rome.
Dohoo I, Martin W and Stryhn H, 2010. Veterinary epidemiologic research. 2nd Edition. VER Inc., Canada.
EFSA (European Food Safety Authority), 2018. Technical report of the methodology and work-plan for developing plant pest survey guidelines. EFSA supporting publication 2018: EN-1399. 36 pp. doi:10.2903/sp.efsa.2018.EN-1399
FAO (Food and Agriculture Organization of the United Nations), 2016. ISPM (International Standards for Phytosanitary Measures) 27. Diagnostic protocols for regulated pests. FAO, Rome, Italy. Available online: https://www.ippc.int/en/publications/593/
FAO (Food and Agriculture Organization of the United Nations), 2018. ISPM (International Standards for Phytosanitary Measures) 5. Glossary of phytosanitary terms. FAO, Rome, Italy. Available online: https://www.ippc.int/en/publications/622/
McMaugh T, 2005. Guidelines for surveillance for plant pests in Asia and the Pacific. ACIAR Monograph No.119, 192 pp.
Montgomery DC and Runger GC, 2010. Applied statistics and probability for engineers. Fifth Edition, John Wiley & Sons. 792 pp.