

**SUBSIDIARY LEGISLATION 433.16****CONTROL OF POTATO CYST NEMATODES  
REGULATIONS**

1st July, 2010

*LEGAL NOTICE 332 of 2010.*

- 1.** The title of these regulations is the Control of Potato Cyst Nematodes Regulations. Title.
- 2.** The scope of these regulations is to transpose Directive 2007/33/EC and consequently establish the measures taken by the Director (Plant Health) against *Globodera pallida* (Stone) Behrens (European populations) and *Globodera rostochiensis* (Wollenweber) Behrens (European populations), hereinafter referred to as 'potato cyst nematodes', in order to determine their distribution, to prevent their spread and to control them. Scope.
- 3.** In these regulations, unless the context otherwise requires: Interpretation.
- "the Act" means the Plant Quarantine Act; Cap. 433.
- "the Commission" means the Commission of the European Community;
- "field" means a plot of agricultural land with defined physical boundaries whereby the latter is subject to the same cultivation practices, same biotic and abiotic factors of that particular area and same phytosanitary regime. It is also the area from which a soil sample will be drawn and tested and to which the consequences of the results of the tests will be applied;
- "investigation" means a methodical procedure to determine the presence of potato cyst nematodes in a field;
- "official" or "officially" means established, authorised or performed by the Plant Health Department as the National Plant Protection Organisation;
- "resistant potato variety" means a variety that when grown significantly inhibits the development of a particular population of potato cyst nematodes;
- "survey" means a methodical procedure conducted over a defined period of time to determine the distribution of potato cyst nematodes in Malta.
- 4.** (1) An official investigation for the presence of potato cyst nematodes shall be carried out on the field in which the plants listed in Schedule I, intended for the production of plants for planting, or seed potatoes intended for the production of seed potatoes, are to be planted or stored. Detection.
- (2) The official investigation provided for in sub-regulation (1) shall be carried out in the period between the harvesting of the last

crop in the field and the planting of the plants or seed potatoes mentioned in the said sub-regulation. It may be carried out earlier, in which case documentary evidence of the results of that investigation confirming that potato cyst nematodes have not been found and that potatoes and other host plants listed in point 1 of Schedule I were not present at the time of the investigation and have not been grown since the investigation, shall be made available.

(3) Results of official investigations other than those referred to in sub-regulation (1) and carried out before 1 July, 2010 may be considered as evidence as referred to in sub-regulation (2).

(4) The Director shall ensure that the results of the investigations referred to in sub-regulations (1) and (3) are officially recorded and are accessible to the Commission.

Specific field cases.

**5.** (1) In the case of fields in which seed potatoes or plants listed in point 1 of Schedule I intended for the production of plants for planting, are to be planted or stored, the official investigation referred to in regulation 4(1) shall involve sampling and testing for the presence of potato cyst nematodes in accordance with Schedule II.

(2) In the case of fields in which the plants listed in point 2 of Schedule I, intended for the production of plants for planting, are to be planted or stored, the official investigation referred to in regulation 4(1) shall involve sampling and testing for the presence of potato cyst nematodes in accordance with Schedule II or verification as set out in Section I of Schedule III.

Official surveys.

**6.** (1) Official surveys are to be carried out on fields used for the production of potatoes, other than those intended for the production of seed potatoes, in order to determine the distribution of potato cyst nematodes.

(2) The official surveys shall involve sampling and testing for the presence of potato cyst nematodes in accordance with paragraph 2 of Schedule II and shall be carried out in accordance with Section II of Schedule III.

(3) The results of the official surveys shall be notified, in writing, to the Commission in accordance with Section II of Schedule III.

Official records.

**7.** If, as a result of the official investigation under regulation 4(1) and (3), no potato cyst nematodes are found, the Director shall ensure that such information is officially recorded.

Infested field.

**8.** (1) When a field is found infested with potato cyst nematodes during the official investigation under regulation 4(1), the Director shall ensure that such information is officially recorded.

(2) When a field is found infested with potato cyst nematodes during the official survey referred to in regulation 6(1), the Director shall ensure that this information is officially recorded.

(3) Potatoes or plants listed in Schedule I which come from a

field officially recorded as infested with potato cyst nematodes as referred to in sub-regulations (1) or (2) or have been in contact with soil in which potato cyst nematodes have been found, shall be officially designated as contaminated.

**9.** (1) When a field has been officially recorded as infested as referred to in regulation 8(1) or (2) the following measures shall apply: Control measures.

- (a) no potatoes intended for the production of seed potatoes shall be planted; and
- (b) no plants listed in Schedule I, intended for replanting, shall be planted or stored: howsoever that, plants listed in point 2 of Schedule I may be planted in that field provided that these plants are to be subject to the officially approved measures referred to in Section III(A) of Schedule III, such that there is no identifiable risk of spreading potato cyst nematodes.

(2) Fields to be used for planting potatoes, other than those intended for the production of seed potatoes, officially recorded as infested as referred to in regulation 8(1) or (2), are subject to an official control programme aiming at least at the suppression of potato cyst nematodes.

(3) The control programme shall take into account the particular production and marketing systems for host plants of potato cyst nematodes in Malta, the characteristics of the population of potato cyst nematodes present, the use of resistant potato varieties of the highest levels of resistance available as specified in Section I of Schedule IV and, where appropriate, other measures. It shall be notified in writing to the Commission and to the other Member States.

(4) The degree of resistance of potato varieties, shall be quantified according to the standard scoring notation table set out in Section I of Schedule IV. Testing for resistance shall be carried out according to the protocol set out in Section II of Schedule IV. Testing for resistance.

(5) Without prejudice to any other laws regulating soil movement, the Director may, by notice, establish guidelines and instructions regulating soil movement. Soil movement.

**10.** The following provisions shall apply to potatoes or plants listed in Schedule I, which have been designated as contaminated under regulation 8(3): Contaminated potatoes or plants.

- (a) in the case of seed potatoes and the host plants listed in point 1 of Schedule I, these shall not be planted unless they have been decontaminated under the supervision of the Department using an appropriate method based on scientific evidence that there is no risk of spreading potato cyst nematodes and adopted by notice issued by the Director;
- (b) in the case of potatoes intended for industrial processing or grading, they shall be subject to officially approved measures in accordance with

Section III(B) of Schedule III; and

- (c) in the case of plants listed in point 2 of Schedule I, they shall not be planted unless they have been subject to the officially approved measures as referred to in Section III(A) of Schedule III, such that they are no longer contaminated.

Reporting of suspected cases.

**11.** (1) Any person, entity, authority or any other legally constituted body shall report to the Director, within three working days of its knowledge or discovery, of any suspected occurrence or confirmed presence of potato cyst nematodes in Malta resulting from a breakdown or change in the effectiveness of a resistant potato variety which relates to an exceptional change in the composition of nematode species, pathotype or virulence group.

(2) For all reported cases, under regulation 11(1), the potato cyst nematode species and, where applicable, the pathotype or virulence group involved, are to be investigated and confirmed by appropriate methods.

(3) The Director shall not later than 31st December of each year send the details of the confirmations referred to in regulation 11(2), in writing, to the Commission and the other Member States.

New varieties of potatoes.

**12.** The Director shall communicate to the Commission and to the other Member States before the 31st January of each year, a list of all new varieties of potatoes which have been found by official testing to be resistant to potato cyst nematodes, and shall state the species, pathotypes, virulence groups or populations to which the varieties are resistant, the degree of resistance and the year of its determination.

Update of official records.

**13.** If, after the officially approved measures referred to in Section III(C) of Schedule III have been taken, the presence of potato cyst nematodes is not confirmed, the Director shall ensure that the official record referred to in regulations 4(4) and 8(1) and (2) is updated and any restrictions imposed on the field are revoked.

Derogations from control measures. S.L. 433.03

**14.** Without prejudice to regulation 4 of the Plant Quarantine (Harmful Organisms) Regulations, the Director may authorise derogations from the measures referred to in regulations 9 and 10 of these regulations and in accordance with provisions laid down in regulation 20, 21 and 22 of the said Plant Quarantine (Harmful Organisms) Regulations.

Stricter or additional measures.

**15.** Without prejudice to the provisions of these regulations, the Director is empowered to issue additional and stricter measures, as may be required, and which are conducive to preventing the spread of the pest and its further entry into Malta. These measures shall be notified in writing to the Commission and to the other Member States.

Offences.

**16.** Any person found guilty of an offence against any provision of these regulations shall be liable to penalties established under the Act.

## SCHEDULE I

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

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 Schedule 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.

## SCHEDULE II

1. With reference to the sampling and testing for the official investigation referred to in regulation 5:
  - (a) sampling shall involve a soil sample with a standard rate of at least 1500 ml soil/ha collected from at least 100 cores/ha preferably in a rectangular grid of not less than 5 meters in width and not more than 20 meters 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 regulation 6(2):

(a) sampling shall be:

- the sampling described in point 1 of this Schedule 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 point 1 of this Schedule.

3. By way of derogation the standard sampling rate referred to in point 1 of this Schedule 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 Schedule 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 Schedule I, other than those for which an official investigation is required according to regulation 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 Schedule I, other than those for which an official investigation is required according to regulation 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 sub-paragraphs (b) and (c).

4. By way of derogation the sampling rate referred to at points 1 and 3 of this Schedule may be reduced for fields larger than 8 ha and 4 ha respectively:

- (a) in the case of the standard rate referred to in point 1 of this Schedule, 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 point 3 of this Schedule, 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 points 3 and 4 of this Schedule may be continued in the subsequent official investigations referred to in regulation 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 point 1 of this Schedule 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.

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### SCHEDULE III

#### SECTION I

#### VERIFICATION

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

- (a) either that no history of potato cyst nematodes in the field during the last 12 years, based on the results of appropriate officially approved testing,
- (b) or that a known cropping history in which no potatoes or other hosts plants listed in point 1 of Schedule I have been grown in the field in the past 12 years.

#### SECTION II

#### SURVEYS

The official surveys referred to in regulation 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 regulation 9(1)(b), regulation 10(1)(c) and in point 2b of Schedule I are:

1. disinfection 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 regulation 10(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 regulation 13 are an official resampling of the field officially recorded as infested as referred to in regulation 8(1) or (2), and testing using one of the methods specified in Schedule 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.

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## SCHEDULE 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 regulation 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 liter 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.

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 x (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.

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