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Pest survey card on *Scirtothrips aurantii*, *Scirtothrips citri* and *Scirtothrips dorsalis*

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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+.

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Keywords: plant pest, survey, risk-based surveillance, *Scirtothrips aurantii*, *Scirtothrips citri*, *Scirtothrips dorsalis*

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Introduction

The information presented in this pest survey card was summarised from the three EFSA pest categorisations of *Scirtothrips aurantii*, *S. citri* and *S. dorsalis* (EFSA PLH Panel, 2018a,b, 2014, respectively), the European and Mediterranean Plant Protection Organization (EPPO) global database, the EPPO PM 7/56(1) (EPPO, 2005) and the Centre for Agriculture and Bioscience International (CABI) Crop Protection Compendium datasheets (*S. aurantii* (CABI, 2018a), *S. citri* (CABI, 2018b) and *S. dorsalis* (CABI, 2018c)). The genus of *Scirtothrips* comprises more than 100 species, of which six are present in the EU according to Fauna europaea (online) (*S. bournieri*, *S. canizoi*, *S. dignus*, *S. inermis*, *S. longipennis*, *S. mangiferae*). Since only *S. aurantii*, *S. citri* and *S. dorsalis* are regulated by Council Directive 2000/29/EC¹, this survey card only deals with these three species.

The objective of this pest survey card is to provide the relevant biological information that is needed to prepare surveys in EU Member States (EFSA, 2018). It is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention 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 potato brown rot *Ralstonia solanacearum*.²
- ii. General documents:
 - a. The general survey guidelines (to be finalised in 2019)
 - b. The RiBESS+ manual available online³
 - c. The statistical tools RiBESS+ and SAMPELATOR which are available online⁴ with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Class: Insecta, Order: <i>Thysanoptera</i> , Family: <i>Thripidae</i> , Genus: <i>Scirtothrips</i> Shull		
Scientific names	Synonyms	Common names
<i>Scirtothrips aurantii</i> Faure	<i>Scirtothrips acaciae</i> Moulton	South African citrus thrips
<i>Scirtothrips citri</i> (Moulton)	<i>Euthrips citri</i>	California citrus thrips, citrus thrips
<i>Scirtothrips dorsalis</i> Hood	<i>Heliiothrips minutissimus</i> (Bagnall), <i>Anaphothrips andreae</i> (Karny), <i>Scirtothrips padmae</i> (Ramakrishna), and <i>S. fragaiae</i> (Girault)	Assam thrips, chilli thrips, flower thrips, strawberry thrips, yellow tea thrips

Recent evidence (Dickey et al., 2015) shows that *S. dorsalis* is a complex species that includes a minimum of nine cryptic (= morphologically indistinguishable from each other but with a different molecular profile) and two morphologically distinguishable species (namely, *S. oligochaetus* and *S. aff. dorsalis*), which are endemic to some regions. At least three of the nine cryptic species are invasive. It is not known at this moment which of the nine cryptic species in the complex may correspond to the outbreak of *S. dorsalis* that was declared in south-eastern Spain in 2017 (EPPO, 2017).

¹ 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

² 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.

³ <https://zenodo.org/record/2541541/preview/ribess-manual.pdf>

⁴ 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%2F%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

1.2. EU pest regulatory status

The only three *Scirtothrips* species regulated under Council Directive 2000/29/EC Annex II, Part A, Section I, are:

- *S. aurantii* Faure regulated in point 25 on Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than seeds
- *S. citri* (Moultex) regulated in point 27 on Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than seeds
- *S. dorsalis* Hood regulated in point 26 on Plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds.

1.3. Pest distribution

The distribution of *S. aurantii*, *S. citri*, *S. dorsalis* is illustrated in Figures 1, 2 and 3, respectively.

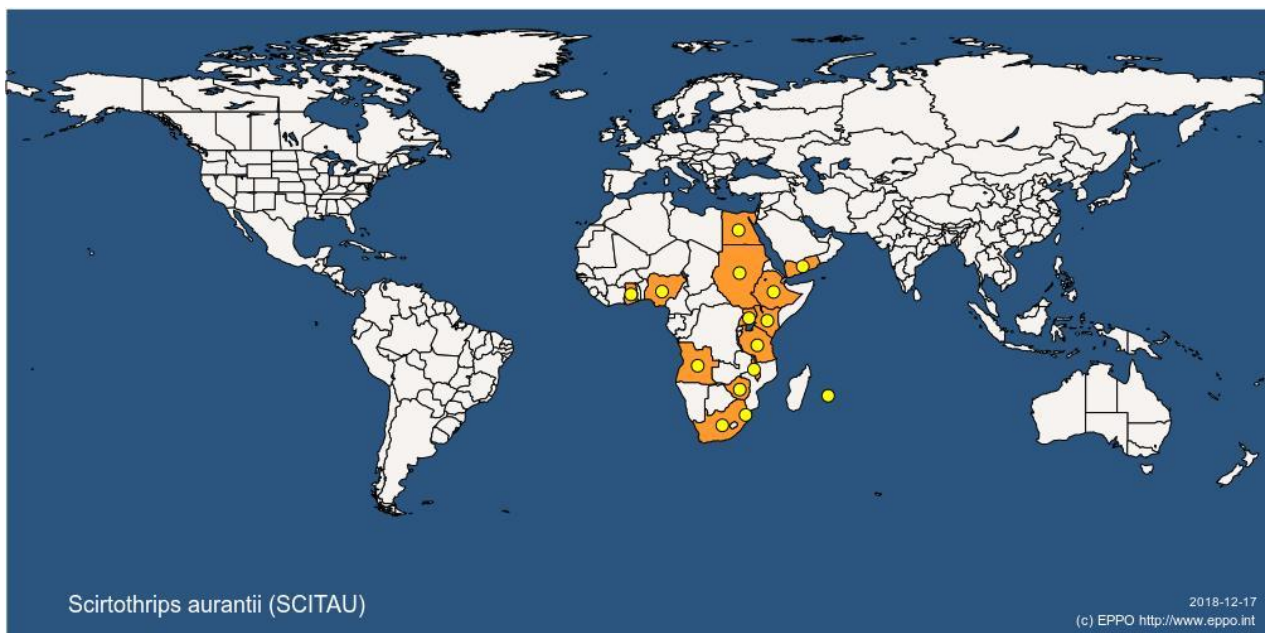


Figure 1: Global distribution of *Scirtothrips aurantii*. The pest status in countries or states is reported as present (yellow dots) (Source: EPPO global database, www.eppo.int)

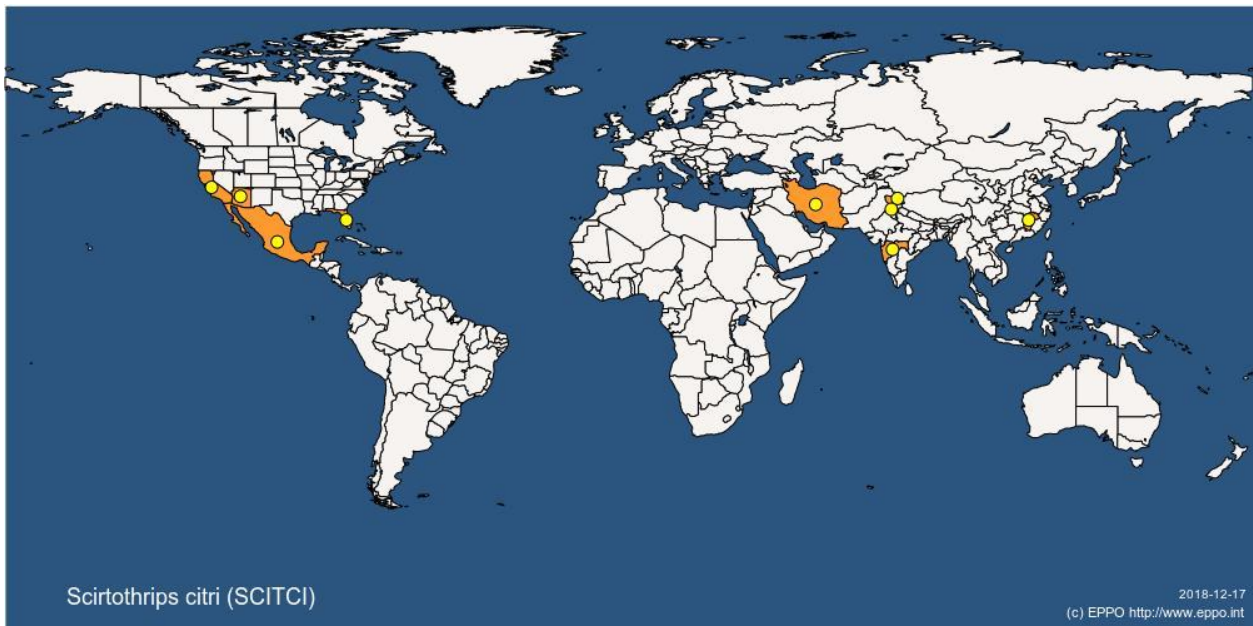


Figure 2: Global distribution of *Scirtothrips citri*. The pest status in countries or states is reported as present (yellow dots) or transient (purple dots) (Source: EPPO global database, www.eppo.int)

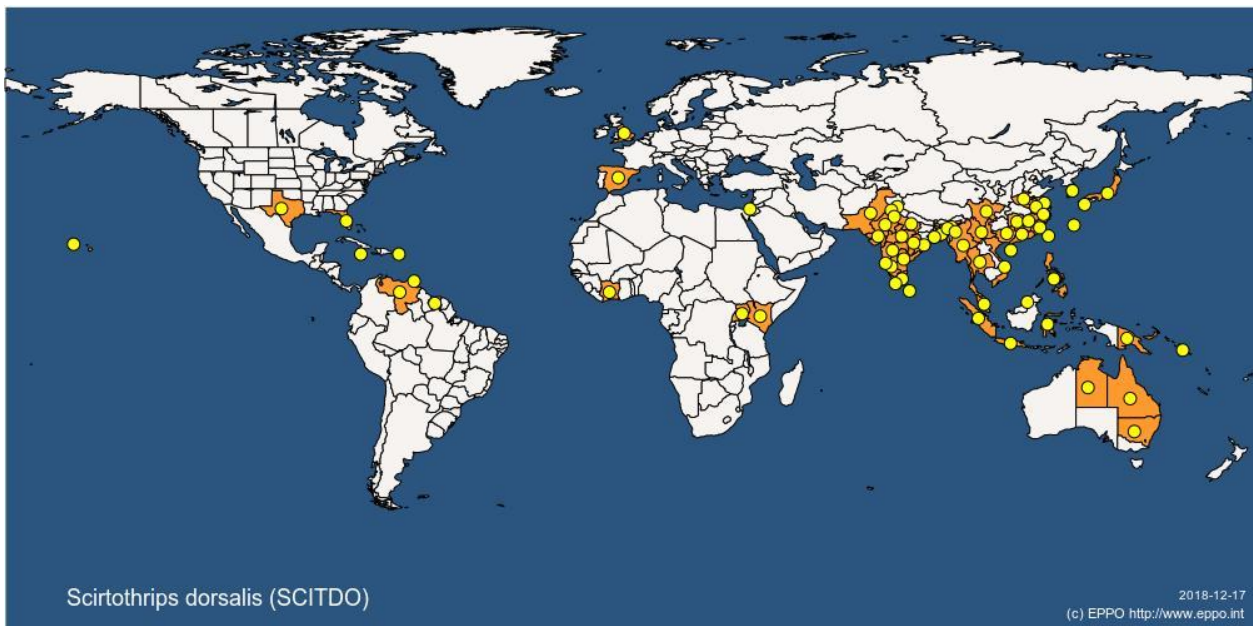


Figure 3: Global distribution of *Scirtothrips dorsalis*. The pest status in countries or states is reported as present (yellow dots) or transient (purple dots) (Source: EPPO global database, www.eppo.int)

1.4. Life cycle

In the citrus-growing areas in Spain where the pest occurs, the thrips is present throughout the year, and the best time to detect it is in the flowers when the citrus trees have their first flush, e.g. for *C. clementina* this is from March to June (Pascual-Ruiz et al., 2014); for other *Citrus* species such as *C. lemon* flushing can occur throughout the year (Figure 4).

For other hosts (e.g. capsicum and other annual crops), the life cycle continues as long as the crop is actively growing.

Moreover, if the pest is present it is more likely to be found on young fruit and therefore at fruit set stage. This timing is variable depending on the *Citrus* species and even on the cultivars used, but in general it is around May–June.

Scirtothrips spp. can be vectors for viruses. For example *S. dorsalis* is an efficient vector for some tospoviruses (e.g. groundnut bud necrosis virus (GBNV), groundnut chlorotic fan-spot virus (GCFSV) and groundnut yellow spot virus (GYSV)). There are also some hints that it may be a vector for tomato spotted wilt virus (TSWV) (Seal et al., 2010). A new strain of *S. dorsalis* infesting capsicum, mango and tea in Japan was identified as a potential vector for TSWV in capsicum (Toda et al., 2014; EFSA PLH Panel, 2012). Host plants for these viruses are not grown in the EU. *Scirtothrips dorsalis* is also mentioned as a vector of the chilli leaf curl virus (CLCV) and tobacco streak virus (TSV) (Prasada Rao et al., 2003).

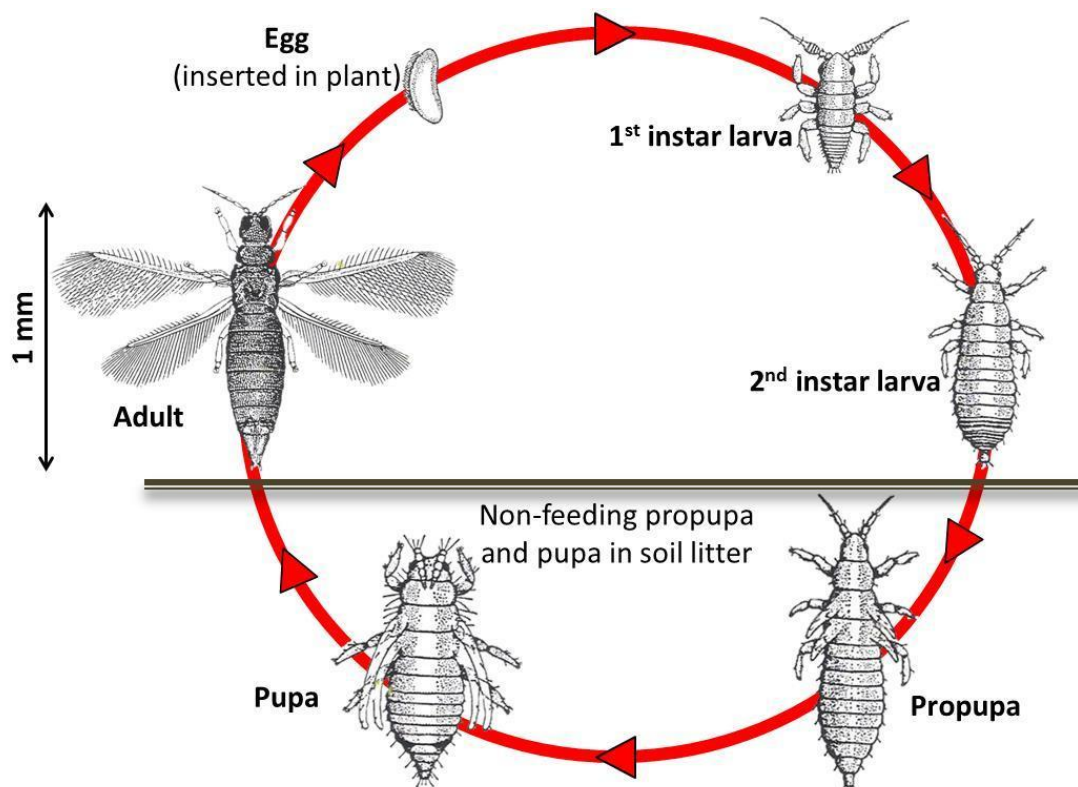


Figure 4: General life cycle of thrips belonging to the suborder Terebrantia (Thysanoptera). Best times for surveying depend on the host plant species and are indicated in the text. (Source: Josep Anton Jaques Miret)

1.5. Host range and main hosts

The three *Scirtothrips* species have a broad host plant range. Comprehensive lists can be found in EFSA PLH Panel (2018a,b, 2014). Table 1 lists the main (cultivated) hosts (according to the EPPO global database and EFSA PLH Panel 2018a,b, 2014) and those for which regulations are in place. *S. dorsalis* is by far the most polyphagous of the three species.

Scirtothrips citri has reached pest status only in highbush blueberries (*Vaccinium corymbosum*) in California and in citrus in south-western USA and Asia. The main hosts at risk in the EU are assumed to be citrus and blueberry plants (EFSA PLH Panel, 2018b).

Table 1: Main host plants for surveillance of the three *Scirtothrips* species. Main hosts according to the EPPO global database and EFSA PLH Panel (2018a,b, 2014), hosts regulated under Council Directive 2000/29/EC and intercepted host plants

Pest Species	Regulated host plants	Main hosts	Main hosts retained relevant for surveillance
<i>Scirtothrips aurantii</i>	<i>Citrus, Fortunella, Poncirus</i>	<i>Citrus sinensis</i>	<i>Citrus sinensis</i>
<i>S. citri</i>	<i>Citrus, Fortunella, Poncirus</i>	<i>Citrus limon, C. paradisi, C. reticulata and C. sinensis, Vaccinium corymbosum</i>	<i>Citrus limon, C. paradisi, C. reticulata and C. sinensis, Vaccinium corymbosum</i>
<i>S. dorsalis</i>	<i>Citrus, Fortunella, Poncirus</i>	<i>Camellia sinensis, Capsicum annuum</i> , Chu et al. (2006) also mention <i>Allium cepa, Gossypium, Solanum lycopersicum, Nicotiana tabacum</i> as primary hosts. Citrus (outbreaks on <i>C. lemon</i> under official control in Valencia (comarca de la 'Vega Baja') in Spain (EPPO, 2017))	<i>Capsicum annuum</i> <i>Citrus</i> spp. <i>Camellia sinensis</i>

1.6. Environmental suitability

Indoors:

Conditions in glasshouses are favourable for the establishment of *Scirtothrips* spp. on their respective hosts, in particular for *S. dorsalis* on *Capsicum annuum* and *Camellia sinensis*. As described in EFSA PLH Panel (2014), in the EU, outbreaks of *S. dorsalis* have been reported, i.e. in the UK in one glasshouse and on ornamental indoor plants in the Netherlands where it was reported and eradicated. Moreover, the thrips continues to be intercepted. The EFSA PLH Panel (2014, 2018a,b) refers to plants for planting or cut flowers as potential pathways for *Scirtothrips* spp. to enter the EU. However, since current EU legislation prohibits the import of citrus plants, and plants for planting should be imported in a dormant stage (no young foliage or fruit present) without any soil or growing medium attached, the likelihood of *Scirtothrips* spp. entering with such plants is limited. Interceptions of *S. aurantii* have occurred on *Eustoma grandiflorum* (Gentianaceae) cut flowers. The cut flower commercial trade of host plants may be the pathway with the highest risk for all the three species, considering the current legislation in place. For *S. citri*, *Vaccinium* plants for planting might be a pathway. For *S. dorsalis*, the pathways are quite diverse but might be focused – apart from cut flowers – on *C. annuum* packing houses.

Outdoors:

- *Scirtothrips aurantii* occurs outdoors and potential areas for establishment in the EU include areas where the climate allows citrus cultivation; in particular the Mediterranean Member States, i.e. Cyprus, Spain, France, Greece, Croatia, Italy, Malta and Portugal.
- *Scirtothrips citri*: the regions in North America where *S. citri* occurs are included within different Köppen–Geiger climatic classes (Peel et al., 2007) that are also found in Europe where citrus and *Vaccinium corymbosum* are grown, corresponding to the majority of the EU territory.
- *Scirtothrips dorsalis*: according to EFSA PLH Panel (2014), *S. dorsalis* is most likely to become established outdoors in the warmer (e.g. southern) regions of Europe (in particular at least one of the species in the complex is becoming established in south-eastern Spain). The climate in central and northern Europe is unfavourable for outdoor establishment.

1.7. Spread capacity

The potential for *Scirtothrips* spp. to spread naturally is relatively limited (EFSA PLH Panel, 2014, 2018a,b). Adults fly actively when the population density peaks in each period (Masui, 2007), but they do not move long distances between hosts and most likely disperse passively downwind. Long distance spread is mostly by trade in plants or plant parts that have actively growing leaf flush or young fruit. As eggs are inserted into the plant tissue, they are quite difficult to detect and very well protected against environmental factors, facilitating spread. Many host plants are frequently traded and widely distributed within the EU, thus these factors present a high risk for new entries followed by spread throughout the EU.

Parthenogenesis and the short generation time facilitate spread due to the high propagule pressure (Derksen, 2009).

The biological characteristics of *Scirtothrips* spp., the association with different pathways, and the passive dispersal by wind, lead to the conclusion that these pests have a high dispersal capacity.

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.

The 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.

The packing houses, nurseries, fresh fruit markets and processing warehouses where the host plants are handled are considered as locations in the production areas with a higher risk, particularly those facilities that process imported commodities originating from areas where the pest is present, i.e. cut flowers, *Vaccinium* plants for planting, *Camellia sinensis* plants for planting and *C. annuum*.

The epidemiological units (host plant cultivations such as citrus orchards, *Camellia sinensis* and *C. annuum* and *V. corymbosum* production sites) contiguous to the above-mentioned high-risk locations present the highest risk of being infested.

The plants for planting pathway deserves special attention and the survey could therefore consider the areas contiguous to nurseries (as the nurseries are already subject to the obligatory regular official examinations to be performed by the Member States under Council Directive 2000/29/EC Article 6 paragraph 5).

2. Detection and identification

2.1. Visual examination

All motile stages of the three *Scirtothrips* species feed on epidermal and occasionally on the palisade cells of young leaves, not mature ones, and on the tip of young fruit, particularly when concealed under the calyx. They could be carried on seedlings or cuttings with young growing leaf buds.

It is difficult to detect *Scirtothrips* spp. when it is only present in low numbers, therefore it might not be detected during transport. Eggs can easily be overlooked as they are inserted into leaves, and quiescent stages (including pupae) can be hidden in leaf axils, leaf curls, under the calyces of flowers and fruit, and in the soil (MacLeod and Collins, 2006). In the past, mature fruit were not considered to be a potential pathway; however, for *S. aurantii* and *S. citri*, though mature citrus fruit are usually thrips-free, damage can be most easily seen in the form of annular scars (rings around the fruit apex) and deformations. Moreover, *S. dorsalis* has been detected in consignments of harvested fruit (MacLeod and Collins, 2006; EFSA PLH Panel, 2014).

As mentioned above (Section 1.4), the young fruit is preferred by the thrips, and the probability of finding *Scirtothrips* spp. on harvested mature fruit is low. However, the damage caused by the insects on the young fruit is more visible and detectable on mature fruit that is no longer infested. These symptoms detected at a late stage could be used as an indicator for triggering further surveillance of the pest.

Therefore, trapping the *Scirtothrips* spp. in the packing houses and other high-risk locations (see Section 1.8) where mature fruit could show symptoms can be considered as a relevant component of the pest survey activity. Placing traps in the fields after tracing back to the production site where the symptomatic fruit come from could also be a target of survey efforts where the probability of finding the pests is higher.

2.1.1. Pest identification

Members of the genus *Scirtothrips* are readily distinguished from all other Thripidae by the following characteristics (for figures see EPPO, 2005):

- surface of pronotum covered with many closely spaced transverse striae
- abdominal tergites laterally with numerous parallel rows of tiny microtrichia
- sternites with marginal setae arising at the posterior margin
- metanotum with a median pair of setae arising near the anterior margin.

Identification of *Scirtothrips* spp. is based on male or female adults. They are pale and minute, and cleared specimens on microscopic slides are needed for identification. A magnification factor of between 100 and 600 is necessary.

Characteristics that allow identification of the genus *Scirtothrips* are shown in Tables 2 and 3 for adult insects.

Table 2: Key for the identification of adults of the genus *Scirtothrips* (adapted from EPPO, 2005)

Abdominal segment X usually conical, not tubular, serrated ovipositor present; wing surface with microtrichia	<i>Terebrantia</i>
Ovipositor downturned at the apex; abdominal sternite VIII not developed; sense cones on antennal segments III and IV emergent, each more than twice as long as wide (Mound & Marullo, 1996: p.41	<i>Thripidae</i>
Head and legs not strongly reticulately sculptured, abdominal tergites may be laterally sculptured; antennal segments III and IV usually with microtrichia; terminal antennal segments rarely elongate; meso- and/ or metathoracic furcae with or without spinula; forewing first vein not fused to costa	<i>Thripinae</i>
Abdominal tergites covered with numerous microtrichia Body often clear yellow 8 antennal segments 3 ocellar setae Posteromarginal pronotal setae B2 usually elongate Pronotum transversely striate, regular with dark internal apodeme	<i>Scirtothrips</i>

Table 3: Morphological characteristics of the adult stages of the three *Scirtothrips* species
(Source: CABI, 2018a,b,c; EPPO, 2005; Grové et al., 2000; EFSA PLH Panel, 2014, 2018a,b)

	<i>S. aurantii</i>	<i>S. citri</i> High levels of morphological intraspecific variation (Mound and Hoddle, 2016)	<i>S. dorsalis</i>
Adults	Winged, reddish-orange, less than 1 mm long	Winged, yellowish, < 0.9 mm long	Winged, almost white on emergence, turning yellowish subsequently; < 2 mm in length
Adult males	Comb of stout setae on the posterior margin of the hind femora. The ninth abdominal tergite of males bears a pair of long curved dark lateral processes (drepanae).	Ocellar setae III situated within ocellar triangle near posterior margin of first ocellus; median metanotal setae situated behind anterior margin; hind vein of the forewing with three setae, fringe cilia wavy; tergites and sternites completely pale, without dark antecostal ridges; tergites laterally with five setae on microtrichia fields; tergites VIII and IX with microtrichia medially; sternites with microtrichia only between postero-marginal setae b2 and b3.	Ocellar setae III situated between posterior margin ocelli; median metanotal setae situated behind anterior margin; forewing hind vein with two setae, posterior fringe cilia straight; tergites with dark patch medially; tergites and sternites with dark antecostal ridge; median tergites each with three setae on lateral microtrichial fields; tergites VIII and IX with microtrichia medially; sternites completely spanned with microtrichia.
Adult females	Median ocellar setae on the head arising close together and in line with the anterior margins of the posterior pair of ocelli; forewing posteromarginal cilia wavy not straight; median abdominal sternites fully covered with microtrichia; abdominal tergites and sternites with transverse anterior dark line; tergites with a dark median area.		



Figure 5: Adult females of *Scirtothrips citri*. (Source: Joseph Morse, University of California - Riverside, Bugwood.org)



Figure 6: *Scirtothrips dorsalis*. (Source: Andrew Derksen, USDA-APHIS, Bugwood.org)

2.1.2. Symptoms

According to EPPO (2005), symptoms are silverying of the leaf surface, linear thickening of the leaf lamina, and brown frass markings on the leaves and fruit. Grey to black markings on fruit often form a conspicuous ring of scarred tissue around the apex. Ultimately, fruit distortion and early senescence of leaves are observed. Examples of some symptoms are presented in Figures 7 and 8.

The larvae of *S. citri* are usually localised on young growing buds, young leaves, sepals and young fruit; therefore, these should be examined carefully. Due to the small size of the thrips, Berlese funnels should be used to find specimens. The funnels are used to extract the insects from the leaf litter with heat and dehydration, into a collecting bottle (EPPO, 2005).



Figure 7: Symptoms caused by *Scirtothrips aurantii* on *Citrus* spp. (Source: Didier Vincenot, SUAD/CIRAD-FLHOR, Bugwood.org)



Figure 8: Symptoms caused by *Scirtothrips dorsalis*. (Source: Florida Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Bugwood.org)

2.1.3. Traps

Detection of the *Scirtothrips* spp. by trapping can be done both in the packing houses and other high-risk locations (see Section 1.8) and in the production places (open fields and glasshouses).

According to Samways (1986), adult winged specimens of *S. aurantii* are highly attracted to fluorescent yellow and saturn yellow sticky traps that were used successfully in monitoring populations. Yellow was preferred to green, red, white and blue variants (Grové et al., 2000). The disadvantage of the saturn yellow sticky traps, however, is that they are more expensive and that they tend to fade in colour (Grout and Richards, 1990). Grout and Richards (1990) used inexpensive non-fluorescent yellow PVC card traps to obtain intervention thresholds.

Moreno et al. (1984) found that *S. citri* also prefer yellow – fluorescent yellow traps were significantly more effective than other available coloured polyvinyl rectangular traps. With regard to the shape of the traps, they found that triangular, elliptical, and rectangular shapes were preferred over circular and square traps.

Yellowish-green traps attracted more adult *S. dorsalis* than other coloured traps (Tsuchiya et al., 1995), and yellow sticky traps caught more adults of *S. dorsalis* than plastic cup (CC) traps (see also Chu et al., 2006). However, they also caught a large number of non-target insects as these traps are not selective. Blue D traps (modified traps from a commercially available dichlorvos strip package for use as a thrips trap (Chu et al. 2006)) in some cases but not consistently collected higher numbers of adults than CC traps. According to Sridhar and Onkara Naik (2015), the capture of *S. dorsalis* was significantly higher in blue traps than in yellow, pink and white traps. Sticky traps were found to be less labour-intensive, required less component assembly and less expertise in trap placement than CC traps (Chu et al., 2006).

For performing a statistically based sample size calculation the knowledge of the trap effectiveness is needed for estimating the sensitivity of the trapping method.

2.2. Laboratory testing (identification methods)

Scirtothrips specimens can be identified to genus level via morphological methods. The three *Scirtothrips* species can be diagnosed by multiplex PCR of the ITS1 and ITS2 regions of rRNA. This has the advantage that the identification is quick, specific, requires only basic laboratory skills, and can be done with the DNA from a single adult individual. Adults from which the DNA has been extracted can then be slide mounted and used for future reference (Rugman-Jones et al. 2006). However, Hoddle et al. (2008), using these molecular tools together with morphometric studies concluded that improved knowledge of taxonomic, including both molecular and morphological identities, together with biogeographic, host plant, and endosymbiont associations, as well as biological compatibility, is still necessary to help provide science-based answers for resolving the identification of *Scirtothrips* spp. when specimens belonging to this genus are detected at ports of entry. In their study, for instance, they concluded that *S. dorsalis* included a complex with at least three separable groups identifiable at the molecular level, but indistinguishable morphologically.

For performing a statistically based sample size calculation, the specificity of the identification method is needed and therefore the analytical specificity of the molecular method for the confirmation of the insect species should be known.

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 4 shows an example of these definitions.

Table 4: Examples of definitions of the target population, epidemiological unit and inspection unit for *Scirtothrips* spp. surveillance

	<i>S. aurantii</i>	<i>S. citri</i>	<i>S. dorsalis</i>	Unit
Target population	Sweet orange (<i>Citrus sinensis</i>) orchards including backyards/gardens in each Member State	<i>Citrus limon</i> , <i>C. paradisi</i> , <i>C. reticulata</i> , <i>C. sinensis</i> orchards including backyards/gardens, and <i>V. corymbosum</i> growing areas in each Member State	<i>Citrus</i> spp. orchards and <i>Capsicum annuum</i> growing areas (outdoors and indoors) in each Member State	Total number of half hectares
Epidemiological units	Orchards, backyards/gardens, with host plants	Orchards, backyards/gardens, and fields with host plants	Glasshouses and fields with <i>Capsicum annuum</i>	Half hectare*
Inspection units	Young plants, fruit (young and mature) and traps			Number of plants, young fruit, mature fruit, and traps

*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 risk-based statistically sound surveillance are presented in a separate document and describe the process of the survey design step by step and include:

- 1/ the choice of the type of survey to develop depending on the objectives of the survey
- 2/ a 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.

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Glossary

TERM	DEFINITION*
Component (of a survey)	<p>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.</p> <p>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).</p>
Confidence	<p>Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).</p>
Design prevalence	<p>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)</p>
Diagnostic protocols	<p>Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).</p>
Epidemiological unit	<p>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).</p>
Expected prevalence	<p>In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.</p>
Identification	<p>Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).</p>
Inspection	<p>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).</p>
Inspection unit	<p>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).</p>
Inspector	<p>Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2018).</p>
Method sensitivity	<p>The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010).</p> <p>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</p>

	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 the 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 on 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: <ul style="list-style-type: none"> • 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 (D _{Sp}) is the probability that a truly negative epidemiological unit will give a negative result 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).
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