

**National regulatory control systems**  
**Systèmes de lutte nationaux réglementaires**

## ***Heterodera glycines*: procedures for official control**

### **Specific scope**

This standard describes the procedures for official control with the aim of eradicating or containing *Heterodera glycines* and contains elements on monitoring.

### **Specific approval and amendment**

First approved in September 2008.

### **Introduction**

*Heterodera glycines* is an A2 pest for the EPPO region. Details about its biology, distribution and economic importance can be found in EPPO/CABI (1997) and PQR (OEPP/EPPO, 2005). *H. glycines* is one of the most destructive pests of soybean world-wide. Although it has a broad host range, *H. glycines* will only establish and become a pest of economic importance where soybeans, are widely cultivated in close rotations or monoculture. It has proven very difficult to eradicate, so preventive measures are essential.

The soybean cyst nematode, *H. glycines* has been present in Japan since 1881 and was first reported in the United States in 1954 and in Canada in 1987. *H. glycines* was found in Italy in 2000<sup>1</sup> and EPPO member countries have considered that official control measures should be recommended by EPPO to prevent further spread of this pest. Prevention of spread of this pest within the region is mainly achieved by international measures, but measures should also be implemented to control the pest in infested places of production.

Soybean, *Glycine max*, is the only major economic crop severely affected. According to FAO statistics around 1 753 000 ha of soybean were grown in the EPPO region in 2005 the largest producers being the Russian federation (656 000 ha) and Ukraine (422 000 ha). Other cultivated hosts, mainly Fabaceae, are *Lespedeza* spp., *Lupinus albus*, *Penstemon* spp., *Phaseolus vulgaris*, *Vicia villosa*, *Vigna angularis* and *V.*

*radiata*. Over 1100 species of plants are reported as potential hosts for the soybean cyst nematode. Most of these species are weeds or crop species not found commonly in soybean fields or soybean cropping sequences. Many species merely allow the nematode to survive until a more susceptible host becomes available.

Soil temperature and moisture are the most important factors determining the survival of the cyst nematode in a field in the absence of the host plant. Slack *et al.* (1972) showed<sup>2</sup> that in the absence of a host, eggs in cysts are capable of remaining viable up to 8 years in moist soil at cool temperature, but the survival period shortens as soil temperature rise. However, this experiment was extended and after 9 years in which no host plants were allowed to grow long enough for a generation of the nematodes to be completed, soybean was planted. Severe damage was recorded during the second year of soybean. No cysts or eggs could be recovered from soil samples taken from the plots. This confirms that the longevity of this pest is not well understood (Riggs, pers. comm., 2007). Even in dry soil, some eggs may survive in cysts as long as three years under cool conditions (Slack *et al.*, 1972). Viability declines more rapidly when dry soil is exposed to high temperatures or when soil is flooded (Slack *et al.*, 1972).

The nematode is sedentary apart from a small amount of independent movement by juveniles and males. However, the durability of the cyst, allows considerable passive transport. New infestation of fields is usually caused by the movement of cysts in soil residues by means such as machinery or wind.

<sup>1</sup>It should be noted that *H. glycines* is not regulated in the European Union and therefore monitoring survey for this pest is not required in Member States.

<sup>2</sup>Although it was not clear from the published paper, Dr Riggs confirmed that the study from Slack *et al.* (1972) was based on a field experiment.

Cysts with viable juveniles have also been recovered from excrement of birds (Epps, 1971).

International transport is most likely to occur with soil or growing medium attached to plants or seeds. *H. glycines* was shown to be viable for up to 8 months in soil particles mixed in with seed stocks (Epps, 1969). Nematodes can also be readily carried in the roots of infested plants.

The EPPO member countries with areas at risk are advised to prepare a contingency plan for surveillance, eradication and containment of this pest.

This standard presents the basis of a national regulatory control system for the monitoring, eradication and containment of *H. glycines* and describes:

- Elements of the monitoring survey that should be conducted to detect a new infestation or to delimit an infested area.
- Measures aiming at eradicating recently detected populations (including an incursion).
- Containment measures to prevent further spread in a country or to neighbouring countries, in areas where the pest is present and eradication is no longer considered feasible.

### Monitoring survey for *H. glycines*

Early detection of *H. glycines* depends on sampling fields where soybean is grown before symptoms become apparent. Surveillance of soybean fields should also include familiarisation with symptoms associated with this pest. Guidance on sampling in the field and symptoms are given in Appendix 1.

### Eradication of *H. glycines*

The eradication programme for *H. glycines* is based on the delimitation of a quarantine area or areas within the country in which measures are applied to prevent further spread of the pest and to eradicate it. These measures are described in Appendix 2. In the case of recently-detected populations, surveillance may indicate the pest is more widespread. In these cases countries may wish to re-evaluate the prospects for eradication and may decide to implement a containment programme rather than an eradication programme.

### Containment of *H. glycines*

The containment programme for *H. glycines* in the case of established populations is based on the rotation with resistant varieties or non-host plants in the infested fields and on measures to prevent spread from these infested fields. These measures are described in Appendix 3.

## References

- EPPO/CABI (1997) *Heterodera glycines. Quarantine Pests for Europe*, 2nd edn, pp. 607–611. CAB International, Wallingford (GB).
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## Appendix 1

### Monitoring of *H. glycines*

Early detection of *H. glycines* is based on sampling soil and roots in fields, as the pest may be present in a field and decrease yields by over 30% without visible above-ground symptoms (Niblack, 1995). Even if plant stunting occurs, it can easily go unnoticed when large field areas are uniformly affected, and if chlorosis does not accompany the stunting. In addition it takes some years for the pest to build up to damaging levels in fields. Preventing this buildup requires early detection and management. Sampling soybean fields, even those with no symptoms, is the only way to detect *H. glycines* before it becomes an economic problem. Nevertheless, inspectors should also be able to identify symptoms which may be associated with *H. glycines*. Guidance on detection and identification of *H. glycines* is given in the EPPO Standard PM 7/89 (1) *Bulletin OEPP/EPPO Bulletin* **38**, 379–389.

### Symptoms

The symptoms caused by *Heterodera glycines* are not specific. General symptoms are for example patches of poor growth in a soybean crop. Sometimes the plants in these patches show yellowing, wilting or loss of leaves with reduced seed production. There is usually a sharp dividing line between affected and non-affected areas of the field. In the affected areas, rows are slow to close and may remain so throughout the season. The most severe damage is often in the centre of the affected area. Damaged areas are frequently located near the field entrance where machinery moves into the field, or in areas where soil from another field is deposited by wind or water. Reduction in seed yield is usually the first sign that an infestation is present. Usually the combination of reduced growth and yellowing is named 'yellow dwarf disease' for soybean infested with *H. glycines*. Root infestation increases the number of lateral roots and reduces the number of *Rhizobium* nodules and nitrogen fixation. Young females and cysts appear white, yellow or brown, about the size of a pin-head just visible with the naked eye on the root-surface. They may be confused with soybean *Rhizobium* nodules. However, soil sampling for cysts is the best method for detecting the presence of the soybean cyst nematode and juveniles may be found in extractions for free-living nematodes.

## Sampling

Selection of soybean fields for sampling should be based on the following criteria

- In areas where the nematode is not known to be present, sampling should be targeted on fields which have been planted with seeds imported from a country where the pest is known to occur. Such fields should be sampled every 4–6 years or each second year after soybean is grown, since nematodes may be lowering yields without symptoms being apparent.
- In areas where the nematode is present, sampling should be targeted toward specific areas such as field entrances, fence lines where wind deposits soil, areas that tend to pond or flood and consistently low-yielding areas, as these are most likely to be the areas where the nematode could be introduced.

Samples may be taken at any time of the year but the most appropriate periods are just before planting or after harvest. Samples can be taken any time when plants show general symptoms of reduced growth or yellowing. It should be noted that *H. glycines* populations are higher at harvest time. If symptoms indicative of *H. glycines* are detected, such as yellow or stunted plants and/or stunted, swollen roots, soil and root samples associated with them should be collected. Roots should be carefully dug and examined for presence of *H. glycines* females (cysts). Infestation on the roots is easier to detect when females are maturing (i.e. not yet cysts) as the cyst can easily drop off the roots when the plant is removed.

### Soil sampling procedure

The probability that a soil sample of any given size will contain one or more cysts depends on the average population density of cysts in the area sampled. Conversely, the probability that a population of given average density will be detected depends on the quantity of soil examined and, to a lesser degree, on the number of points from which the sample is derived and the area over which the sample points are distributed. For practical purposes, to detect relatively low populations, a sufficiently large volume of soil should be taken from the field that can be processed completely for extraction of cysts in the laboratory, or for a bioassay; there should be a sufficient number of field sample points to account for heterogeneity of distribution.

The minimum recommended sampling procedure is to take from each 1 ha of the area, 60 cores (borings) of soil with a half-cylindrical sampling tool to prepare a sample of 500 mL.<sup>3</sup> Soil should be collected to a depth of 15 to 20 cm in the root zone of plants. Representative sampling is best achieved by collecting cores in a zigzag pattern across the entire sample area. For fields bigger than 1 ha, the samples

<sup>3</sup>This sampling regime is adapted from the sampling regime for Potato Cysts Nematodes (PCN) for fields in which seed potatoes are intended to be planted (100 cores of soil to prepare a sample of 1500 mL). The size of the sample and number of cores is reduced compared to PCN as *H. glycines* has more generations per year and also has more host plants.

from each ha should be processed separately; for fields smaller than 1 ha, the same number of cores (60) and the same volume of soil (500 mL) should be taken as for 1 ha. Samples should be collected in a polyethylene bag. Paper bags are not recommended for *H. glycines* as they allow soil to dry excessively. Samples should not be stored in direct sun or where they can overheat.

## Appendix 2 Eradication programme

The eradication process involves four main activities:

- surveillance to fully investigate the distribution of the pest
- containment to prevent the spread of the pest
- treatment and/or control measures to eradicate the pest when it is found
- verification of pest eradication.

### Delimitation of the quarantine area

If an outbreak is detected, measures to prevent spread should be applied and the NPPO should investigate the extent and primary source of the outbreak. This investigation should include at least the testing of all other soybean fields on the farm as well as fields from other farms where equipment has been shared with the contaminated farm.

As long as eradication is not achieved, fields at the place of production should be tested prior to planting with soybean.

### Sampling procedure

The minimum recommended sampling procedure is to take from each 1 ha of the area, 100 cores (borings) of soil with a half-cylindrical sampling tool to prepare a sample of 1000 mL.<sup>4</sup> Overall sample size is higher (1000 mL) than for monitoring programme (500 mL) considering the fact that for eradication purposes a higher detection probability is required. For the process to collect samples see Appendix 1. For fields larger than 1 ha, the samples from each ha should be processed separately; for fields smaller than 1 ha, the same number of cores (100) and the same volume of soil (1000 mL) should be taken as for 1 ha.

### Prevention of spread

Movement of soil from infested fields should be prohibited. Equipment should be cleaned with high pressure water or steam to remove all soil particles and debris before leaving the known infested area. Field operations should be conducted last in infested areas if possible. Seed grown on infested land should not be used for sowing in uninfested fields unless the seed has been properly cleaned. *H. glycines* may be spread in soil peds associated with the seed.

<sup>4</sup>This sampling regime is adapted from the sampling regime for Potato Cysts Nematodes (100 cores of soil to prepare a sample of 1500 mL). The size of the sample is reduced compared to PCN as *H. glycines* has more generations per year and also has more host plants.

*Treatment and/or control measures to eradicate the pest when it is found*

No soybean crops or other host plants should be grown in the contaminated fields for a period of 8 years and host weeds should be eliminated during the same period.

**Other options**

In addition to excluding host plants, the eradication programme may include chemical control applied to the soil to reduce nematode populations, use of natural control agents such as *Hirsutella* species or planting *Brassicae* plants as biofumigants.

*Verification of pest eradication*

Given the uncertainty about the viability of the pest, the field should be tested at the end of the eight year period. Eradication can be declared after a minimum period of eight years without host plant crops or weeds in the field and a negative soil test on the field. It is recommended to include a bioassay in the testing procedure.

**Appendix 3 Containment programme**

In the case of an established population eradication is difficult to achieve, but a containment programme would reduce economic losses and delay further spread.

*H. glycines* requires a combination of management strategies for effective control. Rotation and resistant varieties, prevention of spread, as well as any practice which promotes good soybean health and growth, will all help to prevent damage by *H. glycines*.

**Rotation**

In all fields where *H. glycines* has been detected (see Appendix 2 for the sampling procedure), a four-year rotation or a six-year rotation with a non-host crop is recommended to reduce pest levels. *Zea mays*, *Medicago sativa*, *Trifolium pratense*, *Brassica* spp. and cereals are the most common non-host crop choices for reducing *H. glycines* population. Rotations which alternate

**Table 1** Recommended rotations for fields infested with *H. glycines*

Four-Year Rotation		Six-Year Rotation	
Year	Crop Sequence	Year	Crop Sequence
1	Non-host crop	1	Non-host crop
2	<i>H. GLYCINES</i> -resist. variety 1*	2	<i>H. GLYCINES</i> resist. variety 1*
3	Non-host crop	3	Non-host crop
4	Susceptible variety	4	<i>H. GLYCINES</i> resist. variety 2*
		5	Non-host crop
		6	Susceptible variety

\*Variety 1 is resistant to the predominant race of *H. glycines* in the field. Variety 2 is resistant to the less dominant race.

resistant and non-resistant soybean varieties with one or more non-host crops included between soybeans are most effective.

*Resistant varieties*

Appropriate resistant varieties can increase yields by more than 50% in heavily infested fields. A race test to determine possible cultivar resistance is described in Niblack *et al.* (2002). Because of inevitable shifts in the race type with this pest, resistant varieties alone cannot provide long-term protection. To manage the problem of race shifts, a combination of resistant varieties and rotations is suggested (see Table 1).

**Prevention of spread**

See Appendix 2 for measures to prevent spread.

**Control measures**

Chemical control which is applied to the soil to reduce nematode populations could also be part of the containment programme, as well as the use of natural control agents such as *Hirsutella* species. *Brassicae* plants can also be planted for biofumigation.