PEST SURVEY CARD

APPROVED: 10 July 2020

doi:10.2903/sp.efsa.2020.EN-1904

Pest survey card on tomato leaf curl New Delhi virus

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Abstract

This pest survey card was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137) at the request of the European Commission. Its purpose is to guide the Member States in preparing data and information for tomato leaf curl New Delhi virus (ToLCNDV) surveys. These are required to design statistically sound and risk-based pest surveys, in line with current international standards. Tomato leaf curl New Delhi virus is a clearly defined taxonomic entity. Although the main potential pathway of entry and spread of this virus via plants for planting of host plants is subject to prohibitions and specific measures designed to prevent entry and movement, the widespread occurrence and polyphagous nature of its vector *Bemisia tabaci* constitutes a risk for introduction and further spread of ToLCNDV given its persistent transmission biology. The virus is currently present in parts of the EU, and surveys in Member States can have several objectives, e.g. substantiation of disease freedom for countries or pest-free areas, or disease monitoring. The major hosts of ToLCNDV belong to the Solanaceae and Cucurbitaceae families, and detection surveys should thus target cultivated species belonging to these families. ToLCNDV is expected to be able to become established in most or all areas of the EU where *B. tabaci* is able to become established – outdoors or under protected cultivation – and where Solanaceae and Cucurbitaceae crops are grown. Surveillance should target symptomatic host plants in a fully grown crop. Because of the severity of its symptoms, surveillance of ToLCNDV can rely on visual examination. However, because its symptoms are similar or identical to those of other begomoviruses, its identification requires the use of validated laboratory assays.

Keywords: *Bemisia tabaci*, Cucurbitaceae, plant pest, risk-based surveillance, Solanaceae, survey, ToLCNDV

Requestor: European Commission

Question number: EFSA-Q-2019-00289

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Acknowledgements: EFSA wishes to acknowledge the Netherlands Food and Consumer Product Safety Authority (NVWA) for the support provided to this scientific output in the context of grant GP/EFSA/ALPHA/2017/02. EFSA also wishes to thank Stephan Winter for reviewing this document. EFSA also wishes to thank especially Giulia Mattion (trainee in the Animal and Plant Health Unit – Plant Health Team) for her support to the finalisation and publication of this survey card.

Suggested citation: EFSA (European Food Safety Authority), van Gemert J, Schenk M, Candresse T, Bottex B, Delbianco A and Vos S, 2020. Pest survey card on tomato leaf curl New Delhi virus. EFSA supporting publication 2020:EN-1904. 26 pp. doi:10.2903/sp.efsa.2020.EN-1904

ISSN: 2397-8325

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Introduction

The information presented in this pest survey card was summarised from various scientific publications and other documents, including the EFSA pest categorisation of tomato leaf curl New Delhi virus (EFSA PLH Panel, 2020), and the EFSA risk assessment on *Bemisia tabaci* and the viruses it transmits (EFSA PLH Panel, 2013).

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for tomato leaf curl New Delhi virus (ToLCNDV) in EU Member States (MSs) following the methodology in 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 standards (ISPMs) and guidelines for surveillance (FAO, 2016a,b, 2018). 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 tomato leaf curl New Delhi virus[1].
ii. General documents:
   a. The general survey guidelines
   b. The RiBESS+ manual[2]
   c. The statistical tools RiBESS+ and SAMPELATOR[3].

1. The pest and its biology

1.1. Taxonomy

Scientific name: Tomato leaf curl New Delhi virus

Realm: Riboviria, **Family**: Geminiviridae, **Genus**: *Begomovirus*, **Species**: Tomato leaf curl New Delhi virus (ToLCNDV)

Synonym(s): Bitter gourd yellow vein virus

EPPO Code: TOLCND

Common name: tomato leaf curl New Delhi virus

Tomato leaf curl New Delhi virus (ToLCNDV) is a begomovirus causing leaf curl disease in tomato (tomato leaf curl disease) and diseases in a number of other crops, most significantly in cucurbits (cucumber, zucchini, melon) and solanaceous plants (chilli, eggplant, sweet pepper) but also in cotton. The virus is uniquely transmitted by *Bemisia tabaci* whiteflies. It has a bipartite genome that consists of two genome components: DNA-A and DNA-B (Padidam et al., 1995). Outside Europe, ToLCNDV is often associated with alpha- and betasatellites (Anwar, 2017; Jyothsna et al., 2013; Singh et al., 2016). To discriminate virus species, complete DNA-A genome sequences are considered and distinct virus species have <90% pairwise nucleotide sequence identities with other members of the genus (Brown et al., 2015). Most ToLCNDV DNA-A sequences have more than 90% sequence identity (EFSA PLH Panel, 2020) and despite uncertainties about the ambiguous status of a few virus isolates with diverging genome sequences, ToLCNDV is a well-established virus species with a clear taxonomic status.

The genomes of ToLCNDV isolates are most diverse in Asia (Moriones et al., 2017) and as shown for many other geminiviruses, the genomes are shaped by recombination events comprising sequences of

[1] The Pest Survey Card will be updated in the form of Story Map that will be available in the Plant Pests Story Maps Gallery available online: https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://shiny-efsa.openanalytics.eu/

other begomoviruses or sequences of unknown origin (Lefeuvre and Moriones, 2015). In contrast to the diversity of ToLCNDV genomes reported from India and Pakistan, the ToLCNDV isolates that are currently found in the EU are genetically uniform (99% nucleotide identity) and form a distinct cluster, well separated from isolates occurring outside the EU (Juárez et al., 2019; Panno et al., 2019). Because these isolates are <94% identical to world ToLCNDV isolates, these EU isolates comprise a specific strain, ToLCNDV-ES (Fortes et al., 2016; Ruiz et al., 2017).

Conclusions on taxonomy

Tomato leaf curl New Delhi virus (ToLCNDV) is a clearly defined taxonomic entity.

1.2. EU pest regulatory status

Tomato leaf curl New Delhi virus and non-European populations of its vector *Bemisia tabaci* are Union quarantine pests listed in Annex II Parts B and A, respectively, of Commission Implementing Regulation (EU) 2019/2072[4].

In addition, specific import requirements and internal movement requirements for plants for planting of Cucurbitaceae and Solanaceae, other than seeds, in relation to ToLCNDV are detailed in Annexes VI, VII and VIII, respectively, of Commission Implementing Regulation (EU) 2019/2072.

Jasminum multiflorum, one of the ornamental host plants for ToLCNDV is listed in Annex I (as *Jasminum* L.) of Commission Implementing Regulation (EU) 2018/2019[5], as a high-risk plant. As such, its introduction into EU territory is prohibited, pending risk assessment.

The general requirements for surveys of quarantine organisms in the EU are laid down in Regulation (EU) 2016/2031[6].

Overview of the EU regulatory status

Tomato leaf curl New Delhi virus and non-European populations of its vector *Bemisia tabaci* are both Union quarantine pests. Host plants for ToLCNDV are subject to prohibitions and specific measures are in place to prevent entry and movement of the pest via plants for planting, which is the main potential pathway of entry and spread.

1.3. Pest distribution

Tomato leaf curl New Delhi virus was first reported from India in 1995 (initially as ToLCV-India). Following its discovery, other Asian countries, such as Pakistan and Thailand, soon reported occurrences of the virus (Zaidi et al., 2017), which is now known to be present in several other Asian countries (Bangladesh, Indonesia, Iran, Philippines, Sri Lanka and Taiwan) (Figure 1). In Africa, the virus was first detected in 2015 in Tunisia and is now is present in northern Africa (Algeria, Morocco and Tunisia) as well as in the Seychelles. *Bemisia tabaci* – the insect vector of ToLCNDV – occurs in almost all countries with a suitable climate (tropics and subtropical regions, Mediterranean region). The insect can also be present in greenhouses in temperate regions, amounting to a widespread global distribution.

[4] Commission Implementing Regulation (EU) 2019/2072 of 28 November 2019 establishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019. OJ L 319, 10.12.2019, p. 1–279.

[5] Commission Implementing Regulation (EU) 2018/2019 of 18 December 2018 establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which phytosanitary certificates are not required for introduction into the Union, within the meaning of Article 73 of that Regulation. OJ L 323, 19.12.2018, p. 10–15.

[6] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L 317 23.11.2016, p. 4.

In the EU, ToLCNDV is currently known to be present in Estonia, Greece, Italy, Portugal and Spain (EPPO, online). Firstly, the virus was detected in Spain in 2012 on cucurbit crops (Juárez et al., 2014, 2019; Fortes et al., 2016) and in tomato (Ruiz et al., 2015), followed by detections in Italy (Panno et al., 2016), Greece (Orfanidou et al., 2019), Portugal – including the Azores – (EPPO, 2019a) and Estonia (under protected cultivation) (EPPO, 2019b). The virus was also found on Gran Canaria (the Canary Islands) in 2018 (Espino de Paz et al. 2019). In Italy and Greece, ToLCNDV was detected in zucchini and the virus isolates identified in Italy were closely related to those detected in Spain (Parrella et al., 2018). It has also recently been detected in eggplants (*Solanum melongena*) in Campania, Italy (Parrella et al., 2020).

ToLCNDV has a wide host range as is evident by the many diseases associated with it in crops grown in Asia. However, in Europe ToLCNDV is mostly found in cucurbit crops.

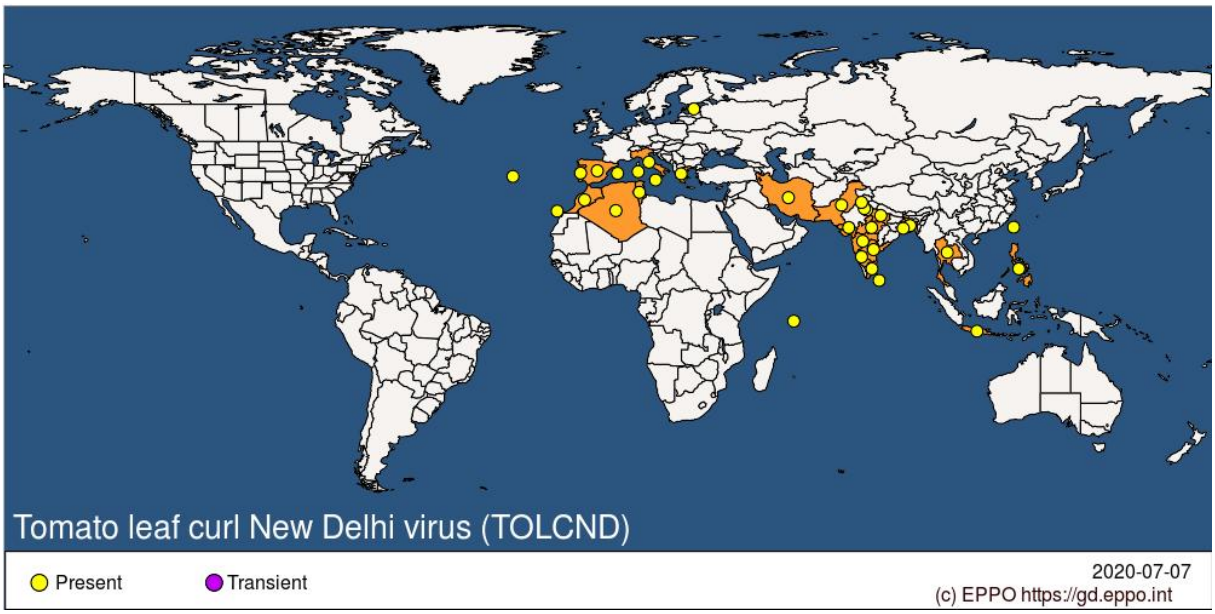

Figure 1: Global distribution of tomato leaf curl New Delhi virus (ToLCNDV) (Source: EPPO Global Database, htpps://gd.eppo.int)

Conclusion on pest distribution

Tomato leaf curl New Delhi virus is currently present in parts of the EU where the climate is suitable for its vector *Bemisia tabaci*. The finding of ToLCNDV in Estonia shows that virus outbreaks can occur in plants grown under protected cultivation.

1.4. Life cycle

Tomato leaf curl New Delhi virus is a virus species transmitted by the whitefly *Bemisia tabaci* (order Hemiptera, family Aleyrodidae). This insect is a very serious pest and a major threat to agricultural and horticultural crops grown worldwide in open fields and under protected cultivation (EFSA PLH Panel, 2013). *Bemisia tabaci* is a cryptic species complex composed of several morphologically indistinguishable species (Xu et al., 2010). The *B. tabaci* species MEAM1 (Middle East-Asia Minor 1) and MED (Mediterranean) occurring in Mediterranean regions of Europe and around the world are highly polyphagous and invasive and very efficient at transmitting begomoviruses and other viruses (EFSA PLH Panel, 2013).

Bemisia tabaci acquires virus particles with the phloem sap of an infected host plant (Rosen et al., 2015) (Figure 2). In the insect vector, the virus passes through the filter chamber and midgut and translocates to the haemolymph before entering the primary salivary glands and stylet and being introduced to new host plants (Hogenhout et al., 2008). Begomoviruses are transmitted in a persistent circulative manner (Moriones et al., 2017) and once acquired, the whitefly vector remains viruliferous

and able to transmit the virus throughout its lifespan (Czosnek and Ghanim, 2012). Even in the temporary absence of host plants, the viruliferous whiteflies remain competent for transmission once susceptible host plants become available. Transmission efficiency decreases with the age of the vector and is negatively correlated with the amount of virus detectable in the vector (Rosen et al., 2015). The transmission efficiency of begomoviruses can vary between different members of the *B. tabaci* cryptic species complex (Rosen et al., 2015) and even between different *B. tabaci* populations (Kollenberg et al., 2014). The virus cannot multiply in *B. tabaci* and only adult insects, not nymphs, can acquire the virus. Once injected into a new host plant, viral replication starts. Under experimental conditions, whitefly transmission of ToLCNDV resulted in symptoms in tomato and zucchini plants after 8–15 days (Ruiz et al., 2017).

While the seed transmissibility of begomoviruses is still debated (Pérez-Padilla et al., 2020), seed transmission of ToLCNDV has been reported for a distinct isolate found in chayote (*Sechium edule*) (Sangeetha et al., 2018) and was recently reported in zucchini from Italy (Kil et al., 2020). As reported by the EFSA PLH Panel (2020), 'ToLCNDV infections of seedlings may arise from contaminated seeds and while this is not very likely in commercial production processes, this mode of transmission exists for ToLCNDV.'

Insect transmission is the primary mode of ToLCNDV spread; thus the occurrence of the virus is tightly linked to its insect vector, as for all begomoviruses (Ruiz et al., 2017). Because of its broad host range, weed and wild hosts may play an important role in the ecology and epidemiology of the virus, serving as virus reservoirs for overwintering or during host-free seasons (Juárez et al., 2019).

Figure 2: ToLCNDV infections begin with *Bemisia tabaci* finding host plants suitable for the virus. The virus is ingested from an infected plant by sap sucking, then passes through the insect to be injected for transmission into a healthy plant. Once acquired, a viruliferous *B. tabaci* is transmission-competent throughout its lifespan

Conclusion on life cycle

Tomato leaf curl New Delhi virus is transmitted in a persistent circulative manner by the insect vector *Bemisia tabaci*. Once the virus is injected into a new host plant, viral replication starts. Surveillance should target symptomatic host plants (e.g. reduced plant and fruit size) in a fully grown crop.

1.5. Host range and main hosts

Tomato leaf curl New Delhi virus has a wide natural host range that includes more than 58 different plant species (Anwar et al., 2020; Ashwathappa et al., 2020; Fortes et al., 2016; Juárez et al., 2019; Pant et al., 2018; Pratap et al., 2011; Ruiz et al., 2017; Sangeetha et al., 2018; Venkataravanappa et

al., 2018, 2019; Anwar et al., 2020; Ashwathappa et al., 2020; Zaidi et al., 2017). However, the actual number of hosts is likely to be larger. Natural hosts mainly belong to the Solanaceae and Cucurbitaceae families and include both economically important crops and wild plant species. The species and the number of hosts that ToLCNDV can infect also depends on the preferences of the local *Bemisia tabaci* population (Zaidi et al., 2017; Moriones et al., 2017). The most polyphagous MEAM1 and MED species occur in the EU and MED was identified as being associated with ToLCNDV (Janssen et al., 2017). Wild plants can serve as reservoirs for the virus and may also be visited by *B. tabaci* (Gill and Brown, 2009), thus enabling transfer from weed plants to cultivated hosts and back.

On the Indian sub-continent, ToLCNDV was initially reported to cause significant damage in tomato (*Solanum lycopersicum*) (Padidam et al., 1995) and later reported from a wide range of hosts that includes crops species that are grown on a large scale in the EU, such as melon (*Cucumis melo*), zucchini (*Cucurbita pepo)*, cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita maxima*), pepper (*Capsicum annuum*), carrot (*Daucus carota*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*) and soybean (*Glycine max*) (Moriones et al., 2017). In addition, various ornamental plants (EFSA PLH Panel, 2020) including species that are grown in the EU (e.g. *Dahlia pinnata*) and species that are imported as cut flowers (e.g. *Chrysanthemum indicum*) are susceptible to the virus. It also infects crops that are mostly grown in tropical regions; the full list is provided in EFSA PLH Panel (2020).

In Europe, all plant infections reported so far were caused by the ToLCNDV-ES strain. In the Mediterranean region, ToLCNDV was only sporadically reported in tomato, sweet pepper and eggplant (Fortes et al., 2016; Zaidi et al., 2017; Juárez et al. 2019; Luigi et al., 2020, Parrella et al., 2020), but it was mostly found in Cucurbitaceae: melon, zucchini, cucumber and pumpkin, but not in watermelon (Parrella et al., 2018, 2020, Orfanidou et al., 2019). Thus, the ToLCNDV-ES strain appears to be adapted to Cucurbitaceae plants as the preferred hosts.

The selection of host plants for surveillance is linked to the objective of the survey. Detection surveys should target both cucurbitaceous and solanaceous crops. When the main objective is to detect or delimit outbreaks of the ToLCNDV-ES strain that is currently circulating in the Mediterranean region, cucurbit crops should be the main target of the survey. Alternatively, when the main objective is to detect ToLCNDV strains that are not present in the EU, tomato seems the best candidate crop.

Conclusion on host range and main hosts

The major hosts of ToLCNDV belong to the Solanaceae and Cucurbitaceae families, and detection surveys should thus target cultivated species belonging to these families. The choice of the plant species depends on the objective of the survey, e.g. inclusion of wild species in delimiting surveys.

1.6. Environmental suitability

The main susceptible host crops of ToLCNDV are grown either in open fields (in southern EU countries) or under protected cultivation. Given that ToLCNDV is dependent on *Bemisia tabaci* for spread, its distribution is intrinsically linked to the areas of *B. tabaci* establishment (EFSA PLH Panel, 2014). In southern EU countries, climatic conditions are suitable for *B. tabaci* establishment in open fields, whereas *B. tabaci* is able to survive outdoors for only a short period of the year in northern Europe. It is therefore considered unlikely that ToLCNDV will become established and spread for extended periods outdoors in northern parts of the EU. However, conditions in greenhouses do allow for the year-round presence of *B. tabaci* and thus the virus could still become established and spread indoors in northern EU countries.

Conclusion on environmental suitability

Tomato leaf curl New Delhi virus is expected to become established in most or all areas in the EU where *Bemisia tabaci* is able to become established – outdoors or under protected cultivation – and where suitable wild and cultivated host plants are present.

1.7. Spread capacity

Natural spread

Natural spread of ToLCNDV occurs via *Bemisia tabaci*. The spread of the virus to new host plants is thus linked to the dispersion capacity of *B. tabaci*. According to the EFSA PLH Panel (2013): 'Only *B. tabaci* adults can have directional and active flights. Whiteflies seldom need to fly more than a few centimetres to a few metres to find suitable host plants. They may cover distances of a few kilometres. *B. tabaci* adults can spread over longer distances by passive transport with wind. Passive long-distance dispersal by wind may result in spread of whitefly to uninfested fields and to wild host plants, to other areas in the same country or to neighbouring countries.'

Large adult whitefly populations will favour efficient spread of the virus. Once introduced into an area, it will be very difficult to eradicate the virus should it be transferred to weeds or wild hosts.

Human-assisted spread

The EFSA PLH Panel (2020) stated that long-distance spread of ToLCNDV occurs via the transport of viruliferous *B. tabaci* and the trade of infected plants for planting, of parts of infected plants (e.g. cut flowers) and possibly of seeds (EFSA PLH Panel, 2020). The risk of entry via plants for planting and seeds is in part mitigated by the specific requirements that are laid down at import in the EU. However, there are currently no requirements for the internal movement of plants for planting of ornamental host plants. Long-distance spread may also occur by viruliferous vector insects carried with or infesting plant material (e.g. vegetables, cut flowers or ornamental plants for planting) of hosts and non-host plants.

As stated in the recent EFSA pest categorisation of ToLCNDV (EFSA PLH Panel, 2020), 'ToLCNDV infections of seedlings may arise from contaminated seeds and while this is not very likely in commercial production processes, this mode of transmission exists for ToLCNDV.' Seeds therefore constitute a possible spread pathway.

ToLCNDV infections can be propagated with scions and cuttings used for propagation or grafting. While mechanical transmission is possible under experimental conditions, it is likely ineffective and unlikely to play a relevant role during crop management practices (e.g. plant injuries and pruning) in a field situation.

Conclusion on spread capacity

Natural spread of ToLCNDV is determined by the presence and spread capacity of *Bemisia tabaci*. Long-distance spread of ToLCNDV occurs via the transport of infected plants for planting, infected plant material carrying viruliferous vector insects and possibly seeds.

1.8. Risk factor identification

Identification of risk factors and their relative risk estimation is essential for performing risk-based surveys. A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest in the area of interest. The risk factors that are relevant for surveillance need to be characterised by their relative risk (should have more than one level of risk for the target population) and the proportion of the overall target population to which they apply. The identification of risk factors needs to be tailored to the situation in each Member State. This section presents two examples of a risk factor for ToLCNDV and is not necessarily exhaustive (Table 1).

To identify risk areas, it is necessary to identify the activities that could contribute to the introduction or spread of ToLCNDV. These activities should then be connected to specific locations. Risk areas can be defined around these locations; their size depends on the spread capacity of the target pest and the availability of host plants around these locations.

Example 1: Host plants

ToLCNDV has a broad host range which includes several agricultural crops. So far, the ToLCNDV-ES strain currently present in Europe is adapted to Cucurbitaceae crops and thus cultivated cucurbit species (melon, zucchini, cucumber and pumpkin) are the main hosts, compared with Solanaceae crops (tomato, eggplant and pepper). Thus, the ToLCNDV-ES strain poses a higher risk to cucurbits than to other solanaceous crops. However, if other ToLCNDV strains were to be introduced to Europe, all other crop hosts of the virus would be at risk and therefore surveys would need to include those plant species. The suitability of integrating this risk factor depends on the specific survey objective(s) (see Section 1.5).

Example 2: *Bemisia tabaci* insects

ToLCNDV is vectored by *Bemisia tabaci*. The most efficient insect species transmitting the virus (MEAM1 and MED) are present in open fields in southern Europe. Therefore, trade and movement within the EU of plant commodities from regions and areas where the virus and its vector are present are considered risk activities. In addition, even though the pathways of entry for the virus and *B. tabaci* into the EU are tightly regulated, around 5,000 interceptions of *B. tabaci* were reported in the period 2000–2020 (EUROPHYT, online), suggesting that the measures in place might not be sufficient to prevent introduction of ToLCNDV with viruliferous *B. tabaci* from third countries. The entry of those insects poses a risk of introducing new ToLCNDV isolates.

Table 1: Examples of a risk activity and corresponding risk locations relevant for the surveillance of ToLCNDV

Risk activity	Risk locations	Risk areas
Cultivation of susceptible crops (Cucurbitaceae)	Open fields or greenhouses where Cucurbitaceae crops are grown and where *Bemisia tabaci* is present	Areas surrounding risk locations, within the spread capacity of the vector and where host plants are present
Trade and movement within the EU of plant commodities infested by the vector from areas where the virus is present	Nurseries and garden centres trading in plant commodities of horticultural crops	Areas surrounding risk locations, within the spread capacity of the vector and where host plants are present
Import of plant commodities from third countries where ToLCNDV and *B. tabaci* are present	Entry points and nurseries	Areas surrounding risk locations, within the spread capacity of the vector and where host plants are present

2. Detection, sampling and identification

2.1. Detection

2.1.1. Visual examination

The goal of the visual examination is to detect the symptoms caused by ToLCNDV. Detection surveys should focus on both cucurbits and tomato. If there is an outbreak in a new area, the identification of the ToLCNDV strain would help to target the survey to the specific host plants. When the objective is to detect or delimit an outbreak of the ToLCNDV-ES strain, cucurbit crops should be the main focus of the survey.

In cucurbits (Figures 3 and 4), ToLCNDV causes pronounced leaf curl-like disease symptoms such as short internodes, curling, vein swelling on young leaves, and severe yellow mosaic on young leaves.

Besides leaf distortion and mosaic, the fruit has a rough skin and reduced size (Juárez et al., 2014; Panno et al., 2016).

Figure 3: Symptoms of ToLCNDV in zucchini (top) and cucumber (bottom) 18 days after whitefly transmission. The virus infecting the zucchini was isolated from a tomato plant (DSM PV1111), the virus infecting the cucumber was isolated from a cucumber plant in Murcia, Spain (DSMZ PV 1009). Leaf curling, blistering and leaf distortion symptoms are found on the young uppermost leaves, while symptoms on older leaves are less pronounced (Source: Stephan Winter, DSMZ Plant Virus Department, Germany)

Figure 4: Symptoms of ToLCNDV in a field-grown zucchini plant. Leaf curling, blistering and leaf distortion of the young uppermost leaves are typical for begomovirus infections. Chlorosis, vein banding and upwards rolling of older leaves and plant stunting, reduced flower setting and fruit dropping mark a severe ToLCNDV infection incurred in the very early stages of plant development (Source: Raffaele Giurato, EPPO Global Database, htpps://gd.eppo.int)

In tomato, ToLCNDV causes tomato leaf curl disease (Figure 5). Symptoms include leaf curling, crinkling and distortion of leaves, mottling and veinal yellowing. Plant infections at an early development stage before the first flowering are most severe, resulting in stunted plants, reduced flowering, termination of flowering, flower dropping and sterile flowers (Chakraborty, 2009). Symptoms in sweet pepper, habanero pepper and eggplant include chlorotic mottle, vein clearing and upward and downward curling of young leaves (Ruiz et al., 2017), similar to those that could be caused by a range of begomoviruses.

Figure 5: Symptoms of tomato leaf curl disease caused by ToLCNDV in tomato. The virus (DSMZ PV 1111) was isolated from a tomato plant from Almeria, Spain. Chlorotic spots and mottling symptoms can be observed on older leaves, and leaf curling, crinkle and upwards rolling on the young uppermost leaves. Stunting of the entire plant. (Source: Stephan Winter, DSMZ Plant Virus Department, Germany)

Risk of misidentification

ToLCNDV is one of the begomoviruses that causes tomato leaf curl disease. However, there are more than 50 distinct begomovirus species associated with this disease worldwide (see EFSA PLH Panel (2013), Appendix B), thus the viruses cannot be discriminated from the symptoms they cause (Figures 6 and 7).

Leaf curl symptoms on young freshly unfolding leaves and stunted crinkled apical portions of the plant are characteristic for many begomovirus infections of plants, particularly tomato. However, while symptoms are indicative of the presence of begomoviruses, virus identification requires laboratory testing. Given the widespread presence of the tomato yellow leaf curl virus (TYLCV) in the Mediterranean region (EFSA PLH Panel, 2014), there is a high probability that symptoms in tomato are caused by TYLCV.

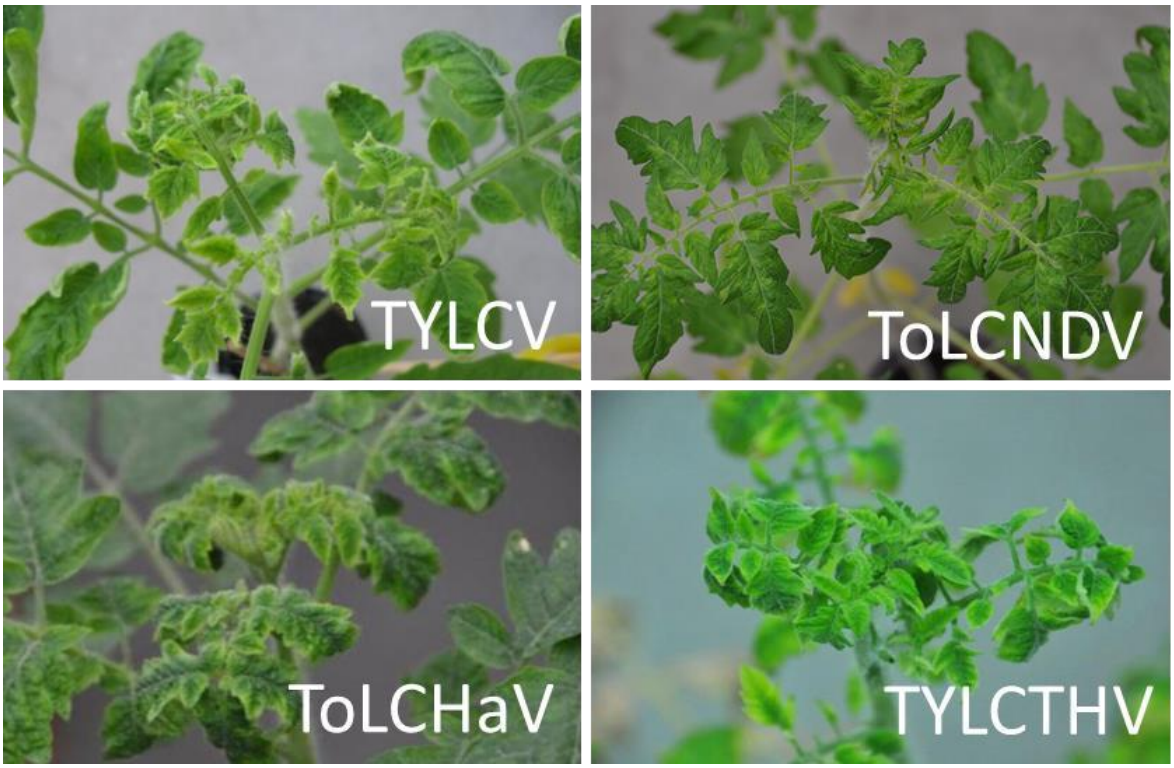

Figure 6: Symptoms of leaf curl diseases in tomato caused by four distinct begomovirus species (i.e. tomato yellow leaf curl virus (TYLCV) (top left), tomato leaf curl Hanoi virus (ToLCHaV) (bottom left) and tomato yellow leaf curl Thailand virus (TYLCTHV) (bottom right)) infecting the tomato variety Linda. Symptoms of Tomato leaf curl New Delhi virus (ToLCNDV) (top right) are highly similar to those caused by other begomoviruses. TYLCV is present in the EU. (Source: Stephan Winter, DSMZ Plant Virus Department, Germany)

There are no cucurbit-infecting begomoviruses in the EU. However, viruses of cucurbits often cause severe symptoms and mixed infections are common. Thus, begomoviruses and ToLCNDV require laboratory testing for identification.

Figure 7: Symptoms of begomoviruses infecting cucurbits. Blistering and upwards rolling of leaves are indicative for begomovirus infections, but are not informative for identification. Symptoms of ToLCNDV (left) can resemble other begomoviruses or even viruses from different genera, including those of cucumber vein yellowing virus (CVYV) (right) (Source: Stephan Winter, DSMZ Plant Virus Department, Germany)

Conclusions for detection methods

Tomato leaf curl New Delhi virus causes clear symptoms in infected plants which are more severe when plants are infected in the early developmental stages. Surveillance of ToLCNDV can thus rely on visual examination, while virus identification requires laboratory testing.

2.2. Sampling

Plants infected with ToLCNDV generally show symptoms that are most prominent on the youngest freshly unfolded leaves and apical plant parts. For begomovirus testing, these young leaves are to be selected for laboratory analysis, since older leaves are less reliable for testing.

Conclusions for sampling

Young leaves from the uppermost plant parts should be sampled for laboratory testing.

2.3. Identification

2.3.1. Laboratory testing

Following visual inspection and the selection of samples from symptomatic plants, ToLCNDV needs to be confirmed through laboratory analysis. It can be identified either through PCR or through ELISA. ELISA provides a good basis for virus detection and confirmation in virus surveys (Juárez et al., 2019), while PCR-based tests overall have higher sensitivity and provide a better discrimination between TYLCV and ToLCNDV in tomato (Figàs et al., 2017). PCR has been demonstrated to be more (usually 100 times) sensitive for plant virus detection than ELISA (Alfaro-Fernández et al., 2009, 2016). However, the unit costs and scale of testing may limit the use of the tests for large-scale surveys. Thus, ELISA can be used to detect and confirm ToLCNDV in cucurbits and tomato followed by discriminatory PCR for identification of ToLCNDV.

For the detection of ToLCNDV by PCR, Sifres Cuerda et al. (2018) used two primer pairs, To-A1F and To-A1R from the DNA-A, and To-B1F and To-B1R from the DNA-B (Sáez et al. 2016) to amplify 505 bp and 677 bp fragments of the two viral DNAs. Panno et al. (2016) performed PCR with the A1F/A1R primer pair (Mizutani et al., 2011) for the DNA-A component and the pair described by Ruiz et al. (2015) for the DNA-B component to amplify a ~1200-bp fragment of DNA-A and a ~890 bp fragment of DNA-B, respectively. PCR assays by Figàs et al. (2017) were carried out with primers To-1F and To-1R (López et al., 2015).

Luigi et al. (2020) designed a new primer and probe set, targeting the BC1 region of component B of the ToLCNDV genome, and developed a real-time PCR protocol (validated according to the guidelines reported in EPPO standard PM 7/98 (EPPO, 2019c)) able to detect ToLCNDV from both host plants and its vector *Bemisia tabaci*.

However, only sequencing of the entire DNA-A genome of ToLCNDV can provide the information necessary to identify ToLCNDV-ES and discriminate new or diverse ToLCNDV isolates (Moriones et al., 2017; Panno et al., 2019).

Conclusion for pest identification

Both ELISA and PCR-based tests are available for the identification of ToLCNDV in host plants. Identification of new ToLCNDV isolates and confirmation of the ToLCNDV-ES strain require a complete sequence analysis of the DNA-A genome component.

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 that the same epidemiological and inspection units are used for each pest in order to optimise the survey programme. This would optimise field inspections since they are organised per crop visit and not by pest. Table 2 shows an example of these definitions.

Table 2: Example of definitions of the target population, epidemiological unit and inspection unit for ToLCNDV

	Definition
Target population	Fields and greenhouses in a Member State where host crops are grown
Epidemiological units	A single field or greenhouse where host crops are grown
Inspection units	Individual host plants

To design a survey on ToLCNDV the general guidelines provide further details on the following steps that will generally be necessary:

1/ Determine the type of survey based on its objectives. For ToLCNDV, the type of survey will depend on the pest status (according to ISPM No. 8 (FAO, 2017)) in the area of interest. The objective could be to substantiate pest freedom, to delimit an outbreak area following an infestation or to determine the pest prevalence. The next steps deal with the example of substantiating pest freedom.

The overall confidence level and design prevalence of the survey have to be decided by the risk managers before designing the surveys as they reflect the acceptable level of the risk of infection of the host plants by ToLCNDV. The general guidelines for pest surveillance provide further details on the choice of these values and the related consequences in terms of pest surveys.

2/ Define the target population and its size. When determining the target population for surveillance of ToLCNDV, the host plants that are relevant for the survey area have to be selected. The size of the target population should be determined. For example, the target population could be all the cucurbit and tomato plants in a Member State.

3/ Define the epidemiological units. The epidemiological units should be single homogeneous areas that each contain at least one individual host plant.

4/ Determine the inspection unit. For a field or greenhouse where host crops are grown, for example, the inspection unit would be a single host plant.

5/ Determine the number of inspection units per epidemiological unit. For a field or greenhouse where host crops are grown this is the average number of plants per epidemiological unit.

6/ Implement the inspections and, if appropriate, the sampling within the epidemiological units, following the procedures suggested by the competent authorities, and estimate the method effectiveness in order to determine the overall method sensitivity (sampling effectiveness × diagnostic sensitivity). A representative number of plants should be examined and if there are suspicious symptoms they should be sampled. RiBESS+ can be used to calculate how many inspection units need to be examined or sampled when using a predefined prevalence level (e.g. 1%) to obtain a particular confidence level. This confidence level is in turn needed to calculate the number of sites to be

inspected (Step 8). Note that the more units are inspected the higher the confidence will be. The competent authorities need to align the survey efforts with the resources available.

7/ Define the risk factors. A risk factor affects the probability that a pest will be present or detected in a specific portion of the target population. It may not always be possible to identify or include a risk factor in the survey design. Risk factors can only be included when both the relative risk and the proportion of the overall plant population to which they apply are known or can be reliably estimated.

8/ Determine the number of epidemiological units that one needs to survey. RiBESS+ can be used to calculate the number of epidemiological units to survey in order to achieve the objectives of the survey set at Step 1 in terms of confidence level (e.g. 95%) and design prevalence (e.g. 1%), while also including the method sensitivity from Step 6 and the risk factors identified in Step 7. For example, RiBESS+ calculates the number of fields where host plants are present that need to be surveyed in a Member State in order to state with 95% confidence that the prevalence of ToLCNDV will be at 1% or below.

9/ Summarise and evaluate the survey design. At this stage, it is necessary to evaluate whether the above steps have resulted in a survey design that matches the available resources, meaning that a feasible number of inspections can be performed within an acceptable time frame per inspection, and resulting in a feasible number of samples. If not, available resources should be adjusted. This adjustment would result in a modified survey design using different input parameters of the statistical tool RiBESS+ (e.g. varying the number of components, method sensitivity, etc.).

10/ Integrate the pest-based survey into a crop-based survey (optional).

11/ Allocate the calculated survey effort. In the survey area, the output of RiBESS+ should be allocated proportionally to the host plant population or to the number of epidemiological units. In addition, the survey sites should be selected from the list of available locations.

12/ Data collection and survey reporting. Consider which data are needed and how these data will be reported together with the related assumptions.

13/ Plan, develop or update the specific instructions for the inspectors. These activities are not addressed by EFSA and fall within the remit of the competent national authorities.

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General glossary for pest survey

Term	Definition*
Buffer zone	An area surrounding or adjacent to an area officially delimited for phytosanitary purposes in order to minimise the probability of spread of the target pest into or out of the delimited area, and subject to phytosanitary or other control measures, if appropriate (ISPM 5: FAO, 2020).
Component (of a survey)	A component is a survey entity which can be distinguished based on its target population, the detection method (e.g. visual examination, laboratory testing, trapping) and the inspection unit (e.g. vectors, branches, twigs, leaves, fruit). A pest survey comprises various components. The overall confidence of the survey will result from the combination of the different components.
Confidence	The sensitivity of the survey is a measure of reliability of the survey procedure (Montgomery and Runger, 2010). The term **confidence level** is used in 'Methodologies for sampling of consignments' (ISPM 31: FAO, 2016b).
Delimiting survey	Survey conducted to establish the boundaries of an area considered to be infested by, or free from, a pest (ISPM 5: FAO, 2020).
Design prevalence *analogous to the term **level of detection** used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	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).
Detection survey	Survey conducted in an area to determine whether pests are present (ISPM 5: FAO, 2020).
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016a).
Epidemiological unit *analogous to the term **lot** used in 'Methodologies for sampling of consignments'*	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 to which statistics are applied (e.g. a

(ISPM 31: FAO 2016b)	tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infested or infested.
Expert knowledge elicitation	A systematic, documented and reviewable process to retrieve expert judgements from a group of experts in the form of a probability distribution (EFSA, 2014).
Host plant	A host plant is a plant species belonging to the host range on which the pest could find shelter, feed or subsist at least for a period of time.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5: FAO, 2020). This definition is limited to an array of host plant species and does not include the commodities other than plants or plant parts.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016a).
Infected versus infested	Infected is used when a pathogen is referred to in relation to its hosts (e.g. the trees are infected by the bacterium). Infested is used when an insect is referred to in relation to its hosts (e.g. the trees are infested by beetles). Infested is used when the pest is mentioned in relation to an area (e.g. an infested zone).
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, 2020).
Inspection unit *analogous to* ***sample unit*** *used in 'Methodologies for sampling of consignments' (ISPM 31: FAO 2016b)*	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, 2020).
Method sensitivity *analogous to the term* ***efficacy of detection*** *used in 'Methodologies for sampling of consignments'*	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010). The method sensitivity (MeSe) is defined as the probability that a truly positive host tests positive. It has two components: the sampling effectiveness (i.e. probability of selecting infested plant parts from an infested plant) and the diagnostic sensitivity (characterised by the visual inspection

(ISPM 31: FAO 2016b)	and/or laboratory test used in the identification process). The diagnostic sensitivity is the probability that a truly positive epidemiological unit will result positive and is related to the analytical sensitivity. It corresponds to the probability that a truly positive inspection unit or sample will be detected and confirmed as positive. The sampling effectiveness depends on the ability of the inspector to successfully choose the infested plant parts in a host plant. It is directly linked to the sampling procedure itself and on the training of the inspectors to recognise the symptomatology of the pest. Furthermore, symptom expressions are dependent, among other factors, on the weather conditions as well as on the physiological stage of the host plant when the sample is taken.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2020).
Pest freedom	Pest freedom can be defined, for a given target population, in a statistical framework, as the confidence of freedom from a certain pest against a pre-set design prevalence (threshold of concern).
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 (FAO, 2014).
RiBESS+	Risk-based surveillance systems. This is 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, 2020).
Risk factor	A factor that may be involved in causing the disease (FAO, 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 with 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.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
SAMPELATOR	Sample size calculator. This is an online application that implements statistical methods to estimate the sample size for pest prevalence estimation surveys. Free access to the software with prior user registration is available at https://shiny-efsa.openanalytics.eu/
Sample size	The sample size refers to the output of the statistical tools for survey design (RiBESS+ and SAMPELATOR). 'A well-chosen sample will contain most of the information about a particular population parameter but the relation between the sample and the population must be such as to allow true inferences to be made about a population from that sample.' (BMJ, online). The survey sample consists of the required number of 'inspection units' or samples thereof to be examined and/or tested in the survey to retrieve sufficient information on the pest presence or prevalence in the total population. For risk-based surveys, the sample size is calculated on the basis of statistical principles that integrate risk factors. If the examination for pest presence is performed by laboratory testing, at least one sample is taken from each inspection unit. These samples will undergo relevant laboratory testing.
Sampling effectiveness	For plants, it is the probability of selecting infested plant parts from an infested plant. For vectors, it is the effectiveness of the method to capture a positive vector when it is present in the survey area. For soil, it is the effectiveness of selecting a soil sample containing the pest when the pest is present in the survey area.
Specified plant	The plant species known to be susceptible to the pest. For example, for *Xylella fastidiosa*, the list of specified plants, which includes host plants and all plants for planting, other than seeds, belonging to the genera or species, can be found in Annex I of Decision (EU) 2015/789.
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, 2020).
Target population *analogous to* ***consignment*** *used in 'Methodologies for*	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:

sampling of consignments' (ISPM 31: FAO 2016b)	• definition of the target population: the target population has to be clearly identified; • target population size and geographic boundary. (EFSA, 2018)
Test	Official examination of plants, plant products or other regulated articles, other than visual, to determine whether pests are present, identify pests or determine compliance with specific phytosanitary requirements (ISPM 5: FAO, 2020).
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 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, 2020).

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