APPROVAL
EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

REVIEW
EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Phytosanitary Regulations.

AMENDMENT RECORD
Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

DISTRIBUTION
EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

SCOPE
EPPO Phytosanitary Procedures are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the inspection, testing and treatment of plants and plant products moving in trade, or for the implementation of surveys against quarantine pests.

REFERENCES

DEFINITIONS
Phytosanitary procedure: Any officially prescribed method for performing inspections, tests, surveys or treatments in connection with plant quarantine.
Inspection: Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations.
Survey: An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.
Test: Official examination, other than visual, to determine if pests are present or to identify pests.
Treatment: An officially authorized procedure for the killing, removal or rendering infertile of pests.
OUTLINE OF REQUIREMENTS

EPPO Phytosanitary Procedures describe the methods to be followed for performing inspections, tests, or treatments of commodities moving in trade, or surveys against quarantine pests. For many quarantine pests, a reference to the relevant EPPO Phytosanitary Procedure is made in the corresponding EPPO Specific Quarantine Requirements. The development of EPPO phytosanitary procedures started many years ago, and these methods have been published in the Bulletin OEPP/EPPO Bulletin under several titles: ‘Fumigation standards’, ‘Quarantine Inspection Procedures’ and ‘Quarantine Procedures’. All of them are now appearing under the title ‘EPPO Phytosanitary Procedures’ and are being edited into EPPO Standard format. The numbering of these procedures will continue to follow the sequence described in the Bulletin OEPP/EPPO Bulletin 20(2), 229-233, which corresponds approximately to the chronological order of appearance of the Phytosanitary Procedures.
Phytosanitary procedure

IDENTIFICATION OF LIRIOMYZA SPP.

Specific scope
This standard describes the identification methods for Liriomyza species.

Specific approval and amendment
First approved in September 1991.

Introduction

Three species of Liriomyza are EPPO quarantine pests. L. trifolii is an A2-listed organism, whereas L. sativae and L. huidobrensis are Al quarantine pests. Details about their biology, distribution and economic importance can be found in Data sheet no. 131 (OEPP/EPPO, 1984). Other species, especially L. bryoniae, are widespread in Europe.

The EPPO specific quarantine requirements for Liriomyza spp. (and Amauromyza maculosa covered by the same SQR) (OEPP/EPPO, 1990) demand that consignments from countries where the pests occur should have been inspected and found free from infestation. In the case of L. trifolii, this is a requirement only for plants intended for planting and propagation and is optional for certain named cut flowers, pot plants and raw vegetables; the consignment freedom is required for all plants in the case of the other species. In addition to this, importation of plants for planting of species known to be hosts of the Liriomyza spp. may be prohibited from countries where the pests occur, or, if not prohibited, the plants should have been inspected at monthly intervals prior to dispatch and found free. In the case of L. trifolii, the option is allowed to perform an approved treatment of the host plants (see EPPO Quarantine procedure for treatment against Liriomyza spp., in preparation).

The inspection of consignments is initially a visual search for mines on the leaves. If mines are found, then larvae and/or pupae are collected from within or on the leaves for precise identification. It is necessary to distinguish the Liriomyza spp. which are quarantine pests from those which are not, and also in some circumstances between the quarantine-significant Liriomyza spp.

Methods

Differences exist between the types of mines produced by the different species (for example L. huidobrensis feeds close to the midrib or other vascular tissue of the leaves) but these differences depend very much on the host species and they are not considered to be reliable diagnostic characters. Larvae and pupae are differentiated by the number and arrangement of stigmatal pores (7-12 in L. bryoniae, 6-9 in L. huidobrensis and 3 in L. sativae and L. trifolii).

Rearing to adult allows the possibility of differentiation by means of the wing venation, length and colour of antennal segments, colour of the femora and of the hind margin of the eye, colour of the mesonotum and length of its setation, length of the genitalia of the males
and the ovipositors of the females. The morphological differences are described in Spencer (1973) and Knodel-Montz & Poe (1982). This method is evidently slow.

Gel electrophoresis with enzyme staining (Menken & Ulenberg, 1983, 1986) can be used to distinguish clearly between different species for all life stages and using as few as single individuals. The method obviously requires adequate experience in electrophoresis and the availability of a range of appropriate equipment and reagents. However, once prepared the method can provide an unequivocal identification within 24 h. It is only relevant if there is circumstantial evidence (morphology, host plants etc.) that the specimen to be identified belong to one of the four species *L. bryoniae, L. huidobrensis, L. sativae* or *L. trifolii*. Details are given in Appendix I.

**APPENDIX I**

*Gel electrophoresis*

The method uses horizontal starch gel electrophoresis and staining of the soluble enzymes isocitrate dehydrogenase (Idh), 6-phosphogluconate dehydrogenase (6 Pgdh) and malic enzyme (Me). The patterns obtained are compared with those of a standard sample of *L. bryoniae* and identification is performed by means of a dichotomous key.

*Specimen preparation*

Living or frozen (-20°C) larvae, pupae and/or adults are homogenized individually in 15 µl distilled water in a 1.88 ml Eppendorf tube. Another 30 µl distilled water is added and the mixture stirred. The mixture is stored at between -30 and –80°C (the colder the better) until used for electrophoresis.

For the electrophoretic runs, a small amount of the thawed supernatant is absorbed with one 4 x 5 or 4 x 10 mm piece of Whatman no. 3 filter paper, depending on the thickness of the gel. The remainder of the homogenate is stored at -30°C for further testing. This cycle of freezing-thawing-refreezing has no effect on allozyme mobility and activity provided that the temperature of the sample does not rise above 5°C. This can be achieved by working in melting ice and ice water.

*Gel preparation*

The horizontal starch gel is a 12% Connaught starch gel, i.e. 12 g starch hydrolyzed in 100 ml gel buffer.

*Electrophoresis*

The buffer system used for Idh assay is: electrode buffer – 0.23 M Tris and 0.086 M citric acid, pH 6.3; gel buffer – 0.008 M Tris and 0.003 M citric acid, pH 6.7; electrophoresis at 2.5 h at 20 V cm⁻¹ using a gel 0.5 cm thick.

For 6 Pgdh and Me, the buffer system is: electrode buffer – 0.01 M EDTA, 0.25 M boric acid, 0.45 M Tris, pH 8.7; gel buffer – 1:20 diluted electrode buffer; electrophoresis at 5 h at 20 V cm⁻¹ using a gel 1 cm thick.

The gel is cut into two at 2 cm from the cathode end. Between the two parts of the gel the pieces of filter paper with supernatant are placed, using forceps, 5 mm from each other. Each gel must be run together with replicates of two control samples from a reference population.
(e.g. *L. bryoniae*) and a piece of filter paper with the marker dye bromocresol green (0.2%).

The electrophoresis is carried out at 5°C (e.g. in a refrigerator).

**Staining**

All stains are prepared just before use. All incubations are carried out in the dark at 37°C. To stain for 6 Pgdh and Me, the gel has to be cut into two slices of 0.5 cm thickness.

Stain buffer pH 8.4: distilled water 2 l, Tris 24.7 g, HCl (concentrated) 5.6 ml.

Stain for Idh: stain buffer 300 ml, isocitric acid 0.40 ml, MnCl$_2$ 0.20 mg, MTT 0.05 g, NADP 0.04 g, PMS pinch. Incubate for 2-12 h.

Stain for 6 Pgdh: stain buffer 300 ml, 6-phosphogluconic acid 0.075 g, NADP 0.025 g, MTT 0.025 g, PMS pinch. Incubate for 2 h.

Stain for Me: stain buffer 300 ml, malic acid 0.55 g, MTT 0.03 g, NADP 0.03 g, MnCl$_2$ 0.15 g, PMS pinch. Incubate for 2 h.
Interpretation

Biochemical key to the species of Liriomyza (L. bryoniae is used as standard):

1. 6 Pgdh mobility same as or slower than standard…………… 2
2. 6 Pgdh mobility faster than standard………………………... 3

2. Idh mobility same as standard………………………………. bryoniae
- Idh mobility slower than standard…………………………... huidobrensis
3. Idh mobility same as standard. Me mobility notably slower than standard……………………………… trifolii
- Idh mobility faster than standard. Me mobility same or somewhat slower than standard…………………………... sativae

References

OEPP/EPPO (1990) Specific quarantine requirements. EPPO Technical Documents no. 1008.

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