

ANNEX I

TEST SCHEME FOR DIAGNOSIS, DETECTION AND IDENTIFICATION OF THE RING ROT BACTERIUM, *CLAVIBACTER MICHIGANENSIS* (Smith) Davis *et al.* ssp. *SEPEDONICUS* (Spieckermann et Kotthoff) Davis *et al.* SCOPE OF THE TEST SCHEME

10. CONFIRMATION TEST

The pathogenicity test must be performed as final confirmation of a diagnosis of *C. m.* subsp. *sepedonicus* and for assessment of virulence of cultures identified as *C. m.* subsp. *sepedonicus*:

- 10.1. Prepare an inoculum of approximately 10^6 cells per ml from three day cultures of the isolate to be tested and an appropriate positive control strain of *C. m.* subsp. *sepedonicus*.
- 10.2. Inoculate 5 to 10 eggplant stems of young seedlings at leaf stage 3 (section 7.3 or 7.4).
- 10.3. Incubate at 18 to 24 °C with sufficient light and high relative humidity with appropriate watering to avoid waterlogging or drought stress (section 7.7). With pure cultures, typical wilting should be obtained within two weeks but plants not showing symptoms (see section 7.8) after this time should be incubated up to three weeks at temperatures conducive to eggplant growth but not exceeding 25 °C (Appendix 8). If after three weeks symptoms are not present, the culture cannot be confirmed as being a pathogenic form of *C. m.* subsp. *sepedonicus*.
- 10.4. Isolate from symptomatic plants by removing a section of stem 2 cm above the inoculation point. Comminute and suspend in a small volume of sterile distilled water or 50 mM phosphate buffer (Appendix 3). Isolate from the suspension by dilution spreading or streaking onto MTNA and YPGA (Appendix 5), incubate for three to five days at 21 to 23 °C and observe the formation of colonies typical of *C. m.* subsp. *sepedonicus*.