Council Directive 98/57/EC of 20 July 1998 on the control of Ralstonia solanacearum (Smith) Yabuuchi et al.

[F1 ANNEX II

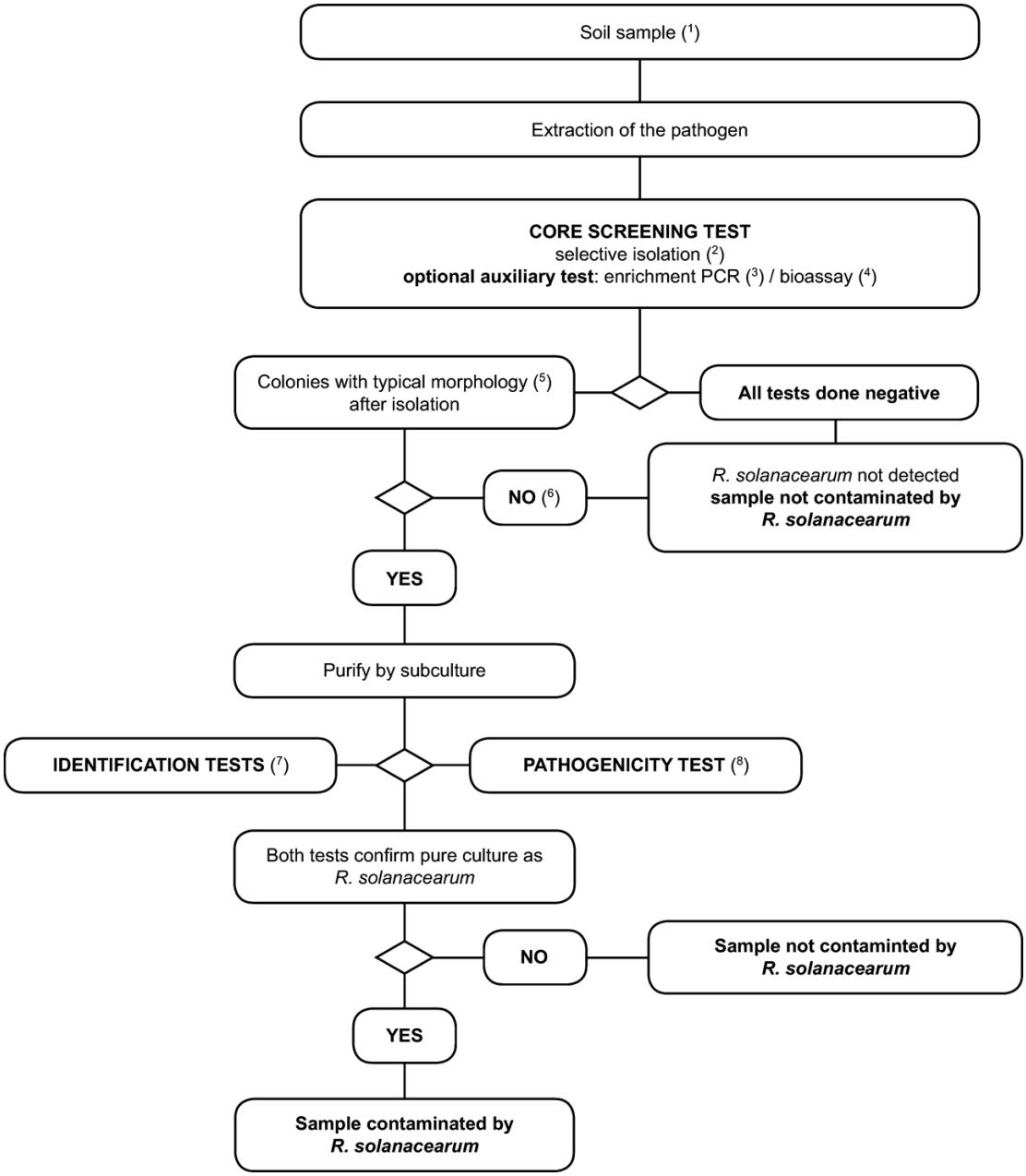
TEST SCHEME FOR DIAGNOSIS, DETECTION AND IDENTIFICATION OF *RALSTONIA SOLANACEARUM* (SMITH) YABUUCHI *ET AL.*

Textual Amendments

F1 Substituted by Commission Directive 2006/63/CE of 14 July 2006 amending Annexes II to VII to Council Directive 98/57/EC on the control of Ralstonia solanacearum (Smith) Yabuuchi et al..

SECTION V

1. Scheme for detection and identification of *R. solanacearum* in soil



(1) See Section V.2.1. for recommended sampling procedures.
(2) The selective isolation test is described in Section VI.A.4.
(3) Enrichment PCR methods are described in Section VI.A.4.2 and Section VI.A.6.
(4) The bioassay is described in Section VI.A.9.
(5) Typical colony morphology is described in Section II.3.d.
(6) Culturing can fail due to competition or inhibition by saprophytic bacteria. If high saprophyte populations are suspected to affect the reliability of the isolation, then repeat the isolation tests after further dilution of the sample.
(7) Reliable identification of pure presumptive *R. solanacearum* cultures is achieved using the tests described in Section VI.B.
(8) The pathogenicity test is described in Section VI.C.

2. Methods for detection and identification of *R. solanacearum* in soil

Principles

The validated detection scheme, described in this section, is applicable for pathogen detection in soil samples but can also be used to test samples of solid potato processing waste or sewage

sludge. However, it should be noted that these methods are insufficiently sensitive to guarantee detection of low and/or irregularly dispersed populations of *Ralstonia solanacearum* that may occur in naturally infested samples of these substrates.

The limitations in sensitivity of this test scheme should be considered when assessing the reliability of any negative results obtained and also when used in surveys to determine presence or absence of the pathogen in soils or sludges. The most reliable test for presence of the pathogen in a field soil is to plant a susceptible host and monitor it for infection, but even with this method low levels of contamination will escape detection.

2.1. Sample preparation

2.1.1. Sampling of field soil should follow standard principals used for nematode sampling. Collect 0,5 to 1 kg of soil per sample from 60 sites per 0,3 ha from a depth of 10 to 20 cm (or in a grid of 7 x 7 metres) If the pathogen is suspected to be present, increase the number of collection points to 120 per 0,3 ha. Maintain samples at 12 to 15 °C prior to testing. Sample potato processing and sewage sludges by collecting a total of 1 kg from sites representing the total volume of sludge to be tested. Mix each sample well before testing.

2.1.2. Disperse sub-samples of 10 to 25 g of soil or sludge by rotary shaking (250 rpm) in 60 to 150 ml extraction buffer (Appendix 4) for up to two hours. If required, addition of 0,02 % sterile Tween-20 and 10 to 20 g sterile gravel may assist dispersion.

2.1.3. Maintain the suspension at 4 °C during testing.

2.2. Testing

See flow chart and description of the tests in the relevant appendices.**]**