

## ANNEX IV

### SECTION II

#### PROTOCOL FOR RESISTANCE TESTING

1. The test shall be performed in a quarantine facility either outside, in glasshouses, or in climate chambers.
2. The test shall be performed in pots each containing at least one litre of soil (or suitable substrate).
3. The soil temperature during the course of the test shall not exceed 25 °C and adequate watering shall be provided.
4. When planting the test or control variety one potato eye plug of each test or control variety shall be used. Removal of all stems except one is recommended.
5. The potato variety 'Désirée' shall be used as a standard susceptible control variety in every test. Additional fully susceptible control varieties of local relevance may be added as internal checks. The standard susceptible control variety may be changed if research indicates that other varieties are either more suitable or more accessible.
6. The following standard populations of potato cyst nematodes shall be used against pathotypes Ro1, Ro5, Pa1 and Pa3:  
Ro1: population Ecosse  
Ro5: population Harmerz  
Pa1: population Scottish  
Pa3: population Chavornay

Other potato cyst nematode populations of local relevance may be added.

7. The identity of the standard population used shall be checked using appropriate methods. It is recommended that at least two resistant varieties or two differential standard clones of known resistance capacity are used in the test experiments.
8. The potato cyst nematode inoculum (Pi) shall consist in total of five infective eggs and juveniles per ml of soil. It is recommended that the number of potato cyst nematodes to be inoculated per ml of soil is determined in hatching experiments. The potato cyst nematodes may be inoculated as cysts, or combined as eggs and juveniles in a suspension.
9. The viability of the potato cyst nematode cyst content used as the inoculum source shall be at least 70 %. It is recommended that the cysts are 6-24 months old and are kept for at least four months at 4 °C immediately prior to use.
10. There shall be at least four replicates (pots) per combination of potato cyst nematode population and potato variety tested. It is recommended to use at least 10 replicates for the standard susceptible control variety.
11. The duration of the test shall be at least three months and the maturity of developing females shall be checked before breaking up the experiment.
12. Potato cyst nematode cysts from the four replicates shall be extracted and counted separately for each pot.

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13. The final population (Pf) on the standard susceptible control variety at the end of the resistance test shall be determined by counting all cysts from all replicates and the eggs and juveniles from at least four replicates.
14. A multiplication rate of at least  $20 \times (Pf/Pi)$  on the standard susceptible control variety shall be achieved.
15. The coefficient of variation (CV) on the standard susceptible control variety shall not exceed 35 %.
16. The relative susceptibility of the tested potato variety to the standard susceptible control variety shall be determined and expressed as a percentage according to the formula:  
$$Pf_{\text{test variety}}/Pf_{\text{standard susceptible control variety}} \times 100 \%$$
17. If a tested potato variety has a relative susceptibility of more than 3 %, cyst counts will suffice. In cases where the relative susceptibility is less than 3 %, eggs and juveniles shall be counted in addition to cyst counts.
18. Where the results of tests in the first year indicate that a variety is fully susceptible to a pathotype, there is no requirement to repeat these tests in a second year.
19. The results of the tests shall be confirmed by at least one other trial performed in another year. The arithmetic mean of the relative susceptibility in the two years shall be used to derive the score according to the standard scoring notation.