◆ EPPO Standards ◆

PHYTOSANITARY PROCEDURES

SYNCHYTRIUM ENDOBIOTICUM: SOIL TESTS AND DESCHEDULING OF PREVIOUSLY INFESTED PLOTS

PM 3/59(2) English

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REVIEW

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AMENDMENT RECORD

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

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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

CABI/EPPO (1997) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

OEPP/EPPO (1996) Glossary of phytosanitary terms. *EPPO Technical Documents* no. 1026.

OEPP/EPPO (1998) EPPO Standards PM 2 Specific Quarantine Requirements. Available as electronic documents from the EPPO Web Site www.eppo.org.

DEFINITIONS

Phytosanitary procedure

Any officially prescribed method for performing inspections, tests, surveys or treatments in connection with regulated pests.

Inspection

Official visual examination of plants, plant products or other regulated articles to determine whether pests are present and/or to determine compliance with phytosanitary regulations.

Survey

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 whether pests are present or to identify pests.

Treatment

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 (EPPO Standards PM 2).

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

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Phytosanitary procedures

SYNCHYTRIUM ENDOBIOTICUM: SOIL TESTS AND DESCHEDULING OF PREVIOUSLY INFESTED PLOTS

Specific scope

This standard describes soil tests for *Synchytrium endobioticum* and a procedure for descheduling plots previously infested by this pest.

Specific approval and amendment

First approved in 1999-09.
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Introduction

Synchytrium endobioticum is an A2 quarantine pest, and details of its biology, distribution and economic importance can be found in the data sheet covering the pest (EPPO/CABI, 1996). In the EPPO specific quarantine requirements (SQR) for *S. endobioticum*, no inspection procedures are considered for the pathogen on potatoes as such, because the wart disease regulations of most countries subject any plot known to be wart-infested to long-term official control and prohibit the growing of potatoes on it; in addition, many countries allow only resistant cultivars to be grown in a surrounding zone. The SQR instead requires that exported potatoes, plants with roots, flower bulbs and tubers should come from a plot in which *S. endobioticum* has never occurred, or else from a plot found free from *S. endobioticum*. As EPPO countries have, in general, kept detailed records of the distribution of wart incidence since the beginning of the twentieth century, the second requirement principally concerns the procedure of releasing previously infested plots from official control (revoking the demarcation of the contaminated plot, in the language of EU Directive 69/464 (EU, 1969), or "descheduling"). It could, however, also be applied to plots for which records provide no information.

The methods described here are for testing the soil in a plot that has been "scheduled", i.e. demarcated as contaminated (in the language of EU Directive 69/464), because symptoms of potato wart disease have been detected at an earlier date. There is a presumption that resting sporangia of *S. endobioticum* survive on the plot. The soil test, combined with the interval since the previous infection, may be used as a criterion for complete or partial descheduling of that plot; complete descheduling removes all official limitation on the types of crops that may be grown, whereas partial descheduling removes only some of the limitations on use and allows the growing of resistant ware potatoes. Caution should be applied when growing resistant cultivars after partial descheduling in case of possible breakdown of resistance.

Descheduling relates to the entirety of the plot that was originally scheduled. However, for partial descheduling, there may be conditions in which a subunit of the plot may be considered separately, provided that the NPPO can guarantee the phytosanitary security of the subplot.

Methods

Methods are of three types: (1) direct examination of soil for presence of viable resting sporangia; (2) bioassay methods; (3) field test. For the latter two methods, it is essential to know the pathotype of *S. endobioticum* involved in the original infestation, so that suitably susceptible cultivars may be chosen for the tests. Details of methods are given in Appendix I.

Direct examination

The methods of Mygind (1961), Pratt (1976), Potocek (1977) and Laidlaw (1985), among others, allow the determination of numbers of resting sporangia per unit weight of soil, but experience is needed to confirm reliably whether the sporangia are viable or not. There is a small possibility of confusion with other *Synchytrium* spp.

The methods are somewhat laborious to apply but give a rapid result. A negative result is not considered as a sufficient criterion to deschedule a plot, so that the test would have to be followed by a confirmatory bioassay. A positive result could, however, avoid the necessity for undertaking the relatively slow bioassay procedure for the sample concerned, so the direct examination method may be useful as a first screen for samples.

Bioassay

Bioassay methods with susceptible potatoes include a pot test (Rintelen *et al.*, 1983; Browning, pers.commun.) and Potocek's tube test (Potocek *et al.*, 1991).

Field test

The plot to be tested is planted with a susceptible potato cultivar. Such a field test gives a high level of security, provided it is conducted under suitable climatic conditions. However, if a positive result is obtained, the practical consequence is that the infection level of the plot is increased and, therefore, the period of official prohibition of planting must start again. For this reason, and also because of its dependence on climatic conditions, a field test is not included in the descheduling procedure described here.

Criteria for descheduling

A plot that has been infested with *S. endobioticum* and has been kept under official control with limitations on the crops allowed to be grown may be descheduled either completely or partially, depending on the interval since the last symptoms of the disease and on the results of soil tests. Because of the very long persistence of sporangia of *S. endobioticum* in soil and the fact that it is not yet possible to declare with certainty that, after a specified number of years, all residue of infestation has disappeared, it is not recommended that a plot that has once been infested should be descheduled simply because of the passage of time, without being tested.

Complete descheduling

A plot that has previously been infested with *S. endobioticum* can be descheduled after a minimum of 20 years since the last infection, provided that it is sampled, tested and found free from viable sporangia and from any evidence of infection (Fig. 1). As a general principle, the plot should have been cultivated during the period of scheduling; it should not have been under permanent grassland. In practice, the plot should be subdivided into units of 0.33 ha, from each of which 60 subsamples are taken; each sample is either:

1. subjected to direct examination by microscope for sporangia and tested for infestation using a bioassay. From a practical point of view, it is preferable to perform the direct examination before a bioassay, since direct examination is quicker. If any viable sporangia are observed in the direct examination, the overall result is positive, and there is no need to proceed with a bioassay or
2. tested for infestation by two successive bioassays. The soil should be sampled on separate occasions for the two bioassays, with a cultivation (rotavation/ploughing) of the field between the two occasions.

If positive results are obtained in the direct examination or bioassays, further testing should wait until at least a further 3 years have passed. If negative results are obtained for all samples, the plot can be descheduled. After descheduling, there are no official limitations on the types of crops that may be grown on the plot, apart from the strong recommendation that the first crop of potatoes of a susceptible cultivar should be inspected at harvest by the NPPO for any infection.

Partial descheduling

A plot can be partially descheduled after a shorter period of time (at least 10 years) so that resistant cultivars of ware potatoes may be grown; however, the plot may not be used for growing other types of potatoes, or for plants for planting, until complete descheduling (Fig. 1). As in the case of complete descheduling, it is a general principle that the plot should have been cultivated during the period of scheduling; it should not have been under permanent grassland. For partial descheduling, the plot should be sampled and tested as above, with the requirement that bioassays should give negative results and that fewer than five viable sporangia per g soil should be found by direct examination. If more than five viable sporangia per g soil are detected by direct examination, or a positive result is obtained in bioassays, a further test may be performed after 2 or more years depending on the level of infection and/or number of viable sporangia detected in the present test(s).

In certain cases, where there is reason to believe that the soil is not conducive to long-term survival of sporangia (e.g. a plot with optimum aeration and water conditions that has been continuously cultivated, higher ambient temperatures) or after direct control measures have been applied (e.g. treatment of soil with a plant protection product), partial descheduling may be obtained after only 5 years since the last infection. In such a case, the sampling intensity should be increased to 10 samples per hectare, each of 60 subsamples. The bioassays should give negative results and fewer than five viable sporangia per g soil should be found by direct examination (as for partial de-scheduling after 10 years).

Appendix I

Description of methods

Sampling scheme for soil

From each unit of the plot to be sampled (0.1 ha or 0.33 ha depending on the descheduling procedure – see section above on "Criteria for descheduling"), one sample should be taken, composed of 60 subsamples. The subsamples should be taken with an auger or other suitable tool to a depth of 20 cm and evenly distributed throughout the area. Each sample should be thoroughly mixed before being tested. If it is possible to determine the precise position of the infested focus or foci in the field, separate samples should be taken from these foci and analysed separately from those taken from the rest of the plot; in this case, the sampling intensity in the rest of the plot may be reduced. The amount of soil to be taken (and therefore the size of the sampling tool) will depend on the test to be applied.

Direct examination

A brief description of the procedure of Pratt (1976), with some recent modifications, is as follows. Two air-dried soil samples of 100 g are suspended in 900 mL of tap water for 24 h, and all soil aggregates thoroughly broken up. The suspension is wet-sieved through an electromagnetic sieve shaker (e.g. Fritsch Analysette 3, A. Christian Ltd, Gateshead, GB) with successive mesh sizes of 500, 250, 125, 71, 40 and 25 µm. The fractions held on the 40- and 25-µm sieves are washed onto filter paper, dried, and transferred to 50-mL centrifuge tubes. Chloroform (15 mL) or a saturated solution of $CaCl_2$ is added to each tube, stirred and the tubes centrifuged at 800 *g* for 15 min. The supernatant is filtered through hardened filter paper. The washing with chloroform or $CaCl_2$ is repeated, normally two more times, or until no more material can be floated off. The residue collected on the hardened filter paper is resuspended in 1 mL of lactoglycerol (or more if from highly organic soil) and examined under the microscope for the resting sporangia of *S. endobioticum*. These are counted and, by appropriate calculation, the number per g of soil can be determined.

Sporangia filled with greyish granular contents or slightly rounded-off cytoplasm (if germination is occurring) are considered viable, while those permanently plasmolysed or with no apparent contents are considered dead. Some viable sporangia also fluoresce with blue-light illumination.

Bioassays

In bioassays, cultivars of potatoes are used that are highly susceptible to the pathotype of *S. endobioticum.* It is therefore essential to know the pathotype involved in the original infestation, so that suitably susceptible cultivars may be chosen. If the original pathotype is not known, the bioassays should test for all pathotypes.

Pot test with potatoes

Samples of soil are placed in pots (2-5 L) and each planted with three tubers of a highly susceptible cultivar. There should be at least 10 pots per ha; in the case of partial descheduling however when only 5 years have passed since the last infection, this should be increased to 20 pots per ha. The pots may be placed in the open air in early summer and irrigated automatically by droplet irrigation, controlled by a tensiometer at a matrix potential of – 90 mb. Alternatively, the pots may be kept in a glasshouse in winter with supplementary lighting (16 h per day) and a misting system to maintain suitable soil moisture. After about 100 days, when new tubers have formed, plants are lifted and examined for warts.

Potocek's tube test

Thirty tubers of a strongly susceptible cultivar with their buds out of dormancy should be used for the testing of each soil sample. Soil samples are placed in conical plastic tubes (3-4 cm in diameter at the upper end, 3.5-4.5 cm at the lower end and 8 cm long) attached to the crown of test tubers by a simple system of clips and elastic bands. Sprouts are allowed to grow up through the soil for 5-7 weeks, with appropriate watering. Any sprouts that grow quickly through the soil should be cut to stimulate the growth of further sprouts. The soil is then removed, and the sprouted tubers are held for 3-4 weeks in covered plastic boxes. They are finally scored for wart disease. In all the bioassays, it is essential to include negative controls of healthy soil and positive controls of infested soil.

Field test

A crop of potatoes of a susceptible cultivar is planted on the plot, according to local agricultural practice. The harvested tubers are examined for symptoms.

References

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Enquiries

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Fig. 1. Diagram of procedure for complete or partial descheduling of plots previously infested with *Synchytrium endobioticum*.