

## ANNEX I

List of plants referred to in Articles 4(1), 4(2), 4(4), 5(1), 5(2), 8(3), 9(1)(b), and 10(1)

1. Host plants with roots:
  - Capsicum* spp.,
  - Lycopersicon lycopersicum* (L.) Karsten ex Farw.,
  - Solanum melongena* L.
2. (a) Other plants with roots:
  - Allium porrum* L.,
  - Beta vulgaris* L.,
  - Brassica* spp.,
  - Fragaria* L.,
  - Asparagus officinalis* L.
- (b) Bulbs, tubers and rhizomes, not subjected to the officially approved measures as referred to in Section III(A) of Annex III, grown in soil and intended for planting, other than those for which there shall be evidence by their packaging or by other means that they are intended for sale to final consumers not involved in professional plant or cut flower production, of:
  - Allium ascalonicum* L.,
  - Allium cepa* L.,
  - Dahlia* spp.,
  - Gladiolus* Tourn. Ex L.,
  - Hyacinthus* spp.,
  - Iris* spp.,
  - Lilium* spp.,
  - Narcissus* L.,
  - Tulipa* L.

## ANNEX II

1. With reference to the sampling and testing for the official investigation referred to in Article 5(1) and 5(2):
  - (a) sampling shall involve a soil sample with a standard rate of at least 1 500 ml soil/ha collected from at least 100 cores/ha preferably in a rectangular grid of not less than 5 metres in width and not more than 20 metres in length between sampling points covering the entire field. The whole sample shall be used for further examination, i.e. extraction of cysts, species identification and, if applicable, pathotype/virulence group determination;
  - (b) testing shall involve methods for the extraction of potato cyst nematodes described in the relevant Phytosanitary Procedures or Diagnostic Protocols for *Globodera pallida* and *Globodera rostochiensis*: EPPO standards.
2. With reference to the sampling and testing for the official survey referred to in Article 6(2):

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- (a) sampling shall be:
- the sampling described in paragraph 1 with a minimum sampling rate of soil of at least 400 ml/ha,
  - or
  - targeted sampling of at least 400 ml of soil after visual examination of roots where there are visual symptoms,
  - or
  - sampling of at least 400 ml of soil associated with the potatoes after harvesting provided that the field where the potatoes were grown is traceable;
- (b) testing shall be the testing referred to in paragraph 1.
3. By way of derogation the standard sampling rate referred to in paragraph 1 may be reduced to a minimum of 400 ml of soil/ha provided that:
- (a) there is documentary evidence that no potatoes or other host plants listed in point 1 of Annex I have been grown and were present in the field in the six years prior to the official investigation;
- or
- (b) no potato cyst nematodes have been found during the last two successive official investigations in samples of 1 500 ml soil/ha and no potatoes or other host plants listed in point 1 of Annex I, other than those for which an official investigation is required according to Article 4(1), have been grown after the first official investigation;
- or
- (c) no potato cyst nematodes or potato cyst nematode cysts without live content have been found in the last official investigation which must have consisted of a sample size of at least 1 500 ml soil/ha and no potatoes or other host plants listed in point 1 of Annex I, other than those for which an official investigation is required according to Article 4(1), have been grown in the field since the last official investigation.
- Results of other official investigations carried out before 1 July 2010 may be considered as official investigations as referred to in subparagraphs (b) and (c).
4. By way of derogation the sampling rate referred to at paragraphs 1 and 3 may be reduced for fields larger than 8 ha and 4 ha respectively:
- (a) in the case of the standard rate referred to in paragraph 1, the first 8 ha shall be sampled at the rate specified therein, but may be reduced for each additional hectare to a minimum of 400 ml of soil/ha;
- (b) in the case of the reduced rate referred to in paragraph 3, the first 4 ha shall be sampled at the rate specified therein, but may be further reduced for each additional hectare to a minimum of 200 ml of soil/ha.
5. The use of the reduced sample size as referred to at paragraphs 3 and 4 may be continued in the subsequent official investigations referred to in Article 4(1) until potato cyst nematodes have been found in the field concerned.
6. By way of derogation the standard size of the soil sample referred to in paragraph 1 may be reduced to a minimum of 200 ml of soil/ha provided that the field is situated in an area declared free from potato cyst nematodes, and designated,

maintained and surveyed in accordance with the relevant International Standards for Phytosanitary Measures. Details of such areas shall be officially notified in writing to the Commission and the other Member States.

7. The minimum size of the soil sample in all cases shall be 100 ml of soil per field.

## ANNEX III

### SECTION I

#### VERIFICATION

With reference to Article 5(2), the official investigation referred to in Article 4(1) shall establish that at the time of verification one of the following criteria are met:

- no history of potato cyst nematodes in the field during the last 12 years, based on the results of appropriate officially approved testing,

or

- a known cropping history in which no potatoes or other hosts plants listed in point 1 of Annex I have been grown in the field in the past 12 years.

### SECTION II

#### SURVEYS

The official surveys referred to in Article 6(1), shall be conducted on at least 0,5 % of the acreage used in the relevant year for the production of potatoes, other than that intended for the production of seed potatoes. The results of the surveys shall be notified to the Commission by 1 April for the previous 12 month period.

### SECTION III

#### OFFICIAL MEASURES

- (A) The officially approved measures referred to in Article 4(4)(c), 9(1)(b) Article 10(1) (c) and in paragraph 2b of Annex I are:
  1. disinfestation by appropriate methods such that there is no identifiable risk of the potato cyst nematodes spreading;
  2. removal of soil by washing or brushing until practically free of soil such that there is no identifiable risk of the potato cyst nematodes spreading.
- (B) The officially approved measures referred to in Article 10(1)(b) are delivery to a processing or grading plant with appropriate and officially approved waste disposal procedures for which it has been established that there is no risk of the potato cyst nematodes spreading.
- (C) The officially approved measures referred to in Article 13 are an official re-sampling of the field officially recorded as infested as referred to in Article 8(1) or 8(2), and

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testing using one of the methods specified in Annex II, after a minimum period of six years counting from the positive confirmation of potato cyst nematodes, or counting from the growing of the last potato crop. This period may be reduced to a minimum of three years if appropriate officially approved control measures have been taken.

## ANNEX IV

### SECTION I

#### DEGREE OF RESISTANCE

The degree of susceptibility of potatoes to potato cyst nematodes shall be quantified according to the following standard scoring notation as referred to in Article 9(2).

The score 9 indicates the highest level of resistance.

Relative susceptibility (%)	Score
< 1	9
1,1-3	8
3,1-5	7
5,1-10	6
10,1-15	5
15,1-25	4
25,1-50	3
50,1-100	2
> 100	1

### 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

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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.
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.

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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.