Bulletin OEPP/EPPO Bulletin (2016) **46** (1), 40–48 ISSN 0250-8052. DOI: 10.1111/epp.12269

Phytosanitary procedures
Procédures phytosanitaires

PM 3/77 (1) 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: *Brassica* spp. (cabbage), *Capsicum* spp. (sweet pepper), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Cucurbita pepo* (zucchini/courgette), *Lactuca* spp. (lettuce), *Solanum lycopersicum* (tomato), *Solanum melongena* (eggplant/aubergine).[1]

Herb plants and potatoes are not covered by this Standard, nor are procedures for inspecting imported plants from outside the EPPO region. The Standard includes relevant pests and sampling. The Standard focuses on pests which are present in the EPPO region or which might be encountered in the region in future. Evidence gathered from inspections carried out according to this Standard may be used to help demonstrate freedom from relevant pests.

Specific approval

This Standard was first approved in 2015-09.

Introduction

Vegetable plants for planting are an important production sector in the EPPO region and represent an important pathway for the entry and spread of pests, since contaminated vegetable plants for planting from a single lot 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 that only healthy plants for planting are placed on the market.

Inside a nursery, an outbreak can be further spread through activities such as grafting, removal of old material by cutting tools, de-heading or pinching out, physical contact between different lots and spread by vectors. Delivering those plants for planting to a large number of vegetable producers may result in the 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.

[1]This Standard forms part of a new series of EPPO Inspection Standards and will be reviewed by the end of 2017. Comments to be taken into account during that review should be sent to the EPPO Secretariat at hq@eppo.int.

Phytosanitary inspections

General background information on phytosanitary inspection of places of production is given in EPPO Standard PM 3/72 *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* and in ISPM no. 23 *Guidelines for inspection*.

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

Visual 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 EPPO Standard PM 5/2 *Pest risk analysis on detection of a pest in an imported consignment* should be followed to allow the NPPO to make a decision as to what phytosanitary action to take.

For an indication of the status of these pests, consult the latest version of the Plant Quarantine Data Retrieval System (PQR; http://www.eppo.int/DATABASES/pqr/pqr.htm) or EPPO Global Database (https://gd.eppo.int/).

Sampling for visual inspection should be done 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 inspection 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 crop (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, for example weeds of host plants that grow in the nursery.

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

The size of the unit of inspection or sample (the minimum number of individuals to be examined) should be determined on the basis of lots undergoing inspection, taking into account the statistical background provided in ISPM no. 31 *Methodologies for sampling of consignments*. Inspection of a sample of 4600 plants selected at random provides at least a 99% confidence of detecting a level of infection present at 0.1%.

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. 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.

Trapping for the monitoring of pests such as leaf miners, thrips or whiteflies is also an important supplement to the visual inspection. Specific details are listed in Appendix 1.

Inspections and sampling can themselves be a pathway for spreading infestations. Therefore inspectors should take all necessary precautions during inspection and sampling, such as wearing protective clothes: coat, overshoes, gloves, etc. Gloves must be changed between different lots. All sampling equipment used must be disinfected between different lots.

Production sector concerned

Vegetable plants for planting grown under protected conditions (i.e. not grown in the open air) are in the scope of this Standard.

The production of these plants takes place in specialized nurseries. The plants are grown from seeds usually imported by the nurseries. Large seeds lots are used for the production of numerous lots of vegetable plants for planting. For grafted plants, different seed lots (for rootstock and variety grafts) are used.

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.

Pests of concern for the EPPO region

This Standard mainly relates to pests in EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests. The pests covered in this Standard are recognized to be of primary importance for vegetable plants for planting. The phytosanitary procedures described in this Standard are primarily aimed at preventing the spread of these specific pests in the EPPO region through trade of vegetable plants for planting. 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 on all of these pests can be found in *Quarantine Pests for Europe*, 2nd edition (EPPO/CABI, 1997) and in EPPO Datasheets. For additional up-to-date information the relevant scientific literature should be consulted, as well as the PQR and EPPO Global Database.

EPPO Lists of A1 and A2 pests are subject to additions and deletions. The present lists (Tables 1–9) will therefore need to be revised whenever relevant new quarantine pests are identified.

Table 1 Polyphagous pests

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Insects	**Insects**
	Bemisia tabaci	*Liriomyza bryoniae* [Turkey (‘A2 List’), European Union ‘Annexe I/B’]
	Liriomyza huidobrensis	
	Liriomyza sativae	
	Liriomyza trifolii	

Table 2 Specific pests of *Brassica* spp. (cabbage)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Viruses	
	Tomato spotted wilt virus	

Table 3 Specific pests of *Capsicum* spp. (sweet pepper)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects	**Bacteria (including phytoplasmas)**	
Leucinodes orbonalis	'*Candidatus* Phytoplasma solani'	
	Ralstonia solanacearum	
	Xanthomonas axonopodis pv. *vesicatoria*	
	Viruses and viroids	
	Potato spindle tuber viroid	
	Tomato infectious chlorosis virus	
	Tomato spotted wilt virus	
	Tomato yellow leaf curl virus	

Table 4 Specific pests of *Cucumis melo* (melon)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Viruses	
	Cucumber vein yellowing virus	
	Cucurbit yellow stunting disorder virus	
	Tomato spotted wilt virus	

Table 5 Specific pests of *Cucumis sativus* (cucumber)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Viruses	
	Cucumber vein yellowing virus	
	Cucurbit yellow stunting disorder virus	
	Tomato ringspot virus	
	Tomato spotted wilt virus	

Table 6 Specific pests of *Cucurbita pepo* (zucchini/courgette)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Viruses	
	Cucumber vein yellowing virus	
	Cucurbit yellow stunting disorder virus	
	Tomato spotted wilt virus	

Table 7 Specific pests of *Lactuca* spp. (lettuce)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
	Viruses	
	Cucurbit yellow stunting disorder virus	
	Tomato infectious chlorosis virus	
	Tomato spotted wilt virus	

Table 8 Specific pests of *Solanum lycopersicum* (tomato)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects	**Insects**	
Keiferia lycopersicella	*Tuta absoluta*	
Leucinodes orbonalis		
	Bacteria (including phytoplasmas)	
	Clavibacter michiganensis subsp. *michiganensis*	
	'*Candidatus* Phytoplasma solani'	
	Ralstonia solanacearum	
	Xanthomonas axonopodis pv. *vesicatoria*	
	Viruses and viroids	
	Potato spindle tuber viroid	
	Tomato infectious chlorosis virus	
	Tomato ringspot virus	
	Tomato spotted wilt virus	
	Tomato yellow leaf curl virus	
	Pepino mosaic virus	

Table 9 Specific pests of *Solanum melongena* (eggplant/aubergine)

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects	**Insects**	
Keiferia lycopersicella	*Tuta absoluta*	
Leucinodes orbonalis		
	Bacteria (including phytoplasmas)	
	'*Candidatus* Phytoplasma solani'	
	Ralstonia solanacearum	
	Viruses and viroids	
	Potato spindle tuber viroid	
	Tomato spotted wilt virus	

Sampling for laboratory testing

Samples should be taken from plants on which pests or signs of them are present and cannot be immediately identified by the inspector, and from plants showing suspicious symptoms or deformations. In these cases the sample consists of the suspect plant(s).

Visual examination of vegetable plants for planting alone is not considered to be 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 freedom from pests.

The size of the sample to be taken 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 preferably 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 the complete plants in order to give a possibility of testing for the whole range of potential pests.

Nevertheless, 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 follow-up action can be taken if necessary.

In order to identify the pest, sampled material such as plants and plant parts should be kept in good condition and

placed 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 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 a 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 kept in alcohol in order to prevent decomposition during transport.

If a pest found during inspection is suspected by the inspector 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. the 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.

Acknowledgements

This Standard was first drafted by Ms S. Devlieghere, from the Federaal Agentschap voor de Veiligheid van de Voedselketen (BE).

References

EPPO/CABI (1997) *Quarantine Pests for Europe*, 2nd edition. Edited by Smith IM, McNamara DG, Scott PR, Holderness M. CAB International, Wallingford (UK).

EPPO (2002) Pest risk analysis on detection of a pest in an imported consignment. *EPPO Bulletin* **32**, 231–233.

EPPO (2005) *Liriomyza* spp. *Bulletin OEPP/EPPO Bulletin* **35**, 271–273.

EPPO (2009) Standard PM 3/72 *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification. Bulletin OEPP/EPPO Bulletin* **39**, 260–262.

EPPO (2015a) *Global Database*. https://gd.eppo.int/ [accessed on 25 February 2015].

EPPO (2015b) *Plant Quarantine Data Retrieval System* (PQR). http://www.eppo.int/DATABASES/pqr/pqr.htm [accessed on 25 February 2015].

FAO (2005) *Guidelines for inspection*. ISPM no. 23. IPPC Secretariat, FAO, Rome (IT).

FAO (2008) *Methodologies for sampling of consignments*. ISPM no. 31. IPPC Secretariat, FAO, Rome (IT).

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 *Quarantine Pests for Europe*, 2nd edition (EPPO/CABI, 1997), as well as in EPPO Datasheets and EPPO Diagnostic Standards. Illustrations are available on the EPPO website (http://www.eppo.int). 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 there is no diagnostic method 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 the purposes of illustration.

(A) Insects

(1) *Bemisia tabaci* (EPPO A2 List)

Symptom description. Chlorotic spots develop on the leaves of affected plants, 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. In severe infestations, numerous small white adult whiteflies will flutter out and quickly resettle when the plant is shaken.

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. Yellow sticky plates can be used for the detection and monitoring of *B. tabaci*. These traps should be placed 20–30 cm above the crop. Where appropriate, samples for laboratory testing should be taken for final identification of the pest. Details on identification of *B. tabaci* are included in EPPO Standard PM 7/35 Bemisia tabaci.

(2) *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 the final instar entering 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 is high.

Sampling and identification. Inspectors should look for adults. Delta traps with specific pheromones and sticky plates can be used for the detection and monitoring of *L. orbonalis*, along with funnel traps and light traps. Where appropriate, samples for laboratory testing should be taken for final identification of the pest.

(3) *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* (EPPO A2 List) and *Liriomyza bryoniae*

Symptom description. Leaves of infested plants have small feeding/oviposition punctures and/or serpentine, irregular mines. The mines are usually white with damp black and dry brown areas.

Sampling and identification. Inspect the leaves for mines and inspect the mines for larvae. Yellow sticky plates can be used for the detection and monitoring of *Liriomyza* spp. These traps should be hung 20–30 cm above the crop. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Details on the identification of *Liriomyza* spp. are included in EPPO Standard PM 7/53 Liriomyza *spp*.

(4) *Keiferia lycopersicella* (EPPO A2 List)

Symptom description. Adults are mottled brown with a length of 9–12 mm. In foliage, the first and second instars of larvae feed as leafminers, producing a blotch mine. Later instars typically fold leaves or attach pairs of leaves with silk to create sheltered feeding sites, but may enter stems.

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

(5) *Tuta absoluta* (EPPO A2 List)

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

Infected plants show conspicuous mines and galleries. Leaf mines are irregular and may later become necrotic. On young plants, the pest prefers apical buds, on which the black frass can be visible.

Sampling and identification. Look for adults between the leaves and inspect the apical buds thoroughly for mines and black frass. Delta traps with specific pheromones and sticky plates can be used for the detection and monitoring of *T. absoluta*. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest.

(B) Bacteria (including phytoplasmas)

(1) *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.

Sampling and identification. Visual inspection of young plants is not feasible. Representative samples of different lots should be taken and subjected to laboratory testing. Complete plants should be sampled. The plants must be tested at the height of the graft and the rootstock should also be analysed. One hundred plants should be sampled per 20 000 plants or per lot (except for small lots, where it is preferable to refer to the tables in ISPM no. 31 *Methodologies for sampling of consignments*). Details on the identification of *C. michiganensis* subsp. *michiganensis* are included in EPPO Standard PM 7/42 Clavibacter michiganensis *subsp.* michiganensis.

(2) '*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 infested (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 sites of infection as a result of abnormal phloem formation. Lateral shoots develop, giving the plant a bushy aspect.

Sampling and identification. Plants, especially the leaves, should be inspected 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.

(3) *Ralstonia solanacearum* Race 1 and Race 3 (EPPO A2 List)

Symptom description. 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 35–37°C for Race 1 and 27°C for Race 3), wilting of the whole plant may follow rapidly. 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.

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. 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. Where appropriate also samples of irrigation (drainage) water (500 mL) should be taken for laboratory testing. Water samples should be ideally transported and stored between 10 and 20°C.

Details on identification of *R. solanacearum* are included in EPPO Standard PM 7/21 Ralstonia solanacearum.

(4) *Xanthomonas axonopodis* pv. *vesicatoria* (EPPO A2 List)

Symptom description. Lesions appear on leaves as irregular water-soaked areas, at first green, later becoming brown and necrotic.

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 of *X. axonopodis* pv. *vesicatoria* are included in EPPO Standard PM 7/110 Xanthomonas *spp*. *(*Xanthomonas euvesicatoria*,* Xanthomonas gardneri*,* Xanthomonas perforans*,* Xanthomonas vesicatoria*) causing bacterial spot of tomato and sweet pepper*.

(C) Viruses and viroids

(1) Pospiviroids

Relevant pospiviroids: *Columnea latent viroid*, *Potato spindle tuber 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.

Sampling and identification. Visual inspection 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. Take 2 samples for lots of 201–5000 plants, and 3 samples for lots of more than 5000 plants. Samples should be transported and stored cooled, but not colder than 4°C. Details on the identification of *Chrysanthemum stunt viroid* are included in EPPO Standard PM 7/6 Chrysanthemum stunt pospiviroid. Details on the identification of *Potato spindle tuber viroid* are included in EPPO Standard PM 7/33 Potato spindle tuber pospiviroid. A generic EPPO Diagnostic Protocol on pospiviroids is in preparation.

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

Symptom description. There is a wide range of symptoms, from chlorotic mottling to vein yellowing, vein clearing and stunting, or no symptoms. *Cucumber vein yellowing virus* is transmitted by the whitefly *B. tabaci.*

Sampling and identification. Look for symptoms such as vein yellowing and vein clearing. Pay attention to the presence and damage caused by the vector *B. tabaci* (see Appendix 1, Section A1). 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 *Cucumber vein yellowing virus* are included in EPPO Standard PM 7/81 (1) Cucumber vein yellowing virus *(Ipomovirus).*

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

Symptom description. Affected plants 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. *Cucurbit yellow stunting disorder virus* is transmitted by the whitefly *B. tabaci.*

Sampling and identification. Look for interveinal mottle on the oldest leaves. Pay attention to the presence and damage of *B. tabaci* (see Appendix 1, Section A1). 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) *Pepino mosaic virus* (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. Take 2 samples for lots of 201–5000 plants and 3 samples for lots of more than 5000 plants. Samples should be transported and stored cooled, but not colder than 4°C. Samples taken for laboratory testing of pospiviroids can also be analysed for *Pepino mosaic virus*. Details on the identification of *Pepino mosaic virus* are included in EPPO Standard PM 7/113 Pepino mosaic virus.

(5) *Tomato infectious chlorosis virus* (EPPO A2 List)

Symptom description. The first indication of infection is a bright interveinal yellowing symptom 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 *Trialeuroides 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 *T. 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 *Tomato infectious chlorosis virus* are included in EPPO Standard PM 7/118 Tomato chlorosis virus *and* Tomato infectious chlorosis virus.

(6) *Tomato ringspot virus* (EPPO A2 List)

Symptom description. There is a conspicuous curling and necrosis of the terminals of one or more actively growing shoots. The basal portions of younger leaves develop brown, clearly defined necrotic rings and sinuous lines.

Sampling and identification. Inspect the shoots and leaves for symptoms. Where appropriate, samples for laboratory testing should be taken for the final identification of the pest. Details on the identification of *Tomato ringspot virus* are included in EPPO Standard PM 7/49 Tomato ringspot nepovirus.

(7) *Tomato spotted wilt virus* (EPPO A2 List)

Symptom description. Tomato spotted wilt virus 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 in comparison with healthy plants. On *C. annuum*, symptoms are mainly stunting and yellowing of the whole plant. Leaves may show chlorotic line patterns or mosaic necrotic spots. *Tomato spotted wilt virus* 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 and 8°C. Details on the identification of *Tomato spotted wilt virus* are included in EPPO Standard PM 7/34 Tomato spotted wilt tospovirus, Impatiens necrotic spot tospovirus *and* Watermelon silver mottle tospovirus.

(8) *Tomato yellow leaf curl 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 virus* is transmitted by the whitefly *B. 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 *B. tabaci* (see Appendix 1, Section A1). 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 complete plants. Take a minimum of 10 leaves per lot. Samples should be transported and stored cooled, but not colder than 4°C. Details on the identification of *Tomato yellow leaf curl virus* are included in EPPO Standard PM 7/50 Tomato yellow leaf curl *and* Tomato mottle begomoviruses.

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 must be changed between different lots. All equipment used for sampling must 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 sown, 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.

Visual 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 plates (*Bemisia*, *Liriomyza*), delta traps with specific pheromones (*L. orbonalis*, *K. lycopersicella*, *T. 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 *B. tabaci*.
- Inspection of the leaves (upper and lower leaf surface) for:
  (i) mines (*Liriomyza* spp., *T. absoluta*, *K. lycopersicella*)
  (ii) honeydew and associated sooty moulds (*B. tabaci*)
  (iii) lesions, chlorotic spots, yellowing, etc. (bacteria, viruses)
  (iv) 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 (vectors 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 is found that is suspected to be a pest recommended for regulation as a quarantine pest, 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 *C. michiganensis* subsp. *michiganensis*, pospiviroids and viruses.
- A sample for the detection of *C. michiganensis* subsp. *michiganensis* and *R. solanacearum* should consist of complete 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 should be used (to avoid rotting of the plant parts).
- Samples of complete 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.