

Pursuant to Article 12, paragraphs 5 and 6 of the Law on Plant Health Protection („Official Gazette of the Republic of Montenegro“ 28/06), the Ministry of Agriculture, Forestry and Water Management adopted

**RULEBOOK  
ON PHYTOSANITARY MEASURES FOR DETECTION, PREVENTION OF  
SPREADING AND CONTROL OF POTATO CYST NEMATODES \***

**(Official Gazette of Montenegro 43/10 from 29 Jul 2010)**

**I. BASIC PROVISIONS**

Article 1

This rulebook prescribes phytosanitary measures conducted for the purpose of establishing of presence, prevention of introduction and spreading as well as control of potato cystic nematodes (hereinafter: PCN), *Globodera pallida* (Stone) Behrens (European population) and *Globodera rostochiensis* (Wollenweber) Behrens (European population).

Article 2

Terms used in this rulebook shall have the following meanings:

- a) **officially** means established, authorized or performed by the Phytosanitary Administration or on the basis of its authorization, in accordance with the Law on Plant Health Protection;
- b) **resistant potato variety** is potato variety by growing of which development of certain PCN populations is significantly prevented;
- c) **investigation** methodological procedure conducted for the purpose of detection of PCN presence on a lot;
- d) **supervision (permanent or special)** is systematic research, i.e. methodological procedure which is conducted in certain time period for the purpose of detection of presence or dispersion of PCN in the territory of Montenegro;
- e) **lot** is every production unit within the same place of production which has clearly set natural or artificial boundaries, where PCN host plants are grown or stored or intended to be grown or stored;
- f) **contaminated field** is lot where, by laboratory analysis of soil, presence of PCN is established;
- g) **positive finding** is finding by which presence of PCN in samples of soil or plants is established;
- h) **Safety zone** is area surrounding contaminated field, and it is established in accordance with standards on phytosanitary procedures of the European and Mediterranean Plant Protection Organization, depending on assessment of risk from spreading of harmful organism.

## Article 3

Phytopsanitary Administration establishes, on the basis of risk assessment, the boundaries of lot and phytopsanitary requirements for the purpose of reduction of risk from occurrence of PCN, which ensuring of homogenous conditions within the lot, adherence to scientific and statistical principles, PCN biology, system of lot processing and special systems of production of PCN host plants, in accordance with the annual programme of supervision within the Phytopsanitary Measures Programme.

Measures for establishment of lot referred to in paragraph 1 of this Article shall be reported by the Phytopsanitary Administration to the European Commission and other European Union member states.

PCN host plants (including root) to which this rulebook applies are: *Solanum tuberosum* L. (potato), *Capsicum* spp. (paprika), *Lycopersicon lycopersicum* (L.) Karsten ex Farw. (tomato) and *Solanum melongena* L. (aubergine).

In addition to plants referred to in paragraph 3 of this Article, this rulebook also applies to:

- plants (including root): *Allium porrum* L. (leek), *Beta vulgaris* L. (beet), *Brassica* spp. (cabbages), *Fragaria* L. (strawberry) i *Asparagus officinalis* L. (asparagus);
- cloves, tubers and rhizome intended for planting, except for those for which there are proof that on there package or otherwise it has been stated that they are intended for sale to end-user and that they are not intended for professional platn produicion or for production of cut flowers: *Allium ascalonicum* L. (chives), *Allium cepa* L. (onion), *Dahlia* spp. (dahlias), *Gladiolus Tourn Ex* L. (sword lily), *Hyacinthus* spp. (hyacinths), *Iris* spp. (irises), *Lilium* spp. (lillies), *Narcissus* L. (dandelions) and *Tulipa* L. (tulips).

## II. ESTABLISHMENT OF PCN PRESENCE

### Article 4

Lots at which it is intended to plant or storage plants referred to in Article 3 paragraphs 3 and 4 of this rulebook, and which are intended for plant production for further production of seeds potato shall be officially examined to PCN presence.

Seeds potato may be produced only at lots where it has been established by official investigations that there is no PCN presence.

Phytopsanitary Administration shall be informed on lots at which it is intended to grow seeds potato, by 10th September of the current year for the next year, at the latest.

Official investigation to PCN presence is carried out in the period form harvest of the previous crop to initiation of planting of plants or seeds potato referred to in paragraph 1 of this Article.

Notwithstanding paragraph 4 of this Article, official investigations can be conducted earlier, on condition that there is written proof that, in these lots, no PCN were found and that potato and other PCN host plants referred to in Article 3 paragraph 3 of this rulebook were not present in the time of investigation and that they were not grown on the lot.

Investigations referred to in paragraphs 1-5 of this Article shall be carried out in accredited laboratories in accordance with the Law.

When the Phytopsanitary Administration establishes, on the basis of report on conducted supervision, that the PCN are not present and that there is no risk form

spreading of PCN, investigations referred to in paragraph 1 of this Article are not necessarily conducted for planting of:

- 1) plants referred to in Article 3 paragraphs 3 and 4 of this rulebook, except for potato, which is aimed at production of plants for planting which will be used at the same place of production;
- 2) seeds potato, aimed at production of seeds potato, in the same place of production;
- 3) plants referred to in Article 3 paragraph 4 indent 2 of this rulebook, aimed at production of plants for planting, on condition that the following measures are conducted on picked or extracted plants:
  - disinfestations by appropriate methods in a manner so that there is no risk from spreading of PCN;
  - removal of soil by washing or brushing until plants are free from soil and there is no risk from spreading of PCN;
  - other cleaning measures (cleaning and disinfection of machines, vehicles and their parts etc.) which can be undertaken for the purpose of prevention and control of spreading of PCN.

Results of investigation referred to in paragraph 6 of this Article shall be submitted to the Phytosanitary Administration in accordance with the annual supervision programme, i.e. Phytosanitary Measures Programme.

Results of investigation referred to in paragraph 8 of this Article shall be recorded and published by the Phytosanitary Administration in the annual report and submitted to the European Commission.

## Article 5

At lots where seeds potato or other plants referred to in Article 3 paragraph 3 of this rulebook, aimed at production of plants for planting, is intended to be sown or stored, investigations are conducted in accordance with Article 4 of this rulebook, by sampling and testing of samples to presence of PCN.

Sample referred to in paragraph 1 of this Article should contain, as standard size, at least 1500 ml of soil/ha, gathered from at least 100 punctures/ha on a rectangular net, so that distance between insertions must not be bigger than 20 meters in length and 5 meters width, covering the entire lot.

Sample referred to in paragraph 2 of this Article shall be used in later check entailing extraction of cysts, identification of strain and identification of pathotype, i.e. virulent group of PCN.

Testing referred to in paragraph 1 of this Article comprises methods of extraction of PCN in accordance with diagnostic procedures for *Globodera pallida* and *Globodera rostochiensis* of the European and Mediterranean Plant Protection Organization., (EPPO).

In procedure of official investigation of lots to presence of PCN, for plants referred to in Article 3 paragraph 4 of this rulebook aimed at production of plants for planting, which are intended for planting or storage, the testing procedure referred to in paragraphs 2, 3 and 4 of this Article shall be applied.

By investigation referred to in Article 4 paragraph 1 of this rulebook, in accordance with paragraph 2 of this Article, fulfilment of one of the following requirements shall be established:

- that, on the basis of results of corresponding officially approved testing in the course of last 12 years, there was no confirmed presence of PCN in the last 12 years on the lot or
- that it is known that in last 12 years, potato nor other host plants referred to in Article 3 of this rulebook have not been grown.

## Article 6

In addition to investigation referred to in Article 5 of this rulebook, the Phytosanitary Administration, within the annual phytosanitary measures programme, conducts supervision over lots for production of mercantile potato, for the purpose of establishment and spreading of PCN.

Supervision referred to in paragraph 1 of this Article shall comprise sampling and testing of samples to presence of PCN which must be conducted on at least 0.5% surface in year of production of mercantile potato.

Testing referred to in paragraph 2 of this Article shall comprise methods of extraction of PCN in accordance with diagnostic procedures for *Globodera pallida* and *Globodera rostochiensis* of the European and Mediterranean Plant Protection Organization.

Results of supervision referred to in paragraph 2 of this Article, shall be published by the Phytosanitary Administration in annual report by 1<sup>st</sup> April of the current year for the next 12 months and inform the European Commission.

Sampling referred to in paragraph 2 of this Article shall comprise taking of at least:

- 400 ml soil/ha in the sample; or
- 400 ml soil/ha in sample after visual check of root in the place where symptoms are visible; or
- 400 ml of soil along with potato tubers after harvest, if it is known that potato was grown on given lot.

Standard quantities of soil in sample referred to in paragraph 5 of this Article can be reduced to at least 400 ml of soil/ha in cases if:

- there are proofs that no potato nor other plants referred to in Article 3 paragraph 3 of this rulebook was grown for six years prior to investigation ; or
- PCN were not found in two last consecutive investigations in 1500 ml of soil/ha and that potato and other plants referred to in Article 3 paragraph 3 of this rulebook has not been grown after the first investigation, save for those for which investigation referred to in Article 4 paragraph 1 of this rulebook is envisaged; or
- there were no PCN found nor cysts of PCN without live material in them in the course of last investigation in which 1500 ml of soil/ha of sample was taken and that potato and other plants referred to in Article 3 paragraph 3 of this rulebook were not grown after the last investigation, except for those for which investigation referred to in Article 4 paragraph 1 of this rulebook is envisaged.

Notwithstanding Article 5 paragraph 2 of this rulebook and paragraph 5 of this Article, size of sample can be reduced in lots are bigger than eight ha, i.e. four ha i cases:

- if it is standard size of sample referred to in Article 5 paragraph 2 of this rulebook, first eight ha must be sampled in accordance with that

Article, and samples for every following ha can be reduced to 400 ml of soil/ha;

- if it is reduced quantity of sample referred to in paragraph 5 of this Article, first four ha must be sampled in accordance with that paragraph, and samples for every following ha may be reduced to 200 ml of soil/ha.

Taking of reduced size of sample referred to in this Article can be continued in further investigations referred to in Article 4 paragraph 1 of this rulebook, until presence of PCN is established.

Notwithstanding Article 5 paragraph 2 of this rulebook, standard size of soil sample can be reduced to at least 200 ml of soil/ha, if the lot is in area established by the Phytosanitary Administration and which is marked as free from PCN and which is supervised in accordance with corresponding international standards for phytosanitary measures.

Smallest size of soil sample taken from a lot is 100 ml.

Sampling is conducted by phytosanitary inspector or authorized institution, i.e. accredited laboratory.

Testing of samples is carried out by an accredited phytosanitary laboratory, and testing results and reports on testing are submitted to the Phytosanitary Administration.

In case of a positive testing findings, the accredited phytosanitary laboratory informs, without delay the Phytosanitary Administration.

#### Article 7

IN case that results of investigation referred to in Article 4 paragraphs 1, 4 and 5 of this rulebook show that there is no PCN presence, the Phytosanitary administration marks the designated area as free from PCN.

#### Article 8

In case that results of investigation on lots, referred to in Article 4 paragraph 1 and Article 6 paragraph 1 of this rulebook show presence of PCN, the Phytosanitary Administration shall record them and designate lots as contaminated.

Potato or plants referred to in Article 3 paragraphs 3 and 4 of this rulebook, originating from lots which were designated as contaminated with PCN or were in touch with soil in which PCN were found, the phytosanitary inspector shall designate as contaminated.

Phytosanitary Administration, on the basis of supervision and investigations performed referred to in Article 7 and paragraphs 1 and 2 of this Article regarding presence of PCN in Montenegro, shall establish the status of PCN in accordance with the International standards for phytosanitary measures.

### **III. MEASURES IN CASE OF A POSITIVE FINDING**

#### Article 9

On lots which are marked as contaminated in accordance with Article 8 paragraph 1 of this rulebook, the phytosanitary inspector shall order the following phytosanitary measures:

- prohibit growing of seeds potato (by assessment, mercantile as well);

- prohibit planting and storage of plants referred to in Article 3 paragraphs 3 and 4 of this rulebook.

Notwithstanding paragraph 1 of this Article, plants referred to in Article 3 paragraph 4 of this rulebook can be planted on contaminated lot if the following measures have been ordered for these plants:

- disinfestation by corresponding methods in such manner that there is no risk from spreading of PCN;

- removal of soil by washing or brushing until plants are free from soil and that there is no risk from spreading of PCN;

In case when lots are used for planting of potato, except for lots which are aimed at production of seeds potato, labelled as contaminated with PCN, the phytosanitary Administration shall conduct on these lots the measures established by the control programme for control of PCN, in which it informs the European Commission and other European Union member States.

On the occasion of preparation of programme referred to in paragraph 3 of this Article, specificity of production and trade in PCN host plants is taken into account, the characteristics of PCN population which is present, use of resistant varieties of potato with the biggest level of resistance and if possible, in other circumstances, as well.

Level of resistance of potato varieties to PCN is quantitatively established by table of standard values (while level 9 denotes the highest level of resistance):

- to 1 % level 9;
- 1.1-3 % level 8;
- 3.1-5 % level 7;
- 5.1-10 % level 6;
- 10.1-15% level 5;
- 15.1-25% level 4;
- 25.1-50% level 3;
- 50.1-100 % level 2;
- above 100 % level 1.

Testing of resistance of potato varieties shall be conducted in the following manner:

- 1) test must be carried out in quarantine conditions in an open field, in greenhouse or air-conditioned chamber
- 2) test must be carried out in receptacles with volume of at least one litre of soil or corresponding substrate;
- 3) soil temperature during testing must not exceed 25°C and adequate water supply must be provided ;
- 4) on the occasion of planting potato varieties, one eye of every variety which is tested or control variety must be taken, and for all other shoots, it is recommended to remove them;
- 5) potato variety "Désirée" is used as a standard for control of varietal sensitivity in every test. Local varieties with high sensitivity can be used and control in internal checks. Standard sensitive varieties for control can be replaced with other varieties if it is scientifically proven that the other varieties are more sensitive or suitable;
- 6) for comparison of pathotypes Ro1, Ro5, Pa1 and Pa3, the following standard populations of PCN are used:
  - for Ro1: population Ecosse;
  - for Ro5: population Harmerz;
  - for Pa1: population Scottish;
  - for Pa3: population Chavornay, and

- other PCN of local importance can be used.
- 7) identity of used standard PCN populations is checked by use of corresponding methods. In experiments, it is recommended to use at least two resistant varieties or two different standard clones of the known level of resistance;
  - 8) inoculum of PCN ( $P_i$ ) contains totally five infective eggs and larvae in one milliliter of soil. It is recommended to establish the count of PCN by which inoculation of soil by millimetre, by the experiment of hatching. Inoculation can be conducted by use of cysts or combination of eggs and larvae in water area;
  - 9) ability for life of PCN from inoculum must be at least 70%. It is recommended to use cysts from six to 24 months old and that they were kept at the temperature of 4 °C four months before use;
  - 10) for every combination of population of PCN and potato variety, at least 4 consecutive receptacles are prepared. For a standard variety used as control, at least 10 receptacles are recommended;
  - 11) test lasts at least three months and in the end of the month, level of development of females is checked;
  - 12) PCN cysts from four consecutive repetitions are extracted and calculated separately for every receptacle;
  - 13) Final population ( $P_f$ ) on control variety with standard sensitivity, in the end of resistance test, is established by counting of all cysts from all consecutive repetitions, and counting of eggs and larvae from the last four repetitions;
  - 14) Multiplication rate on control of varieties with standard sensitivity should at least amount  $20 \times (P_f/P_i)$ ;
  - 15) Coefficient of variation (CV) for control variety with standard sensitivity must not exceed 35%;
  - 16) reactive sensitivity of tested potato variety is calculated with respect to control variety with standard sensitivity by a formula and it is expressed in percentage:  
$$\frac{P_f \text{ of the tested variety}}{P_f \text{ of control variety with standard sensitivity}} \times 100 \%$$
;
  - 17) if relative sensitivity of the tested potato variety exceeds 3%, only cysts are counted. If relative sensitivity is under 3% in addition to cysts, eggs and larvae are counted;
  - 18) if result of the test in the first year shows that the potato variety is highly sensitive to PCN, it is not necessary to repeat testing in the second year;
  - 19) results of the test must be confirmed by conducting of at least one test in the second year. Average value of relative sensitivity in both years is calculated by calculation of value in accordance with the standard recorded values.

#### Article 10

For potato or plants referred to in Article 3 paragraphs 3 and 4 of this rulebook, designated as contaminated with PCN, the phytosanitary inspector, on the basis of data received from the Phytosanitary Administration, orders one or several measures, as follows:

- prohibition of planting of potato seeds and host plants referred to in Article 3 paragraph 3 of this rulebook, until it is free from PCN, under supervision of the inspector with use of corresponding methods, which are based on scientific proofs that there is no risk from spreading of PCN
- for potato aimed at industrial processing or assortment, dispatching to plants for processing or assortment, in which corresponding prescribed procedures for waste disposal are carried out, so that there is no danger from spreading of PCN, and which are approved by the Phytosanitary Administration;

- prohibition of planting of plants referred to in Article 3 paragraph 4 of this rulebook, except if the following measures are taken on these plants: disinfestation by corresponding methods and removal of soil by washing or brushing until it is free from soil, so that it is no longer considered contaminated and that there is no risk from spreading of PCN and
- other measures if it is necessary for prevention and control of spreading of PCN.

## Article 11

When presence of PCN is suspected or it is established by the laboratory analysis of soil or plant samples it is established that the lot is contaminated with PCN, the phytosanitary inspector shall designate that lot as contaminated and place it under special supervision and inform thereof, without delay, the Phytosanitary Administration for the purpose of establishment of boundary of contaminated lot and safety zone boundary.

Safety zone must be large enough to ensure protection of surrounding surfaces.

Phytosanitary Administration shall establish territorial delimitation between the contaminated lot and safety zone.

In case when contamination occurred on several lots, the phytosanitary inspector shall establish measures of prohibition of growing and planting and measures of disinfestations with corresponding methods and removal of soil, so that it is no longer considered contaminated and that there is no risk from spreading of PCN.

Phytosanitary Administration, in case of suspected occurrence of disruption in resistance of potato varieties in contaminated area, shall publish results on that disruption occurring due to changes in varieties, pathotypes or virulent groups of nematodes.

In cases of suspected occurrence of disruption of resistance of potato varieties in contaminated area, the Phytosanitary Administration, in accordance with programme referred to in Article 9 paragraphs 3 and 4 of this rulebook, shall ensure monitoring of occurrence of disruption in resistance of potato varieties through authorized institution, which confirms disruptions of resistance with regard to PCN strains, i.e. pathotypes and virulent groups, by corresponding methods and investigations.

Data on disruptions referred to in paragraph 5 of this Article and results of monitoring of these disruptions shall be performed by 31<sup>st</sup> December of the current year.

## Article 12

On the basis of testing of resistance of potato varieties referred to in Article 9 this rulebook, the List of varieties of potato which were investigated to resistance to PCN is published once a year, by 31<sup>st</sup> January at the latest.

List referred to in paragraph 1 of this Article contains: name of varieties, pathotypes, virulent groupations or populations of PCN which are resistant, level of resistance and year of their determination.

## Article 13

Phytopsanitary Administration after at least six years from positive finding to presence of PCN or if at least six years from the last potato growing has elapsed on the lot, shall conduct the following measures:

- new taking of samples from a lot which is designated as contaminated in accordance with Article 8 paragraph 1 of this rulebook;
- investigation and sampling in accordance with Article 5 of this rulebook for potato and other host plants aimed at planting and Article 6 of this rulebook for mercantile potato.

Phytopsanitary Administration may reduce the period referred to in paragraph 1 of this Article to at least three years if measures referred to in Article 9 paragraph 1 of this rulebook have been conducted.

If, after conducted measures referred to in paragraph 1 of this Article, absence of PCN is established in the lot designated as contaminated, the Phytopsanitary Administration shall update and record these data, and phytopsanitary inspector shall remove all limitation measures referring to that lot.

Phytopsanitary Administration shall publish data referred to in paragraph 3 of this Article in the annual report and submit it to the European Commission.

## Article 14

PCN must not be grown.

Notwithstanding paragraph 1 of this Article, the Phytopsanitary Administration may allow for introduction, i.e. growing of PCN, only for scientific and research purposes and, for the needs of varietal selection, on condition that by their introduction, and growing there is no danger from their spreading.

## IV. TRANSITIONAL AND FINAL PROVISIONS

### Article 15

Results of investigation of PCN presence, established by entry into force of this rulebook, shall be used in the procedures of examination of presence of PCN in the sense of this rulebook.

### Article 16

By the day of entry into force of this rulebook, the Order on taking of measures for prevention of spreading, control and eradication of potato cystic nematode (*Globodera rostochinensis*) shall not be applied and areas contaminated with this harmful organism shall be declared (Official Gazette of FRYU, 39/02).

## Article 17

This rulebook shall enter into force on the eight day from the day of its publication in the Official Gazette of Montenegro.

No:  
Podgorica, 15 July 2010

MINISTER

Milutin Simović, MA

\*Rulebook is harmonized with Council Directive 2007/33/EC from 11 June 2007 for control of potato cystic nematodes, amending the Directive 69/465/EEC.