



SAMPLING PROCEDURES

(As amended on 24 November 2022)

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1 SAMPLING FOR NEMATODE ANALYSIS

- 1.1 Collected soil samples for the detection and identification of *Xiphinema index* must, as far as possible, include roots.
- 1.2 At least 50 random subsamples per hectare must be collected at a depth of 500 mm with the use of a spade or auger with a diameter of 20 mm.
- 1.3 Mix subsamples thoroughly and provide 1 L soil for analysis.
- 1.4 Samples must not be exposed to extreme heat.
- 1.5 Samples must be handled with care and submitted for analysis within 72 hours after sampling was conducted.
- 1.6 Plastic bags containing samples must be clearly marked with all the necessary information as requested.
- 1.7 Root samples must be taken from all suspect plants for visual inspection.

2 SAMPLING FOR FANLEAF TESTING

- 2.1 Sampling must be conducted as follows:
 - a) Standard material - a representative sample must be collected and pooled as prescribed.
 - b) Suspect material - samples are collected from vines that express suspicious symptoms. Each vine is sampled and tested separately.
- 2.2 Leaf samples are collected from shoots with a length of at least 100 mm and sampling can continue as long as the positive control plant tests positive;
- 2.3 Sampling must preferably be conducted during the cooler period of the day thus during the morning or on cloudy days.
- 2.4 A leaf sample consists of 2-3 small, young, soft developing leaves per vine.
- 2.5 Samples must be placed in plastic bags and kept in a cooler-bag or held at 4 °C and must be submitted for analysis within 72 hours after sampling.
- 2.6 Samples must be clearly labelled and must contain all the necessary information such as variety/clone, row number, vine number, block number, date, owner and farm;

- 2.7 Leaf samples are normally tested for Grapevine fanleaf virus within 1 to 3 days using the ELISA method;
- 2.8 It may be more convenient to bulk up the samples before the ELISA testing. The following procedure is recommended:
 - a) Leaves are sampled in the morning, stored in a cooler bag and grinded as soon as possible before being stored in the freezer.
 - b) Frozen leaf samples must be tested within a month.
 - c) It is of extreme importance for a positive control (leaves from a known infected vine) to be frozen together with the other samples. The control samples must be handled in the same way as the other samples. The control must clearly indicate a positive result to ensure the viability of the test.
- 2.9 Phloem scrapings from dormant winter shoots: Use one ripe shoot base from rootstocks or scions where petioles are no longer available.

3. SAMPLING FOR LEAFROLL ASSOCIATED VIRUSES TESTING

- 3.1 Sampling must be conducted as follows:
 - a) Standard material: a representative sample must be collected and pooled as prescribed in Paragraph 13.5.
 - b) Suspect material: Samples are collected from vines that express suspicious symptoms. Each vine is sampled and tested separately.
- 3.2 This test is only applicable to scion varieties as well as rootstock varieties that are crossings of *Vitis vinifera* namely Jacques, 143 B, US 2-1 and US 8-7.
- 3.3 Period of sampling: **1 March – 31 August**
- 3.4 Collect three basal petioles per plant for scions and rootstocks.
- 3.5 One ripe shoot from the basal area of the scion or rootstock can be sampled in the event of petioles not being available.
- 3.6 Samples can be pooled to a maximum of 12 plants per pooled sample.
- 3.7 Collect fresh petioles from basal leaves as well as suspect leaves.
- 3.8 Place all samples in plastic bags and keep cool at 4°C.
- 3.9 Submit samples for analysis on the same day, if possible.

- 3.10 Samples must be clearly labelled and must contain all the necessary information such as variety/clone, row number, vine number, block number, date, owner and farm.
- 3.11 Processed samples in a buffer solution can be tested immediately or stored at -20 °C for a maximum period of three months.
- 3.12 The positive and negative controls must be handled in the same way as all the other test samples to ensure the reliability of the ELISA test.

4 SAMPLING IN GREENHOUSES AND TUNNELS FOR ELISA TESTS

4.1 Fanleaf

- a) Grapevine fanleaf virus testing can only be reliable when plants are not exposed to high temperatures (> 25°C) for long periods. For this reason, the sampling period is critical.
- b) Sampling after 15 December can only be conducted given that the temperature in the greenhouse or tunnel varies between 20-25°C.
- c) Only young, actively growing leaves must be sampled.
- d) As an alternative, phloem scrapings from dormant winter shoots can be used: Use one ripe shoot base from rootstocks or scions where petioles are no longer available.

4.2 Leafroll associated viruses

- a) This test is only applicable on scion varieties as well as rootstock varieties that are crossings of *Vitis vinifera* namely Jacques, 143 B, US 2-1 and US 8-7.
- b) Period of sampling: 1 **March – 31 August**
- c) For the collection of plant material, petioles can be sampled if available.
- d) Bark material can also be used for testing during winter sampling.
- e) Collect three basal petioles per plant for scions and rootstocks.
- f) One ripe shoot from the basal area of the scion or rootstock can be sampled in the event of petioles not being available.
- g) Samples can be pooled to a maximum of 12 plants per pooled sample.

- h) Sample fresh petioles from basal leaves as well as suspect leaves.
- i) Place all material in plastic bags and keep cool at 4°C.
- j) Submit samples for analysis on the same day, if possible.
- k) Samples must be clearly labelled and must contain all the necessary information.
- l) Processed samples in a buffer solution can be tested immediately or stored at -20 °C for a maximum period of three months.
- m) The positive and negative controls must be handled in the same way as all the other test samples to ensure the validity of the ELISA test.

5 SAMPLE SIZE

5.1 Fanleaf

Unit	Sample	Test
Nucleus unit	Each vine	Each vine
Foundation: Glasshouse	Each vine	Pooling up to 12 vines
Foundation: Field	Each vine	Pooling up to 12 vines
Mother units	5% statistical basis (1 in 20 vines)	Pooling up to 12 vines

5.2 Leafroll Virus

Unit	Sample	Test
Nucleus unit	Each vine	Each vine
Foundation: Glasshouse	Each vine	Pooling up to 12 vines
Foundation: Field	Each vine	Pooling up to 12 vines
Mother units	Each vine	Pooling up to 12 vines