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EPPO STANDARD ON PHYTOSANITARY PROCEDURES

PM 3/77 (2) Vegetable plants for planting under protected conditions - Inspection of places of production

Specific scope: This Standard describes the procedure for inspection of places of production of vegetable plants for planting grown in protected cultivation. The Standard focuses on the most common species where the young plants are grown under protected conditions in the EPPO region: *Brassica* spp. (cabbage), *Capsicum* spp. (sweet pepper), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Cucurbita pepo* (zucchini), *Lactuca* spp. (lettuce), *Solanum lycopersicum* (tomato) and *Solanum melongena* (eggplant). Evidence gathered from inspections carried out according to this Standard may be used for export, for internal country movements of materials, for general surveillance or to help demonstrate freedom from relevant pests. The Standard does not cover the application of phytosanitary measures.

Specific approval and amendment: This Standard was first approved in 2015–09. Revision approved in 2022–09.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

The production of vegetable plants for planting is an important sector in the EPPO region. Vegetable plants for planting represent an important pathway for the entry and spread of pests, since infested vegetable plants for planting from a single place of production can be planted in many different locations. There is a risk of introduction of pests into nurseries through infested seeds. It is thus very important to ensure only healthy plants for planting are placed on the market.

Inside a nursery, an outbreak can be further spread through activities such as grafting, de-heading or pinching out, physical contact between different lots and by vectors. The delivery of vegetable plants for planting to a large number of producers may result in spread of pests over a wide area and to many crops.

Place of production freedom or crop freedom for specified pests is a frequent requirement of vegetable plants for planting in phytosanitary legislation.

2 | PHYTOSANITARY INSPECTIONS

ISPM 5 *Glossary of phytosanitary terms* (IPPC, 2019) defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations'.

General background information on phytosanitary inspection of places of production is given in the EPPO Standard PM 3/72 *Elements common to inspection of places of production*, *area-wide surveillance*, *inspection of consignments and lot identification* (EPPO, 2009) and in ISPM N°23 *Guidelines for inspection* (IPPC, 2016a).

The general elements of the procedure may also be useful to the member countries when they perform inspection of places of production for commodities exported to other countries.

Inspection should also be carried out for the detection of organisms for which the phytosanitary risk has not yet been determined. When an unfamiliar pest or a pest from the EPPO Alert List is detected, the procedures specified in the EPPO Standard PM 5/2 *Pest risk analysis on detection of a pest in an imported consignment* (EPPO, 2002) should be followed to allow the NPPO to make a decision as to whether phytosanitary action should be taken.

3 | PRODUCTION SECTOR CONCERNED

The production of vegetable plants for planting in protected conditions (e.g. glasshouses) takes place in specialized nurseries. Vegetable plants for planting are mainly grown from seeds, which if infested may result in many lots of plants for planting being infested. For grafted plants, there may be an increased risk as different seed lots (for rootstock and variety grafts) are used, and plants are exposed to infestation by handling during grafting.

Some vegetable plants are grafted, and the growing point may be pinched to encourage side shoots (e.g., tomatoes, cucumber, and eggplant).

The plants are intended for commercial vegetable production or for dispatch to the final (private) consumer/gardener.

4 | PESTS OF CONCERN FOR THE EPPO REGION

This Standard relates to pests affecting the listed vegetable plants for planting, mainly those which are listed in the EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests (see Table 1). It also considers those pests which are regulated in specific EPPO countries, if not mentioned in the EPPO A1 or A2 Lists (see Table 2). Pests are included if the vegetable plant is listed as a major host to that pest in EPPO Global database (EPPO, 2022a). This Standard also covers polyphagous quarantine pests which have vegetable plants as economically relevant hosts, and it includes hitchhiker pests which may be introduced as contaminants.

Details of the pests concerned (biology, geographical distribution, and host plants) can be found in the EPPO Global Database, in EPPO Standards regarding the specific pests or vegetable species, in EFSA Plant Pest Survey Cards and in the relevant scientific literature.

The EPPO Lists of A1 and A2 pests are subject to additions and deletions. The present list may need to be revised when new quarantine pests are identified.

5 | LOT IDENTIFICATION

An adequate proportion of plants should be subjected to a systematic examination to detect the presence or signs of pests. If appropriate, samples should be sent to the laboratory for identification.

General background information on lot identification is given in EPPO (2009). A lot should be defined as a number of plants that are produced from the same seed lot(s) and are treated in the same way and at the same time (plants which were sown, grafted, pinched out, etc.). In practice, a lot is often the same as a batch of plants destined for a specific vegetable producer or customer.

6 | SAMPLING

The number of plants that should be selected for inspection to detect a specified level of infection in a specified lot size is indicated in Tables 1, 3 and 4 of ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2016b).[1]

The NPPO should determine the sample size. For example, if 3689 plants are inspected from a lot of 10000 plants, this would provide a 99% confidence of detecting symptoms present in 0.1% of the plants, provided the symptoms are visible and are uniformly distributed and the plants are randomly selected. To reach the same level of confidence for small lots (fewer than 1000 plants), all plants should be inspected.

Sampling for inspection should be conducted at the most appropriate time. For vegetable plants for planting this is in the period from 1 week after placing the plants in the greenhouse up to the delivery of the plants. For grafted and/or pinched out plants (e.g., tomatoes, cucumber, eggplant) the most appropriate time for inspections and sampling is 10 days after grafting and/or pinching out.

Phytosanitary inspection should start with an overall examination of the place of production in order to check the physical condition of the plants. If there is an abnormal die-off in a place or lot, or there are other anomalies within the plants present (e.g., abnormal growth, differences in colour), these lots should be checked with specific attention.

It is also necessary to inspect plants in the vicinity of the place of production, e.g. weeds of host plants that grow in the nursery.

Trapping for the monitoring of pests such as leaf miners, thrips or whiteflies is an important addition to the inspection. Specific details are given for specific pests in Appendix 1.

Inspectors should take all necessary precautions during inspection and sampling, such as wearing protective clothes: coat, overshoes, gloves etc. to avoid pest spread within and between places of production.

7 | SYMPTOMS

Some symptoms are characteristics of certain pests. These symptoms should be looked for during visual examination of the plants (see Appendix 1), such as:

On tomatoes

- Plants with flaccid appearance, wilting plants (*Ralstonia solanacearum* species complex, *Clavibacter michiganensis* subsp. *michiganensis*, *Leucinodes orbonalis*),
- Stunted plants (*Tomato spotted wilt virus*, *Tomato yellow leaf curl virus*, *Tomato yellow leaf curl Sardinia virus*),
- Plants with smaller leaves (*Tomato yellow leaf curl virus*, *Tomato yellow leaf curl Sardinia virus*),
- Greenish yellow leaves, especially at the margins which may roll upwards ('*Candidatus* Phytoplasma solani'),
- Dull green, oily areas between leaf veins, desiccating to form pale brown necrotic areas or mealy appearance on leaves, stems and calyces as if they have been dusted by coarse flour– (*Clavibacter michiganensis* subsp. *michiganensis*),

[1]ISPM 31 provides information on the number of units to be sampled, which is considered useful to determine sample sizes for both consignments and places of production.

TABLE 1 Pests recommended for regulation by EPPO on vegetable plants for planting (pests included in the table are pests for which the host is given as a major host)

Host	EPPO A1	EPPO A2
Brassica oleracea*, *B. rapa		***Insects***: *Spodoptera littoralis*
Capsicum annuum	***Bacteria (including phytoplasmas)***: 'Candidatus *liberibacter solanacearum'*	***Bacteria (including phytoplasmas)***: *Ralstonia solanacearum Species Complex*, *Xanthomonas euvesicatoria* pv. *euvesicatoria*, *Xanthomonas hortorum* pv. *gardneri*, *Xanthomonas vesicatoria*
	Insects*: Anthonomus eugenii*, *Helicoverpa zea*, *Prodiplosis longifila*	***Insectslnematodeslmites***: *Aphelenchoides besseyi*, *Frankliniella occidentalis*, *Platynota stultana*, *Scirtothrips dorsalis*, *Spodoptera littoralis*, *Tetranychus evansi*
		Viruses: *Tomato brown rugose fruit virus*, *Tomato chlorosis virus*, *Tomato leaf curl New Delhi virus*, *Tomato spotted wilt virus*
Capsicum frutescens	***Bacteria (including phytoplasmas)***: 'Candidatus *liberibacter solanacearum'*	***Viruses***: *Tomato leaf curl New Delhi virus*
	Insects: *Anthonomus eugenii*, *Prodiplosis longifila*	
Cucumis melo	***Bacteria (including phytoplasmas)***: *Acidovorax citrulli*	***Viruses***: *Cucurbit yellow stunting disorder virus*, *Tomato leaf curl New Delhi virus;*
	Insects: *Diabrotica undecimpunctata howardi*, *Diabrotica undecimpunctata undecimpunctata*	
	Viruses: *Lettuce infectious yellows virus*	
Cucumis sativus	***Insects***: *Diabrotica undecimpunctata howardi*	***Insects***: *Frankliniella occidentalis*, *Spodoptera littoralis*
		Viruses: *Cucumber vein yellowing virus*, *Cucurbit yellow stunting disorder virus*, *Tomato spotted wilt virus*, *Tomato leaf curl New Delhi virus;*
Cucurbita pepo	***Insects***: *Diabrotica undecimpunctata howardi*, *Diabrotica undecimpunctata undecimpunctata*	***Viruses***: *Squash leaf curl virus*, *Tomato spotted wilt virus*
	Viruses: *Lettuce infectious yellows virus*	
Lactuca sativa	***Viruses***: *Lettuce infectious yellows virus*	***Insects***: *Nemorimyza maculosa*, *Spodoptera littoralis*
		Viruses: *Tomato spotted wilt virus*
Solanum lycopersicum	***Bacteria (including phytoplasmas)***: 'Candidatus *liberibacter solanacearum'*	***Bacteria (including phytoplasmas)***: 'Candidatus *phytoplasma solani'*, *Clavibacter michiganensis subsp. michiganensis*, *Ralstonia solanacearum Species Complex*, *Xanthomonas euvesicatoria* pv. *euvesicatoria*, *Xanthomonas euvesicatoria* pv. *perforans*, *Xanthomonas euvesicatoria* pv. *perforans*, *Xanthomonas hortorum* pv. *gardneri*, *Xanthomonas vesicatoria*
	Insects: *Bactericera cockerelli*, *Ceratothripoides brunneus*, *Ceratothripoides claratris*, *Helicoverpa zea*, *Keiferia lycopersicella*, *Leucinodes africensis*, *Prodiplosis longifila*, *Spodoptera eridania*	***Insectslnematodeslmites***: *Bemisia tabaci*, *Meloidogyne fallax*, *M. chitwoodi*, *Spodoptera littoralis*, *Tetranychus evansi*, *Tuta absoluta*
	Viruses: *Tomato mottle virus*	***Viruses***: *Pepino mosaic virus*, *Tomato brown rugose fruit virus*, *Tomato chlorosis virus*, *Tomato infectious chlorosis virus*, *Tomato spotted wilt virus*, *Tomato yellow leaf curl Sardinia virus*, *Tomato yellow leaf curl virus*, *Tomato leaf curl New Delhi virus;*
Solanum melongena	***Insects***: *Leucinodes africensis*, *Leucinodes orbonalis*, *Leucinodes pseudorbonalis*, *Leucinodes rimavallis*	***Bacteria (including phytoplasmas)***: *Clavibacter michiganensis* subsp. *michiganensis*, *Ralstonia solanacearum Species Complex*
		Insectslmites: *Spodoptera littoralis*, *Tetranychus evansi*
		Viruses: *Tomato spotted wilt virus*, *Tomato leaf curl New Delhi virus;*
Polyphagous pests	***Insects***: *Thrips palmi*	***Insects***: *Bemisia tabaci*, *Liriomyza huidobrensis*, *Liriomyza sativae*, *Liriomyza trifolii*

TABLE 2 Other pests regulated by specific EPPO countries on vegetable plants for planting (pests included in the table are pests for which the host is given as a major host)

Host	Other pests regulated by EPPO countries
***Brassica* spp.**	***Insects***: *Bagrada hilaris* (Israel Quarantine pest: 2009)
Brassica oleracea*, *B. rapa	***Insects***: *Delia floralis* (Israel Quarantine pest: 2009)
Capsicum annuum	***Viruses***: *Eggplant mottled dwarf nucleorhabdovirus* (Jordan A2: 2013); *Pepper mild tigré virus* (Jordan A1 list: 2013; Moldova Quarantine pest: 2017; Turkey A1 list: 2016);
Cucumis melo	***Viruses***: *Eggplant mottled dwarf nucleorhabdovirus; Watermelon chlorotic stunt virus* (Jordan A2: 2013); *Zucchini yellow mosaic virus* (Jordan A2: 2013)
Cucumis sativus	***Viruses***: *Eggplant mottled dwarf nucleorhabdovirus; Zucchini yellow mosaic virus* (Jordan A2: 2013)
Cucurbita pepo	***Viruses***: *Tomato leaf curl New Delhi virus; Zucchini yellow mosaic virus*
Lactuca sativa	***Viruses***: *Lettuce mosaic virus* (Jordan A2: 2013)
Solanum lycopersicum	***Viruses***: *Columnea latent viroid* (United Kingdom Quarantine Pest 2020); *Tomato apical stunt viroid* (Jordan A1 List: 2013; Tunisia Quarantine pest: 2012; United Kingdom RNQP), *Tomato chlorotic dwarf viroid* (Jordan A1 List: 2013; Tunisia Quarantine pest: 2012; United Kingdom RNQP), *Tomato torrado virus* (Tunisia Quarantine pest: 2012; United Kingdom Quarantine pest: 2012)
Solanum melongena	***Insects***: *Hishimonus phycitis* (Turkey A1 list: 2016; EU Quarantine pest Annex II A: 2019)
	Viruses: *Eggplant mottled dwarf nucleorhabdovirus;*
Polyphagous pests	***Insects***: *Liriomyza bryoniae* (Turkey A2 list: 2016; EU PZ Quarantine pest Annex III: 2019)

- Irregular, water soaked areas, initially green and then becoming brown and necrotic (*Xanthomonas* spp.).

On brassicas

- Tunnels in stems of Brassicas (*Delia floralis*)
- Damage to apical stem (*Bagrada hilaris*)

On Capsicum

- Lesions on *Capsicum annuum* leaves are of irregular shape and necrotic, in some cases surrounded by a chlorotic halo (*Xanthomonas euvesicatoria*). When the infection is severe, foliar blight can occur and leaves may fall.

On lettuce

- Irregular shaped seedlings (lettuce mosaic virus)

On a range of plants

- Discolouration of the upper leaf surface; silvering, deformity, growth malfunction and brown bumps on the foliage, thickening of leaf lamina, premature leaf fall (thrips)
- Chlorotic leaves, white to brown spots on leaves, silk webs (mites such as *Tetranychus evansi*)
- Insects at any life stage, including black frass from larvae
- Small punctures or mines on upper and lower leaf surface (*Liriomyza* spp., *Tuta absoluta*, *Keiferia lycopersicella*) or, bore holes in fruit (*Leucinodes orbonalis*)
- Leaves tied together by thread (leafrolling caterpillars such as *Platynota stultana*)
- Holes in leaves (caterpillars such as *Spodoptera littoralis*)
- Lesions, chlorotic spots on upper and lower leaf surface, yellowing of leaves, leaf curling (bacteria, viruses, leafhoppers)
- Vein yellowing, interveinal mottling or chlorosis, deformed or distorted leaves, bubbling on leaf surface. Bronzing, chlorotic streaks, necrotic lesions turning to shot holes (viruses)

Close attention should be given to the presence of vectors such as leafhoppers (vectors of 'Candidatus *Phytoplasma solani*'), the whiteflies *Trialeurodes vaporariorum* (vector of *Tomato infectious chlorosis virus*) and *B. tabaci* (vector of multiple viruses), and *Thripidae* (vector of *Tomato spotted wilt virus*). The presence of honeydew and sooty moulds can be indicative of the presence of some virus vectors such as whiteflies.

8 | SAMPLING FOR LABORATORY TESTING

Visual examination of vegetable plants for planting alone is not considered sufficient for many pests which may be present in a latent stage and/or are difficult to detect on young plants. Laboratory testing should thus be carried out to provide additional assurance of pest freedom.

Plants should be sampled for laboratory testing when they have signs of pests or when they show symptoms, and the pest cannot be immediately identified by the inspector. The same applies to plants showing suspicious symptoms or deformations. In these cases, the sample consists of the suspect plant(s).

The size of the sample to be taken, when sampling is not based on symptoms, depends on the potential distribution of the pests within the lot and on the method selected for diagnosis in the laboratory. Sampling should be done on a lot basis, with plants evenly collected

throughout the lot. In general, since the plant parts most suitable for detection greatly differ for different pests, samples for laboratory testing should contain whole plants in order to provide the option of testing for the whole range of potential pests.

However, in order to take the commercial value of plant pests into account, in some cases only parts of the plants (e.g. leaves) can be taken instead of the complete plants. Appendix 1 specifies the plant part to be sampled for the relevant pests.

Sampling plans should be formulated to determine the frequency of sample submission for laboratory testing.

Each sample should be individually labelled with nursery name (or reference number), sample number, date, plant species, plant variety if relevant and lot number, so that follow-up action can be taken if necessary.

In order to identify the pest, sampled material such as the entire plant and roots, or plant parts should be kept in good condition and placed in plastic bags. For plant parts, this should be together with a piece of absorbent paper. If the plant parts are dry, a piece of slightly damp absorbent paper should be added; for wet plant parts a piece of dry absorbent paper should be added (to avoid rotting of the plant parts). Plants with roots in potting compost, substrate, etc. do not easily dry out; as a consequence, absorbent paper is not needed.

Samples of adult insects, larvae, pupae and eggs should be put in a pot with screw cap. Living organisms should be sent to the laboratory together with plant material of the host plant in a suitable container. Dead organisms should be placed into a tube with alcohol in order to prevent decomposition during transport.

If a pest found during inspection is suspected to be a quarantine pest, the suspect lot should be detained under official control pending a test result. All other lots potentially at risk of infestation and lots which are related to the suspect lot (e.g., same seed lot, contact by means of manipulation of the plants, contact by irrigation etc.) should also be detained under official control.

For further details on symptoms, sampling and identification of the relevant pests of vegetable plants for planting, see Appendix 1.

Appendix 2 provides a short procedure for inspectors.

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APPENDIX 1 - SYMPTOMS AND SAMPLING FOR THE IDENTIFICATION OF QUARANTINE PESTS OF VEGETABLE PLANTS FOR PLANTING

For each of the quarantine pests mentioned below basic information on host range, biology, detection and identification can be found in EPPO Global Database (https://gd.eppo.int/), as well as in EPPO Datasheets, EPPO Diagnostic Standards, and EFSA Plant Pest Survey Cards. Illustrations are available on the EPPO Global Database and the EFSA Plant Pest Survey Cards (https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489). When an EPPO Diagnostic Standard exists, it is mentioned in the text. The fact that there is no EPPO Diagnostic Standard does not mean that no method for diagnosis is available in the scientific literature. In some cases symptoms have been included for information from plants older than those which are usually traded for planting, because symptoms on seedlings are sometimes difficult to spot. As a general rule, symptoms might not be readily visible on very young plants, and they are given in part for illustration purposes.

A. INSECTS AND MITES

1. *Bagrada hilaris* (Israel Quarantine pest)

Symptom description

Bagrada hilaris (Figure A1) feeds by inserting its piercing and sucking mouthparts (stylets) between the epidermal layers of the host (*Brassica* spp.) and releasing a salivary enzyme into the cells. Young seedlings are extremely susceptible to feeding damage, especially at the cotyledon stage. Damage to the apical meristem of head-forming *Brassica* crops results in smaller and unmarketable heads, or no heads at all.

Sampling and identification

There are currently no monitoring techniques specific to *B. hilaris*. Sampling should be performed from mid-morning to late afternoon when activity is highest. *B. hilaris* has been observed feeding on seedlings immediately after emergence. Therefore, sampling is recommended when seedlings begin to emerge. The young seedlings should be checked thoroughly for the presence of *B. hilaris* adults and feeding damage. Sampling of larger or transplanted seedlings (i.e., two- to three-leaf stage or larger) should involve thorough inspection of the undersides of leaves, the stem and the soil surface.

FIGURE A1 *Bagrada hilaris* adults on *Brassica* (Image courtesy Jennifer Carr, University of Florida, Bugwood.org).

2. *Bemisia tabaci* (EPPO A2 List)

Symptom description

Bemisia tabaci is a species of whitefly (Figure A2). Chlorotic spots develop on the leaves of affected plants (e.g. tomato, cucumber), which may also be disfigured by honeydew and associated sooty moulds. Leaf curling, yellowing, mosaics or yellow-veining could indicate the presence of whitefly-transmitted viruses. A close observation of the underside of the leaves will show the tiny yellow/white larval scales (Figure A3). In severe infestations, when the plant is shaken, numerous small white adult whiteflies will flutter out and quickly resettle.

Sampling and identification

The underside of the leaves should be inspected carefully to detect different life stages of the pest (larvae, puparia) or signs of it such as chlorotic spots and honeydew. Adults can be detected by shaking the plants. For the detection and monitoring of *Bemisia tabaci*, yellow sticky traps can be used. These traps should be placed 20 to 30 cm above the plants. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on identification of *Bemisia tabaci* are included in the EPPO Standard PM 7/35 *Bemisia tabaci* (EPPO, 2004a).

FIGURE A2 *Bemisia tabaci* adult (EPPO Global Database: Image courtesy Agroscope FAW Wädenswil, Switzerland).

FIGURE A3 Puparium and larvae (EPPO Global Database: Courtesy: Varga András, Hungary).

3. *Delia floralis* (Israel Quarantine pest)

Symptom description

Delia floralis (Figure A4) mainly affects root brassicas. The larvae tunnel deeply into the stems and there is only one generation each year which is active from mid-summer until early autumn. Damage is usually seen from late September.

Sampling and identification

Look for adults and damage by larvae. For the detection and monitoring of *D. floralis* sticky cards can be used. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

4. *Frankliniella occidentalis* (EPPO A2 List)

Symptom description

Symptoms of *F. occidentalis* infestation vary depending on the host plants (for *Cucumis sativus* see Figure A5). However, the symptoms of infestation with this pest are

FIGURE A4 *Delia floralis* adult (Image courtesy: Whitney Cranshaw, Colorado State University, Bugwood.org).

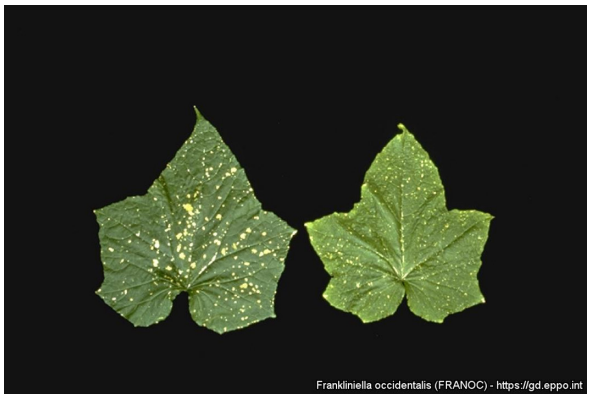

FIGURE A5 Thrips damage on cucumber leaves: *F. occidentalis* with coarser pattern (left); *Thrips tabaci* on right, for comparison (EPPO Global Database image courtesy: P.M.J Ramakers - PTG, Waaldwijk (NL)).

FIGURE A6 Adult *F. occidentalis* EPPO Global Database image courtesy: Blandine Delbourse - Point of Entry Roissy CDG airport (FR).

usually: discolouration of the upper leaf surface; silvering, deformity, growth malfunction and brown bumps on the foliage; halo spotting on leaves, which consists of small dark scars surrounded by whitish tissue; appearance on the surface of leaves of liquid faecal deposits which cause dark-green speckling.

Sampling and identification

Look for adults (Figure A6) and check the upper leaf surface for discoloration. During inspection of plant material for the presence of *F. occidentalis*, aerial parts of plants should be shaken over sheets of white paper. Thrips and other small insects present on the surface of plants fall onto the paper, where they can be collected with small brush-pencils and put in a tube with 10% alcohol plus wetting liquid or by an insect aspirator. Blue sticky traps can be used for the detection and monitoring of *F. occidentalis*. These traps should be placed 20–30 cm above the plants. Morphological identification may be limited for insects collected from sticky traps. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on the identification of *F. occidentalis* are included in the EPPO Standard PM 7/11 *Frankliniella occidentalis* (EPPO, 2003).

5. *Hishimonus phycitis* (Tunisia Quarantine pest : 2012, Turkey A1 list: 2016, EU Quarantine pest Annex II A: 2019)

Symptom description

Hishimonus phycitis is a pest of *Solanum melongena*. Symptoms of leafhopper damage include host leaves with yellow spots, or leaves that curl up at the margin and sometimes drop. As the pest feeds on nutrients from the vascular tissue, sooty mould can develop on the expelled exudate.

Sampling and identification

Adults are small, 3–4 mm long and greenish yellow (Figure A7); the abdomen and legs are brownish. Nymphs are yellow with brown spots on the abdomen. Colouration of the species varies considerably. A detailed description is provided by Distant (1908). Taxonomic keys are available in Dai et al. (2013) and Viraktamath and Anantha Murthy (2014). Sweep nets, suction devices and yellow sticky traps can be used to detect and monitor populations in the field (EFSA, 2017).

6. *Leucinodes orbonalis* (EPPO A1 List)

Symptom description

Flowers often show the first signs of damage which can indicate larval feeding. Bore holes in the fruit can indicate where the final instar entered the fruit in order to pupate within it. The small entrance holes in the fruit are normally closed by dried excrement. Wilting of the foliage can be evident when infestation levels are high (e.g. eggplant).

Sampling and identification

Look for adults (Figure A8). For the detection and monitoring of *Leucinodes orbonalis*, delta-traps with specific pheromones and sticky traps can be used, along with funnel traps and light traps. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

7. *Liriomyza huidobrensis* (EPPO A2), *L. sativae* (EPPO A2), *L. trifolii* (EPPO A2 List) and *Liriomyza bryoniae* (Turkey (A2 list)), EU (PZ Quarantine pest (Annex III: 2019))

Symptom description

Liriomyza species can infest a wide range of vegetable plants. Leaves of infested plants have small feeding/oviposition punctures and/or serpentine, irregular mines. The mines are usually white with dampened black and dried brown areas.

FIGURE A7 *Hishimonus phycitis* adult (J.L. Danet, INRA Centre de Recherches de Bordeaux, Bugwood.org).

FIGURE A8 *Leucinodes orbonalis* adult (EPPO Global Database image *courtesy BfUL – Christina Staudigl Courtesy*).

FIGURE A9 *Liriomyza huidobrensis* adult (EPPO Global Database image courtesy Central Science Laboratory, York (GB) - British Crown).

FIGURE A10 *Liriomyza sativae* (EPPO Global Database image courtesy D. M. Firake and G. T. Behere).

Sampling and identification

Inspect the leaves for mines and inspect the mines for larvae. For the detection and monitoring of *Liriomyza* spp. (Figures A9 and A10), yellow sticky traps can be used. These traps should be hung 20 to 30 cm above the plants. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Details on identification of *Liriomyza* spp. Are included in the EPPO Standard PM 7/53 (2) *Liriomyza* spp. (EPPO, 2022b).

8. *Keiferia lycopersicella* (EPPO A1 List)

Symptom description

Adults are mottled brown with a length of 9-12 mm. In foliage of *Solanum* species, the first and second instars of larvae feed as leafminers, producing a blotch mine (Figure A11). Later instars typically fold leaves (Figure A12) or attach pairs of leaves with silk to create sheltered feeding sites, but may enter stems.

FIGURE A11 *Keiferia lycopersicella* caterpillar (EPPO Global Database Courtesy: Max Badgley, University of California, Riverside (US)).

FIGURE A12 Damage caused by *Keiferia lycopersicella* (EPPO Global Database Courtesy: John Trumble, University of California, Riverside (US)).

Sampling and identification

Look for adults and damage by larvae. For the detection and monitoring of *Keiferia lycopersicella*, delta-traps with specific pheromones and sticky traps can be used. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

9. *Platynota stultana* (EPPO A2 List)

Symptom description

Platynota stultana (Figures A13 and A14) has been recorded on more than 25 plant families with many economically important plants. *Capsicum annum* is a major host. Larvae of *P. stultana* tie leaves together and feed inside.

Sampling and identification

Adults can be detected by using pheromone traps and larvae by visual examination of leaves and in shoot

FIGURE A13 *Platynota stultana* adult (Todd M. Gilligan and Marc E. Epstein, TortAI: Tortricids of Agricultural Importance, USDA APHIS PPQ, Bugwood.org).

FIGURE A14 *Platynota stultana* caterpillar (Barry Freeman, Auburn University, Bugwood.org).

tips. In Spain, damage has exclusively been reported on *Capsicum annuum*. Further details are available via EPPO Global Database (EPPO, 2022a).

10. *Scirtothrips dorsalis* (EPPO A2 List)

Symptom description

Symptoms are silvering of the leaf surface, linear thickening of the leaf lamina and brown excreta on the leaves and fruits of host plants (e.g. pepper, tomato, eggplant). The species can cause distortion to young leaves and premature leaf fall.

Sampling and identification

All stages of *S. dorsalis* (Figures A15 and A16) feed on epidermal and sometimes palisade cells of young leaves. They do not feed on mature leaves. They could be carried on plants for planting, in particular seedlings. Further details are available in the EPPO Global Database (EPPO, 2022a) and in EPPO Standard PM 7/56 *Scirtothrips aurantii, Scirtothrips citri, Scirtothrips dorsalis* (EPPO, 2005).

FIGURE A15 *Scirtothrips dorsalis* nymph.

FIGURE A16 *Scirtothrips dorsalis* adult (Andrew Derksen, USDA-APHIS, Bugwood.org).

11. *Spodoptera littoralis* (EPPO A2 List)

Symptom description

Symptoms of the presence of larvae are holes in leaves with the presence of excrement on a number of species of vegetable plants. Symptoms caused by the larvae are generic for most primarily foliage feeding Lepidoptera. Under natural conditions, pupation takes place in the soil where the pupae are difficult to detect. Pupae can incidentally be found in commodities without soil, since larvae will always start pupating when fully grown, regardless of the presence of soil (Figures A17 and A18).

Sampling and identification

The species can be found on plants or above-ground plant parts. All stages of the pest can be detected visually (with a hand lens for early stages), and specimens can be collected by hand or with a sweep net (adults). Adults can also be detected with the aid of light traps and pheromone-baited traps. Pheromone-baited traps

FIGURE A17 *Spodoptera littoralis* caterpillar (EPPO Global Database Courtesy: Biologische Bundesanstalt (DE)).

FIGURE A18 *Spodoptera littoralis* adult (EPPO Global Database Courtesy: O. Heikinheimo (FI)).

allow adult males to be caught and light traps catch both adult females and males. Adults can sometimes be found and collected by hand, especially when plants are transported or stored in cool conditions. Eggs can be found on all above-ground plant parts, mostly on the underside of leaves. Further details are available in EPPO Standard PM 7/124 (1) *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda, Spodoptera eridania* (EPPO, 2015).

12. *Tetranychus evansi* (EPPO A2 List)

Symptom description

Mites (Figure A19) live on both sides of the leaves with a slight preference for the underside and for the vicinity of the veins. Feeding causes the leaves to become chlorotic (e.g. on tomato leaves Figure A20). White to brown (depending on the plant species) spots caused by the mites' salivary contents appear on both sides and lead to the destruction of parenchyma cells. At high levels of infestation, the leaves dry out and die. Silk webs are also produced. At high infestation levels the dense webs can 'mummify' the plant. In very heavy infestations, which

FIGURE A19 *Tetranychus evansi* male at the top, female at the bottom.

FIGURE A20 Symptoms of *Tetranychus evansi* (EPPO Global Database Courtesy: Alain Migeon (INRAE, Montpellier, FR)).

are frequent (contrary to other *Tetranychus* pests), feeding and webbing cause the death of the plant.

Sampling and identification

The mites can be present on plant material, especially plants for planting (Solanaceae). At low densities, spider mites are extremely difficult to detect. Inconspicuous (less than half a millimetre in length) they can be invisible to the naked eye. Attention should focus on small whitish, brownish or yellow spots (symptoms which could also be caused by viruses or superficial wounds). An examination of the both sides of the leaves under a stereo-microscope will confirm (or not) the presence of spider mites, generally associated with white exuviae and webbing. On hairy plants like eggplant exuviae can be seen on the hairs. *T. evansi* with its orange colouration, its highly aggregative behaviour and its faculty to live on both sides of the leaves is easier to detect than other tetranychid mites. Further details are available in EPPO

Standard PM 7/116 *Tetranychus evansi* (EPPO, 2013a, under revision).

13. *Tuta absoluta* (EPPO A2 List)

Symptom description

Adults are nocturnal and usually hide during the day between leaves.

Infested plants (e.g. tomatoes) show conspicuous mines and galleries. Leaf mines are irregular and may later become necrotic (Figure A21). On young plants, the pest prefers apical buds, on which the black frass can be visible.

Sampling and identification

Look for adults (Figure A22) between the leaves and inspect the apical buds thoroughly for mines and black frass. For the detection and monitoring of *Tuta absoluta*, delta traps with specific pheromones and sticky traps can be used. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest.

FIGURE A21 Leaf mines caused by *Tuta absoluta*.

FIGURE A22 Adult *Tuta absoluta* EPPO Global Database Courtesy: Alain Migeon (INRAE, Montpellier, FR).

B. BACTERIA (INCLUDING PHYTOPLASMAS)

1. *Acidovorax citrulli* (EPPO A1)

Symptom description

On cotyledonal leaves (Cucumis and Cucurbita), during nursery production, lesions initially appear as water-soaked spots, rapidly developing into large rotting and necrotizing areas.

Sampling and identification

Visual inspection can be conducted during the production of seedlings. Early disease detection in transplant nurseries is possible due to large necrotic areas on cotyledonal leaves. Diseased plants are usually grouped in small patches randomly distributed on the production site. The first aim for inspection at nurseries is to try and locate such patches. EPPO Standard PM 7/127 *Acidovorax citrulli* (EPPO, 2016a) provides further information regarding detection and identification of *A. citrulli* in different plant material.

2. *Clavibacter michiganensis* subsp. *michiganensis* (EPPO A2 List)

Symptom description

Damage is not likely to be detected on young plants, symptoms only appear as plants approach maturity (Figure A23). Tomato is a major host of *Clavibacter michiganensis* subsp. *michiganensis*.

FIGURE A23 *Clavibacter michiganensis* subsp. *michiganensis* symptoms (EPPO Global Database: Courtesy: Andrea Minuto CERSAA, Albenga (IT)).

Sampling and identification

Visual examination of young plants is not feasible. Leaves have a flaccid appearance, usually at the warmest time of day. Representative samples of different lots should be taken and subjected to laboratory testing. The complete plants should be sampled. The plants should be tested at the height of the graft, the rootstock should also be analyzed. One hundred plants should be sampled per 20000 plants or per lot (except for small lots, where it is preferable to refer to the tables in ISPM N°31 (IPPC, 2016b). Details on the identification of *Clavibacter michiganensis* subsp. *michiganensis* are included in the EPPO Standard PM 7/42(3) *Clavibacter michiganensis* subsp. *michiganensis* (EPPO, 2016b).

3. '*Candidatus* Phytoplasma solani' (EPPO A2 List)

Symptom description

'*Candidatus* Phytoplasma solani' is naturally dispersed by its leafhopper vectors, such as *Hyalesthes obsoletus*. Young host plants (e.g., seedlings of tomatoes, peppers or eggplants) could carry the phytoplasma, but young plants are unlikely to have become infected (since the disease is not seed-borne). '*Candidatus* Phytoplasma solani' is readily transmissible by grafting.

Leaves developed before infection become greenish-yellow, especially at the margins which may roll upward. Newly formed leaves become more yellow and are smaller. Stems become thin at the apex as growth is stopped, but enlarged at the infection sites as a result of abnormal phloem formation. Lateral shoots develop, giving the plant a bushy aspect.

Sampling and identification

Plants should be inspected, especially the leaves, for symptoms. Attention should be paid to the presence of the leafhopper vectors. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest.

4. *Ralstonia solanacearum* species complex (EPPO A2 List)

Symptom description

Pepper, eggplant and tomato are major hosts of *Ralstonia solanacearum* species complex. The youngest leaves are the first to be affected and have a flaccid appearance, usually at the warmest time of day. If environmental conditions are favourable for the pathogen (temperature optimum of 24-35°C wilting of the whole plant may follow rapidly (Figure A24). Under less favourable conditions, stunting may occur and large numbers of adventitious roots are produced on the stem. The vascular tissues of the stem show a brown discoloration and, if the stem is cut crosswise, drops of white or yellowish bacterial ooze may be visible.

FIGURE A24 Wilted tomato plant (cv. Moneymaker) infected with *Ralstonia solanacearum*. EPPO Global Database Courtesy: John Elphinstone (Defra, Crown Copyright).

Sampling and identification

Look for plants with a flaccid appearance, preferably at the warmest time of day. Cut the stem of suspected plants crosswise and put the stem in a cup with clear water to look for bacterial ooze in the stem. It should be noted that the absence of ooze does not necessarily mean absence of the bacteria. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Complete plants should be sampled. The bacterium can spread in irrigation (drainage) water and wild plants can be reservoirs for the bacteria. Where appropriate samples of irrigation (drainage) water (500 mL) or *Solanum nigrum/dulcamara* should be taken for laboratory testing. Water samples should be ideally transported and stored between 10°C and 16°C.

Details on identification of *Ralstonia solanacearum* are included in the EPPO Standard PM 7/21 (3) *Ralstonia solanacearum, R. pseudosolanacearum* and *R. syzygii* (*Ralstonia solanacearum* species complex) (EPPO, 2022c).

5. *Xanthomonas euvesicatoria* pv. *euvesicatoria* (EPPO A2), *Xanthomonas euvesicatoria* pv. *perforans* (EPPO A2), *Xanthomonas hortorum* pv. *gardneri* (EPPO A2), *Xanthomonas vesicatoria* (EPPO A2 List)

Symptom description

On tomato leaves, lesions appear as irregular, water-soaked areas that are green at first, becoming brown and necrotic later. Lesions are frequently surrounded by large chlorotic haloes. Foliar blight can occur when the lesions coalesce. Necrosis of the petioles and canker-like splits can be observed along the stem. On tomato leaves, bacterial speck lesions (*P. syringae* pv. tomato) look similar initially but are surrounded by a more distinct yellow

FIGURES A25 AND A26 Symptoms of bacterial spot disease caused by *Xanthomonas euvesicatoria* on pepper under greenhouse conditions. EPPO Global Database: Courtesy: Ebrahim Osdaghi.

halo. Lesions on *Capsicum annuum* leaves are of irregular shape and necrotic, in some cases surrounded by a chlorotic halo (Figures A25 and A26). When the infection is severe, foliar blight can occur and leaves may fall.

Sampling and identification

Look for irregular water-soaked lesions on the leaves.

Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Details on identification are included in the EPPO Standard PM 7/110 *Xanthomonas* spp. (*Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Xanthomonas perforans*, *Xanthomonas vesicatoria*) causing bacterial spot of tomato and sweet pepper (EPPO, 2013b, under revision).

C. VIRUSES AND VIROIDS

1. Pospiviroids

Relevant Pospiviroids: *Columnea latent viroid*, *Tomato apical stunt viroid* and *Tomato chlorotic dwarf viroid*.

Symptom description

Damage is not likely to be detected on young plants; symptoms only appear as plants approach maturity. Pospiviroids are generally distributed in most parts of the plants, including seeds. Their propensity to develop symptoms largely depends on the viroid species, strain, host species, cultivar and environmental conditions (EPPO, 2021a).

Sampling and identification

Visual examination of young plants is not feasible. Representative samples of different lots should be taken and subjected to laboratory testing. It is sufficient to sample leaves instead of the complete plants. A maximum of 25 leaves should be taken per sample. Samples should be transported and stored cooled, but not colder than 4°C. Details on the identification of Pospiviroid viruses can be found in PM 7/138 Pospiviroids (genus Pospiviroid) (EPPO, 2021a).

2. *Cucumber vein yellowing virus* (CVYV) (EPPO A2 List)

Symptom description

There is a wide range of symptoms on cucumber, from chlorotic mottling to vein yellowing, vein clearing and stunting, or no symptoms. CVYV is transmitted by the whitefly *Bemisia tabaci*.

Sampling and identification

Look for symptoms as vein yellowing and vein clearing. Pay attention to the presence and damage caused by the vector *Bemisia tabaci* (see a2). Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing. Details on the identification of CVYV are included in the EPPO Standard PM 7/81(1) *Cucumber vein yellowing virus* (*Ipomovirus*) (EPPO, 2007).

3. *Cucurbit yellow stunting disorder virus* (CYSDV) (EPPO A2 List)

Symptom description

Affected plants (e.g. pepper and cucumber) show yellowing symptoms that start as an interveinal mottle on the older leaves and intensify as leaves age. Chlorotic mottling, yellowing and stunting occur on affected plants. CYSDV is transmitted by the whitefly *Bemisia tabaci*.

Sampling and identification

Look for interveinal mottle on the oldest leaves. Pay attention to the presence and damage of *Bemisia tabaci* (see a2). Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing.

4. *Eggplant mottled dwarf nucleorhabdovirus* (EMDV) (Jordan A2 list)

Symptom description

Infected eggplants show deformed leaves of varying intensity, which are crinkled and have chlorotic discolorations

of the veins and adjacent tissues that sometimes turn into a chlorotic mottling. Plants may show stunting.

Sampling and identification

Look for symptoms as chlorotic discolorations of the veins and adjacent tissues. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing.

5. *Lettuce mosaic virus* (LMV) (Jordan A2 list)

Symptom description

Symptoms vary depending on the type of lettuce and cultivar, the growth stage when infected, and environmental conditions. Seedling leaves originating from infected seeds appear irregular in shape and develop a light green mottle or mosaic (EPPO, 2000). The virus is seed transmitted and sap-transmissible. The virus can also be transmitted by many species of aphid.

Sampling and identification

Look for symptoms such as irregular shaped seedlings. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing.

6. *Pepino mosaic virus* (PepMV) (EPPO A2 List)

Symptom description

Affected tomato plants show distorted leaf development, with bubbling of the leaf surface and chlorosis. On *Capsicum annuum*, it causes a yellow mosaic in young leaves.

Sampling and identification

Look for distorted and chlorotic leaves. As damage is not likely to be visually detected on young plants, representative samples of different lots should be taken and subjected to laboratory testing. It is sufficient to sample leaves instead of the complete plants. A maximum of 25 leaves should be taken per sample. Samples should be transported and stored cooled, but not colder than 4°C. Samples taken for laboratory testing of *Pospiviroids* can also be analyzed for PepMV. Details on the identification of *Pepino mosaic virus* are included in the EPPO Standard PM 7/113 *Pepino mosaic virus* (EPPO, 2013c).

7. *Pepper mild tigré virus* (PepMTV) (Jordan, Turkey (A1 list), Moldova (Quarantine pest))

Symptom description

Symptoms on Capsicum include mild interveinal chlorosis, veinal distortion and mild stunting. Symptoms on tomato include leaf curling, mild interveinal chlorosis and moderate stunting.

Sampling and identification

Look for symptoms as chlorotic discolorations of the veins and adjacent tissues. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing.

8. *Squash leaf curl virus* (SLCV) (EPPO A2 List)

Symptom description

SLCV causes severe systemic stunting and leaf curling in most cucurbits. In pumpkin, squash and watermelon, a severe, chlorotic foliar mosaic or mottle accompanies leaf curling and stunting symptoms. Infected young plants may not always show symptoms

Sampling and identification

SLCV is not mechanically transmissible. Recommended indicator plants are *Cucurbita maxima*, *C. moschata* or *C. pepo* or *Phaseolus vulgaris* (systemic green mosaic leaf symptoms and distortion). SLCV can be detected in *B. tabaci* by DNA spot hybridization assay

9. *Tomato brown rugose fruit virus* (ToBRFV) (EPPO A2 List)

Symptom description

There are not always external signs of infection on seedlings (EPPO, 2021b, 2022d). Chlorosis, mosaic pattern (chlorotic/pale patches) and mottling are often observed on younger leaves in the head and side shoots (e.g. tomato and pepper). Younger leaves may also be crumpled, puckered or deformed. Narrowing of leaves (needle-like symptoms) is occasionally observed on tomato. Blistering of the leaf surface is observed. Leaves may wilt, followed by yellowing and death of complete plants.

Sampling and identification

Young plants are usually symptomless before eight weeks of age (7 leaves) (EPPO, 2020). Young leaflets/leaves

should be collected from the top of different plants. Leaflets can be pooled in subsamples and the EPPO Diagnostic protocol *PM 7/146 Tomato brown rugose fruit virus* (EPPO, 2021b, under revision) provides guidance on the number of leaves/leaflets that can be sampled and molecular tests for detection.

10. *Tomato chlorosis virus* (ToCV) (EPPO A2 List)

Symptom description

Affected tomato plants show symptoms consisting of interveinal yellowing beginning on lower leaves. Initially, delimited chlorotic patches evolve with a light-yellow colour; then they coalesce and the whole leaf appears bright yellow except for the veins, which remain green. These symptoms are similar to those produced by nutritional disorders, phytotoxicity or senescence. Symptomatic leaves are also thickened and brittle when hand crushed. Yellowing develops from the base to the top but is rarely visible on younger leaves. In addition, reddening, bronzing, necrosis and rolling of older leaves may be observed. Infected plants show early senescence and less vigour (EPPO, 2022e).

Sampling and identification

ToCV is transmitted locally by whiteflies of the genera *Bemisia* and *Trialeurodes*. Fully developed leaves, showing mild interveinal yellowing, should be sampled. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing. Guidance for detection and identification of this virus are given in the EPPO Standard PM 7/118 (1) *Tomato chlorosis virus* and *Tomato infectious chlorosis* virus (EPPO, 2013d)

11. *Tomato infectious chlorosis virus* (TICV) (EPPO A2 List)

Symptom description

The first indication of infection in tomato is a bright interveinal yellowing on the older leaves. As the disease progresses, the yellowing develops acropetally and the leaves thicken, become brittle and roll. *Tomato infectious chlorosis virus* is transmitted by the whitefly *Trialeurodes vaporariorum*.

Sampling and identification

Look for interveinal yellowing on the leaves. Pay attention to the presence and damage (chlorotic spots, honeydew and sooty moulds) of the whitefly vector *Trialeurodes vaporariorum*. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing. Details on the identification of TICV are included in the EPPO Standard PM 7/118 (1) *Tomato chlorosis virus* and *Tomato infectious chlorosis* virus (EPPO, 2013d).

12. *Tomato leaf curl New Delhi virus* (ToLCNDV) (EPPO A2 List)

Symptom description

ToLCNDV is transmitted by *Bemisia tabaci* whiteflies and persists in the vector. The virus causes systemic infections in its hosts and early symptoms become visible in the youngest, uppermost plant parts showing leaf curling, distortion, and blistering. In cucurbit hosts this is associated with discoloration, chlorotic mottling and vein banding followed by upward rolling and general chlorosis of older leaves. In tomato, leaf curling of the apical portions of the plant are the first signs of a developing begomovirus infection, somewhat similar to symptoms caused by *Tomato yellow leaf curl virus*

Sampling and identification

Inspect the plants for symptoms, especially the apical parts. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing. An EPPO Diagnostic Protocol for Begomoviruses in under development and will cover detection and identification of ToLCNDV.

13. *Tomato spotted wilt virus* (TSWV) (EPPO A2 List)

Symptom description

TSWV can induce a wide variety of symptoms. On tomatoes, plants show bronzing, curling, necrotic streaks and spots on the leaves. Dark-brown streaks also appear on leaf petioles, stems and growing tips. The plants are small and stunted compared to healthy plants. On *Capsicum annuum*, symptoms are mainly stunting and yellowing of the whole plant. Leaves may show chlorotic line patterns or mosaic necrotic spots. TSWV is transmitted and spread by insects of the family *Thripidae* (thrips).

Sampling and identification

Inspect the plants for symptoms, especially the leaves, leaf petioles and growing tips. Pay attention to the presence and damage of *Thripidae*, such as silvery

feeding scars on the leaves. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. As damage is not likely to be detected on young plants, representative samples of different lots should be taken and subjected to laboratory testing. It is sufficient to sample leaves instead of the complete plants. Take leaves from 6% of the plants in the lot with a minimum of 10 and a maximum of 25 leaves per sample. Samples should be transported and stored cooled between 4°C and 8°C. Details on the identification of TSWV are included in the EPPO Standard PM 7/139 Tospoviruses (Genus Orthotospovirus) (EPPO, 2020).

14. *Tomato torrado virus* (ToTV) (Tunisia (Quarantine pest), United Kingdom (Quarantine pest))

Symptom description

Affected plants show necrotic lesions at the base of the leaflets, which later turn into shot holes. Longitudinal necrotic lesions also appear on the stems, necrotic line patterns or blotches followed by deformations appear on the fruits rendering them unmarketable. Affected plants present a general burnt-like appearance. In some cases, ToTV has been detected in mixed infections with other viruses (i.e., Pepino mosaic virus in Spain, and Cucumber mosaic virus in Panama), but it is not known whether this might play a role in the disease severity. Although severe symptoms have been observed on some tomato crops, the economic impact of the disease remains to be determined.

Sampling and identification

ToTV is transmitted by whiteflies of the genera *Bemisia* and *Trialeurodes*. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Representative samples of different lots (including different plant parts or whole plants) should be taken and subjected to laboratory testing.

15. *Tomato yellow leaf curl virus* (TYLCV) (EPPO A2 List) and *Tomato yellow leaf curl Sardinia virus* (EPPO, A2 List)

Symptom description

Tomato plants infected at an early stage are severely stunted: their terminal and axillary shoots are erect, and their leaflets are reduced in size and abnormally shaped. Leaves that develop soon after infection are cupped downward, whereas leaves developing later are prominently chlorotic and deformed, with leaf margins rolled upwards and curling between the veins. Tomato yellow leaf curl viruses are transmitted by the whitefly *Bemisia tabaci*.

Sampling and identification

Look for stunted plants, erect shoots with terminal and auxiliary leaves that are reduced in size. Pay attention to the presence and damage of the vector *Bemisia tabaci* (see a2). Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. As damage is not likely to be detected on young plants, representative samples of different lots should be taken and subjected to laboratory testing. It is sufficient to sample leaves instead of the complete plants. Take a minimum of 10 leaves per lot. Samples should be transported and stored cooled, but not colder than 4°C. An EPPO Diagnostic Protocol for Begomoviruses in under development and will cover detection and identification of these viruses.

16. *Zucchini yellow mosaic virus* (ZYMV) (Jordan (A2))

Symptom description

Symptoms on cucumber may consist of mosaic, leaf yellowing, leaf deformation, leaf curling and growth reduction. Symptoms and their severity vary with the virus isolate causing the infection, the plant species and the cultivar, the plant stage and environmental conditions in which infection takes place (EPPO, 2004b).

Sampling and identification

Symptoms are often not sufficiently characteristic for a reliable diagnosis to be made. Additional diagnostic methods may be needed (e.g. mechanical inoculation to test plants, ELISA test) (EPPO, 2004b).

APPENDIX 2 - SHORT PROCEDURE FOR INSPECTORS

Time of inspection

The most appropriate time for inspections of vegetable plants for planting is in the period from 1 week after placing the plants out in the greenhouse until the delivery of the plants. For grafted and/or pinched out plants (e.g., tomatoes, cucumber, eggplant) the most appropriate time for inspections and sampling is 10 days after grafting and/or pinching out.

Hygiene measures

In order not to spread and increase infestations, adequate precautions should be taken during inspection and sampling such as wearing protective clothes: coat, overshoes, gloves etc. Gloves should be changed between different lots. All used equipment for sampling should be disinfected between different lots.

Lot identification

A lot is a number of plants that are produced from the same seed lot(s) and are treated in the same way and at the same time (plants which were sowed, grafted, pinched out, etc. on the same day). In practice a lot is often the same as a batch of plants destined for a specific vegetable producer or customer.

Inspection

- Overall examination to check the physical condition of the plants.
- Inspection of plants in the vicinity of the place of production, e.g., weeds.
- Examination of traps: yellow sticky traps (*Bemisia*, *Liriomyza*), delta-traps with specific pheromones (*Leucinodes orbonalis*, *Keiferia lycopersicella*, *Tuta absoluta*) etc.
- Thorough examination of lots with an abnormal level of dying off, with differences in colour, plants with an abnormal growth, plants with a flaccid appearance, wilting plants, stunted plants, in size reduced leaves…
- Examination of leaves (upper and lower leaf surface) and the crown of the plants for insects in any stage, including black frass of larvae.
- Plants should be shaken for the presence of adults of *Bemisia tabaci* and tapped upon white paper for the presence of Thripidae.
- Inspection of the leaves (upper and lower leaf surface) for
  - Mines (*Liriomyza* spp., *Tuta absoluta*, *Keiferia lycopersicella*)
  - Honeydew and associated sooty moulds (*Bemisia tabaci*)
  - Lesions, chlorotic spots, yellowing etc. (bacteria, viruses)
  - Vein yellowing (viruses)
- Pay attention to vectors such as leafhoppers (vectors of 'Candidatus *Phytoplasma solani*'), the whitefly *Trialeurodes vaporariorum* (vector of *Tomato infectious chlorosis virus*) and *Thripidae* (vector of *Tomato spotted wilt virus*).

Sampling for laboratory testing

- If suspicious symptoms or signs are detected, samples should be taken and subjected to laboratory testing in order to identify the pest.
- If a pest that is suspected to be a pest recommended for regulation as a quarantine pest is found, the suspect lot should be detained under official control pending a test result.
- Laboratory tests should be done to detect the presence of pests in a latent stage such as *Clavibacter michiganensis* subsp. *michiganensis*, *Pospiviroids* and viruses.
- A sample for the detection of *Clavibacter michiganensis* subsp. *michiganensis* and *Ralstonia solanacearum* species complex or *Acidovorax citrulli* should consist of whole plants. For other pests parts of the plants (e.g. leaves) may be taken.
- Each sample should be individually labelled with nursery name (or reference number), sample number, date, plant species, plant variety if necessary and lot number so follow-up action can be taken if necessary.
- Samples of insects, larvae, pupae and eggs should be put in a pot with screw cap. Living organisms should be sent to the laboratory together with plant material of the host plant in suitable containers. Dead organisms should be kept in alcohol in order to prevent decomposition during transport.
- Samples of plants parts should be put in plastic bags together with a piece of absorbent paper. If the plant parts are dry, a piece of slightly damp absorbent paper should be used; for wet plant parts a piece of dry absorbent paper must be used (to avoid rotting of the plant parts).
- Samples of whole plants with roots in potting compost, substrate, etc. should be put in plastic bags. Absorbent paper is not needed as they will not easily dry out.