[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 IV

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

2.1. Sample preparation

Note:

— Detection of *R. solanacearum* in surface water is most reliable during late spring, summer and autumn seasons when water temperatures exceed 15 °C.

— Repeated sampling at different times in the above mentioned period at designated sampling points will increase the reliability of detection by reducing the effects of climatic variation.

— Take into account the effects of heavy rainfall and the geography of the watercourse to avoid extensive dilution effects that may obscure presence of the pathogen.

— Take surface water samples in the vicinity of host plants if these hosts are present.

2.1.1. At selected sampling points, collect water samples by filling disposable sterile tubes or bottles at a depth if possible below 30 cm and within 2 m from the bank. For processing and sewage effluents, collect samples from the point of effluent discharge. Sample sizes up to 500 ml per sampling point are recommended. If smaller samples are preferred, it is advisable to take samples on at least three occasions per sampling point, each sample consisting of two replicated sub-samples of at least 30 ml. For intensive survey work, select at least three sampling points per 3 km of watercourse and ensure that tributaries entering the watercourse are also sampled.

2.1.2. Transport samples in cool dark conditions (4 to 10 °C) and test within 24 hours.

2.1.3. If required, the bacterial fraction may be concentrated using one of the following methods:

(a) Centrifuge 30 to 50 ml sub-samples at 10 000 g for 10 minutes (or 7 000 g for 15 minutes) preferably at 4 to 10 °C, discard the supernatant and resuspend the pellet in 1 ml pellet buffer (Appendix 4).

(b) Membrane filtration (minimum pore size 0,45 µm) followed by washing the filter in 5 to 10 ml pellet buffer and retention of the washings. This method is suitable for larger volumes of water containing low numbers of saprophytes.

Concentration is usually not advisable for samples of potato processing or sewage effluent since increased populations of competing saprophytic bacteria will inhibit detection of *Ralstonia solanacearum*.**]**