INDUSTRY BIOSECURITY PLAN

FOR THE GRAINS INDUSTRY

Generic Contingency Plan

Exotic nematodes affecting the grains industry

Specific examples detailed in this plan:

Maize cyst nematode (*Heterodera zeae*),
Soybean cyst nematode (*Heterodera glycines*)

and

Chickpea cyst nematode (*Heterodera ciceri*)

Plant Health Australia

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Plant Health AUSTRALIA

GRDC Grains Research & Development Corporation

CRC PLANT biosecurity

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1 Purpose and background of this contingency plan

Developing a pest contingency plan for groups of exotic pests will ensure the industry is prepared for new pest incursions. This generic contingency plan framework is designed to assist the grains industry for an incursion of a nematode that may not already be covered by a pest specific contingency plan. As most nematodes share a common behaviour in terms of their ability to spread to new areas by the movement of soil or water this generic framework has implications for the management of this group of soil pests.

This contingency plan framework provides background information on the pest biology and available control measures to assist with preparedness for an incursion into Australia of a range of nematodes that could impact on the grains industry. Three nematodes have been used as specific examples of exotic nematodes that could potentially enter Australia. It should be noted that some nematodes, such as the cereal cyst nematode *Heterodera avenae*, are already present in Australia. Endemic nematodes are not considered in this contingency plan framework. A pest specific contingency plan for Cereal cyst nematodes (*H. latipons, H. filipjevi* and *H. avenae*) was previously prepared as part of the CRC3009 project.

The contingency plan provides guidelines and options for steps to be undertaken and considered when developing a Response Plan for an incursion of an exotic nematode. Any Response Plan developed using information in whole or in part from this contingency plan must follow procedures as set out in PLANTPLAN and be endorsed by the National Management Group prior to implementation.

The information for this plan has been primarily obtained from documents as cited in the reference section. Information on background, life cycle, host range, distribution and symptoms of three nematodes are given as examples, with the emphasis of this document on the management options in the event of an exotic nematode incursion into Australia.

2 Australian grains industry

The grains industry is the largest plant industry in Australia and grain crops are grown in all states and territories. The grains industry is primarily situated in a narrow crescent running through the mainland states, known as the grain belt. This area stretches from central Queensland, through New South Wales, Victoria and southern South Australia. In Western Australia, the grain belt covers the south-west corner of the state. Wheat is Australia's most widely planted grain crop (Figure 1).

The grains industry consists of 25 leviable crops, all of which are affected by one or more species of nematodes.

Due to Australia's relatively small population and domestic demand, export markets are essential for the viability of Australian grain farms. Australia is one of the world's largest grain exporters. With this reliance on exports, maintaining our current plant health status through appropriate biosecurity measures is essential.

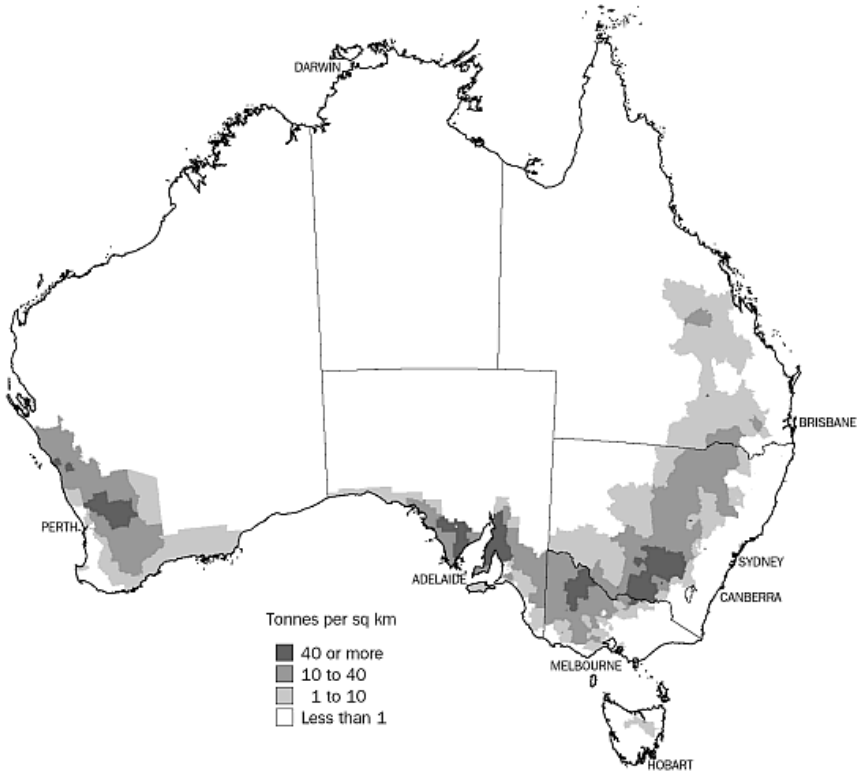

Figure 1 *Map of wheat producing regions in Australia (i.e. the grain belt). (Source ABS 2007)*

2.1 Notification process for the reporting of suspect pests

Early detection and reporting may prevent or minimise the long-term impact of an incursion into Australia of an exotic nematode. The notification process is described in Figure 2.

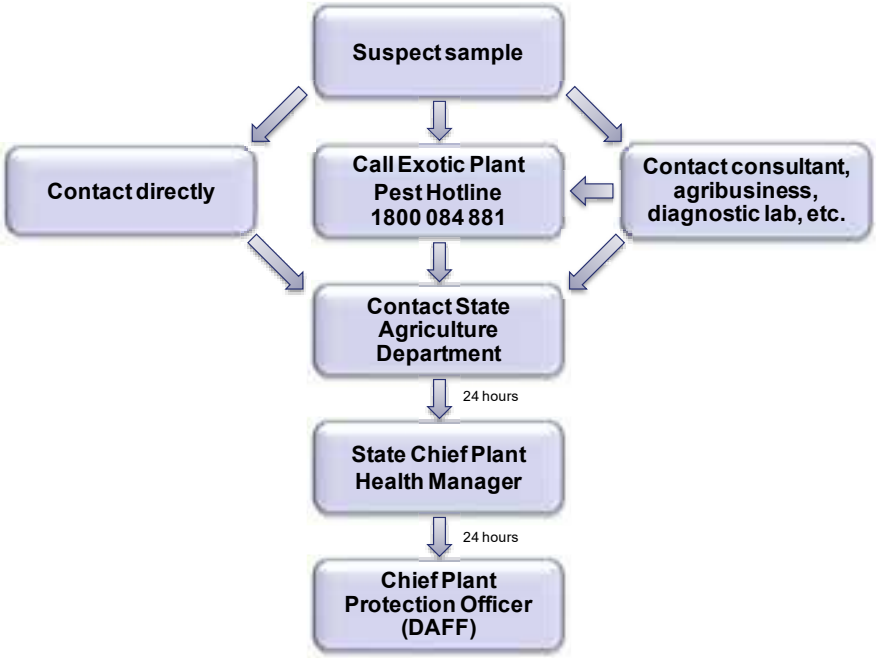

Figure 2. *Notification process for the reporting of suspect pests*

3 Eradication or containment decision matrix

The decision to eradicate should be based on the potential economic impact of host damage resulting from the introduction of an exotic nematode, the cost of eradication and technical feasibility. Eradication costs must factor in long term surveys to prove the success of the eradication program.

Before pest free status can be declared the exact number of years with no detection of the nematode will depend on the survival ability of the nematode concerned.

No specific eradication matrix has been determined for exotic nematodes; however the general decision process as outlined in Figure 3 and Table 1 should be followed in determining if an incursion of a particular nematode will result in eradication or management/containment. The final decision between eradication and management will be made through the National Management Group.

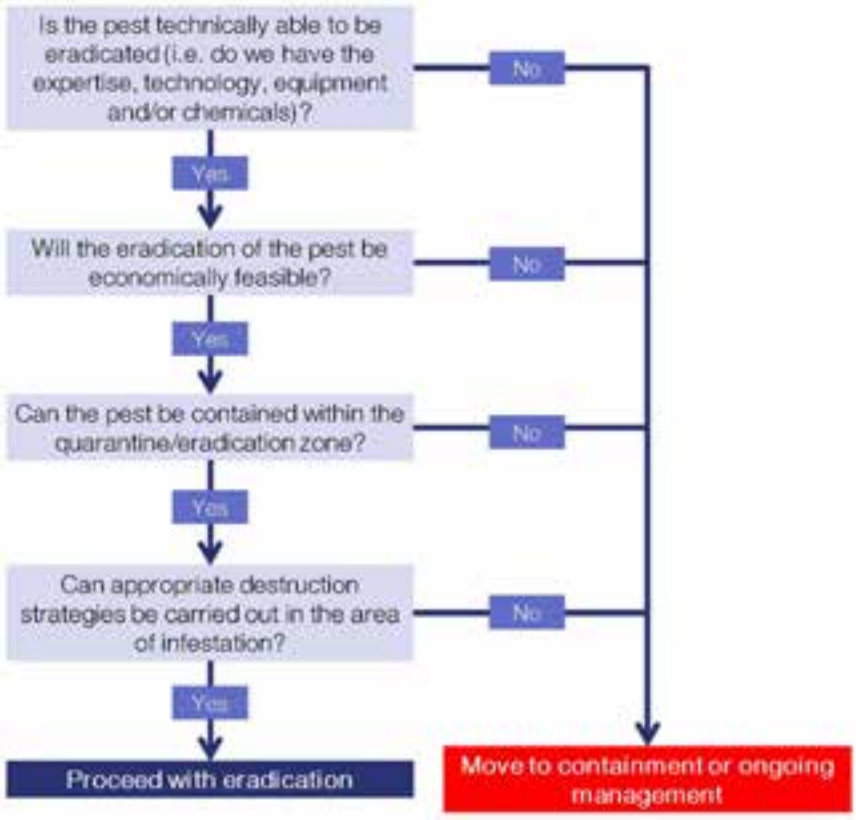

Figure 3. *Decision outline for the response to an exotic pest incursion*

Table 1. *Factors considered in determining whether eradication or alternative action will be taken for an EPP Incident (taken from Table 2; Section 4.16 of PLANTPLAN)*

Factors favouring eradication	Factors favouring alternative action
• Cost/benefit analysis shows significant economic loss to industry or the community if the organism established. • Physical barriers and/or discontinuity of host between production districts. • The generation time, population dynamics and dispersal of the organism favour more restricted spread and distribution. • Vectors discontinuous in distribution and can be effectively controlled. • Outbreaks few and confined. • Trace back information indicates few opportunities for secondary spread. • Weather records show unfavourable conditions for pest development. • Ease of access to outbreak site and location of alternate hosts. • Pathways for reintroduction from international trade closed.	• Cost/benefit analysis shows relatively low economic or environmental impact if the organism establishes. • Major areas of continuous production of host plants. • Short generation times, potential for rapid population growth and long distance dispersal lead to rapid establishment and spread. • Vectors unknown, continuous in distribution or difficult to control. • Outbreaks numerous and widely dispersed. • Trace back information indicates extensive opportunities for secondary spread. • Weather records show optimum conditions for pest development. • Terrain difficult and/or problems accessing and locating host plants. • Pathways for reintroduction from international trade open.

4 Pest information/status – exotic nematodes that affect grain crops

4.1 Background

Some nematodes currently occur in Australia and are able to cause significant damage to Australian crops. Thirty-one exotic nematodes have been identified in the Grains Industry Biosecurity Plan (Plant Health Australia 2009, currently under review) that could have an impact on the grains industry should they become established in Australia (see Table 2). These nematodes represent thirteen genera with different characteristics as summarised in Table 3.

The three species used as examples in this contingency plan have been selected to illustrate the management options available in the event on an incursion of an exotic nematode, as symptoms (e.g. patches of discoloured, unhealthy plants), controls, sampling procedures, the way nematodes are able to disperse (e.g. soil movement), are similar for all nematodes.

Most nematodes will be controlled in a similar manner; however specific chemicals, application rates, biological controls, etc. are likely to vary between species and will have to be considered on a case by case basis. Details such as the general procedures for control (Section 6.3.1), sampling protocols (Section 6.2), quarantine and movement controls (Section 7.3), zoning requirements (Section 7.4) and other components of this contingency plan may be very similar across the different nematode species and genera should they enter Australia and impact on the grains industry.

Table 2. *Exotic nematodes identified in the Grains Industry Biosecurity Plan (Plant Health Australia 2009)*

Common name	Scientific name	Overall risk[1]
Sting nematode	*Belonolaimus gracilis*	Very Low
Sting nematode	*Belonolaimus longicaudatus*	Very Low
Awl nematode	*Dolichodorus heterocephalus*	Very Low
Spiral nematode	*Helicotylenchus pseudorobustus*	Very Low
Cereal cyst nematode[2]	*Heterodera avenae (exotic strains)*	Medium
Cereal cyst nematode	*Heterodera bifenestra*	Very Low
Chickpea cyst nematode	*Heterodera ciceri*	Medium
Cereal cyst nematode[2]	*Heterodera filipjevi*	Medium
Soybean cyst nematode	*Heterodera glycines*	Medium
Cereal cyst nematode	*Heterodera hordecalis*	Negligible
Cereal cyst nematode[2]	*Heterodera latipons*	Medium
Cereal cyst nematode	*Heterodera mani*	Very Low
Cereal cyst nematode	*Heterodera pakistanensis*	Very Low
Maize cyst nematode	*Heterodera zeae*	Medium
Lance nematode	*Hoplolaimus columbus*	Very Low
Lance nematode	*Hoplolaimus galeatus*	Low
Lance nematode	*Hoplolaimus indicus*	Negligible
Lance nematode	*Hoplolaimus seinhorsti*	Negligible
Needle nematode	*Longidorus breviannulatus*	Low
Needle nematode	*Longidorus elongatus*	Negligible
Root-knot nematode	*Meloidogyne chitwoodi*	Very Low
Rice root knot nematode	*Meloidogyne graminicola*	Very Low
Root knot nematode	*Meloidogyne naasi*	Low
False root knot nematode	*Nacobbus dorsalis*	Very Low
Lesion nematode	*Pratylenchus delattrei*	Low

[1] Note: when more than one crop is affected by the same species of nematode the highest overall risk has been included in the table.

[2] A contingency plan has already been created for this species (Plant Health Australia 2012).

Common name	Scientific name	Overall risk[1]
Mexican Corn Cyst nematode	*Punctodera chalcoensis*	Very Low
Grass cyst nematode	*Punctodera punctata*	Negligible
Stubby-root nematode	*Quinisulcius acutus*	Unknown
Reniform nematode	*Rotylenchus parvus*	Very Low
Dagger nematode	*Xiphinema barberchekae*	Very Low
Dagger nematode	*Xiphinema mediterraneum*	Very Low

There are thirteen genera of nematode that were identified in the Industry Biosecurity Plan for the grains industry (Plant Health Australia 2009, currently under review). Each genus has different characteristics regarding its survival in the absence of host plants, where it feeds and how it behaves (summary of information see Table 3). The life cycle/behaviour of the nematode influences how nematodes can be effectively collected from infected areas.

4.2 Generic information on nematode life cycles

The lifecycles of most nematodes are similar. All nematodes lay eggs and pass through a number of juvenile states (designated by a “J” followed by a number designating the stage the nematode has reached) before becoming an adult. Eggs are either laid in the soil, plant tissues, remain inside the females’ body which develops into a cyst, or are laid as an egg mass that surrounds the female nematode.

After hatching the second stage juveniles (J2) generally move towards the roots of host plants where they begin to feed (either internally or externally depending on the genus, see Table 3). After several moults they develop into adults with timing usually dependent on the soil temperature.

Female nematodes of some species are able to reproduce parthenogenetically (i.e. asexually), while others reproduce sexually. Adult males of all genera are usually vermiform (worm-like) and are free living in the soil, even the males of genera where the females remain attached to the plants roots for their entire adult lives (e.g. *Heterodera* spp.). As adult males of many species are rarely observed mature females more frequently used for identification purposes.

4.3 Behaviour

Nematode behaviour is varied. Some nematodes spend their whole life living in the soil around plant roots (termed as migratory) and occasionally feeding on the host plant. Other species move onto the plant at some point in their life cycle (from J2 onwards) and remain there for the rest of their lives (such species are termed as “sedentary”), with the exception of the males which are usually free living. After mating (although there are some species that can reproduce asexually) the females begin to lay eggs.

Nematodes feed in three ways either as ecto-parasites (which feed on roots with their head and body outside of the plant), as endo-parasites (which feed with their head and body inside the host plant) or semi-endoparasites (which feed with only their head inside the host plant).

Table 3. *Characteristics of the nematode genera identified in the Industry Biosecurity Plan for the Grains industry (Plant Health Australia 2009)*

Common name	Scientific name	Survival mechanism	General comments[3]
Sting nematode	*Belonolaimus* spp.	All stages other than egg feed on plant roots. Does not form galls or cysts.	All stages other than egg feed on the exterior of plant roots (i.e. ectoparasites). Prefer sandy soil, mostly found 15-30 cm under soil surface (Bridge and Starr 2007). Collect from soil or root samples.
Awl nematode	*Dolichodorus* spp.	Does not form galls or cysts.	Awl nematodes are fairly large at 1.5-3 mm long and prefer moist/wet soils (Crow and Brammer 2005). All life stages feed on the outside of the host plant's roots (i.e. ectoparasites). Awl nematodes can be collected in soil and root samples.
Spiral nematode	*Helicotylenchus* spp.	Does not form galls or cysts.	Eggs are laid in the soil, J1 juveniles develop in the egg, moult and emerge as J2 juveniles. These nematodes are external feeders (ectoparasites) although there are some that will burrow their heads into roots to feed (i.e. semi-endoparasites). Rarely causes enough damage overseas to warrant chemical control (Crow 2013). Collected in soil and root samples.
Cyst nematode	*Heterodera* spp.	Cysts filled with eggs and J1 juveniles. Cysts very resistant to desiccation and can survive without host plants for many years.	J2 nematodes emerge from cysts and attach themselves to plant roots. Females swell with eggs forming cysts. Cysts can be collected from root samples or in soil samples after they have broken away from the root. Other life stages can be collected from root or soil samples.
Lance nematode	*Hoplolaimus* spp.	Does not form galls or cysts.	Feed in or on plant roots (i.e. endoparasites and ectoparasites), all life stages are infective (Bridge and Starr 2007). All stages of development can be collected in either soil or root samples.
Needle nematode	*Longidorus* spp.	Does not form galls or cysts.	Large external feeding (ectoparasitic) nematodes. Mostly feed on root tips, which often become swollen. Genus has long stylet. Collect in soil or root samples.
Root-knot nematode	*Meloidogyne* spp.	Galls are formed on the roots at the infection site.	Only J2 stage will infect roots. The J2 invade roots and create a feeding site where it stays for the rest of its life (i.e. it is an endoparasite). Males are free living. Females produce an egg mass that darkens overtime. Soil samples likely to only find J2 and males. Root samples more useful to find mature females and identify the pest.

[3] General comments are a summary of the information presented in: Bridge and Starr (2007); Crow and Brammer (2005); Crow (2013); Vovlas (1983).

Common name	Scientific name	Survival mechanism	General comments[3]
False Root knot nematode	*Nacobbus* spp.	Females form galls.	Adult females cause the formation of galls on the host's root. All other stages move between soil and roots. Members of this genus are internal feeders (i.e. endoparasitic nematodes). Soil samples can collect all stages except mature females. Root samples can collect all stages.
Lesion nematode	*Pratylenchus* spp.	Does not form galls or cysts.	Eggs are laid in or near plant roots. Nematodes are internal root feeders (i.e. endoparasites). Most commonly found on roots (Bridge and Starr 2007), so when sampling make sure that roots are collected not just soil samples.
Cyst nematode	*Punctodera* spp.	Female forms a cyst.	Juveniles and males are free living in the soil. Females attach themselves to the root of the host plant and swell with eggs creating a cyst. Collect mature females in root samples, collect other life stages in soil or root samples.
Stubby-root nematode	*Quinisulcius* spp.	Does not form galls or cysts.	Some species such as *Q. acti* feed with head imbedded into the roots of the host plant (i.e. semi-endoparasitic) (Vovlas 1983). Collect in root or soil samples.
Reniform nematode	*Rotylenchus* spp.	Can survive for months by coiling themselves tightly and going dormant. Does not form cysts or galls.	Doesn't feed from J2-J4. Only immature vermiform females infect roots, where they feed with their head in the root and tail in the soil (i.e. semi-endoparasites). Eggs are laid in a mass surrounding the female. Genus is collected in soil and root samples
Dagger nematode	*Xiphinema* spp.	Does not form galls or cysts.	Large (up to 10 mm long) external feeding (ectoparasitic) nematodes. Mostly feed on root tips, which often become swollen. Genus has long stylet. Long lifecycle as some species can take 3 years to mature (e.g. *X. diversicaudatum*) (Bridge and Starr 2007). Collect in soil or root samples.

4.4 Dispersal

The exotic nematodes (from Table 2) are all soil dwelling species with the potential to enter Australia in soil (e.g. on machinery, as a contaminant of other goods, etc.) from infected overseas countries.

Once in Australia the nematodes can spread through movement of soil (e.g. soil adhering to footwear, machinery and equipment, people and potentially livestock). Irrigation water and even the wind can disperse some species (Leham 1994). It has also been suggested that cysts of *Heterodera glycines* have spread long distances in the digestive tracts of birds, as viable cysts of *H. glycines* have been detected in the faeces of Brown headed cowbird (*Molothrus ater*), Grackle (*Quiscalus quiscula*) and Starlings (*Sturnis vulgaris*) in the USA (Epps 1971).

The ability of exotic nematodes to develop and establish in Australia will be determined by soil type, soil temperatures and the availability of host plants.

4.5 Symptoms

All exotic nematodes (from Table 2) affect the roots of their host plants. By feeding on the roots they cause a range of symptoms, including:

- Isolated patches of plants in the field showing yellowing leaves, stunting, or abnormal growth (see Figure 4-6).
- Poor or abnormal root development (this may include the development of galls or cysts) (as shown in Figure 7).
- Poor nodulation may also be observed in legumes affected by nematodes.
- Reduced grain yield due to poorly performing plants.

Figure 4. *Typical nematode symptoms in soybean. The yellow patches (circled) are caused by Meloidogyne spp. source: Edward Sikora, Auburn University, Bugwood.org*

Figure 5. *Dead patches (circled) in a cereal rye field caused by Heterodera filipjevi. Source: Bonsak Hammeraas, Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Bugwood.org*

Figure 6. *Stunted maize plants (circled) caused by Mexican corn cyst nematode (Punctodera chalcoensis). Source: Laurence I. Miller, Virginia Polytechnic Institute and State University, Bugwood.org.*

Figure 7. *Root galls (some arrowed) on soybeans caused by Meloidogyne spp. Source: Edward Sikora, Auburn University, Bugwood.org*

4.6 Sampling

Sampling methodology will vary according to nematode genera. For example sedentary endoparasites, such as female *Meloidogyne* spp. (see Table 3) are most likely to be collected on root samples rather than soil samples. Whereas all life stages of migratory ectoparasites such as *Belonolamimus* spp. can be collected easily in soil samples. Therefore an understanding of the nematodes behaviour is important when sampling crops for nematodes.

For appropriate sampling protocols always seek the advice from nematologist specialists.

In most cases soil samples containing some plant roots will increase the likelihood of collecting mature female nematodes, which are the most useful specimens for identification purposes. Further details are given in Section 6.2.

4.7 Diagnosis

Currently there is not an endorsed National Diagnostic Protocol for any nematode that affects grain crops. Generally nematodes are identified by close examination of adult females as adult males are often rare or rarely collected (Bridge and Starr 2007). The physical size of the nematode, its shape, and examination of its reproductive organs are used to identify specimens to a species level.

For diagnostic facilities and advisory services that can be utilised in the event of an incursion see Section 10.2 Appendix 2.

4.8 General comments on control

If left unchecked nematodes can cause significant damage to host crops. Nematicides can control infestations but are often not considered as an economical control option with crop rotations and host plant resistance the preferred method used to manage most endemic nematodes.

In the event of a pest incursion nematicides may be an option in the eradication of the pest. The chemical required and application rates will be determined on a case by case basis and be tailored to the specific species involved. Currently there are some nematicides registered for the control of endemic nematodes.

However any chemical used in Australia as part of a control or eradication program must be approved for that use by the Australian Pesticides and Veterinary Medicines Authority (APVMA) before it can be used in Australia.

Table 4. *Chemicals currently registered in Australia for the control of plant nematodes*

Active ingredient	Crops chemical is used on
Carbofuran	Sugarcane
Chloropicrin	Pre-planting soil fumigation
Fenamiphos	Banana, carrot, parsnip, crucifers, ginger, ornamentals, pineapple, potato, strawberry, tomato, sugarcane, turf
Metham	Pre-planting soil fumigation, Turf
Methyl bromide	Pre-planting soil fumigation, Turf (only under specific conditions)
Oxamyl	Banana, capsicum, tomato

Host plant resistance and the use of crop rotations incorporating non-host crops are common methods used to manage nematode pests. For example there are a number of soybean cultivars that are resistant to *Heterodera glycines*. Crop rotations between host and non-host crops reduces the risk of the nematode population growing to damaging levels, which would occur if the paddock were planted to successive host crops.

In some situations biological control options have been used to control nematodes (Koenning 2004). Section 6.3.7 provides more information on biological control of nematodes.

5 Specific examples of exotic nematodes affecting the grains industry

5.1 Pest Details – Maize Cyst Nematode (*Heterodera zeae*)

Common name:	Maize cyst nematode
Scientific name:	*Heterodera zeae* Koshy, (Swarup and Sethi, 1971)
Synonyms:	Corn cyst nematode
Taxonomic position:	Kingdom: Animalia
	Phylum: Nematoda
	Class: Secernentea
	Family: Heteroderidea
	Genus: *Heterodera*

The information from this plan has been primarily obtained from documents as cited in the reference section, as well as from a Pest Risk Review (Plant Health Australia 2005a).

5.1.1 Background

H. zeae is a major pest of maize overseas and is restricted to warmer tropical or sub-tropical areas including Virginia and Maryland in the USA (Koenning et al., 1999). This nematode has been associated with significant losses with reports of yield losses of between 21 and 73% in infected maize grown in pots (Krusbec et al., 1997).

The nematode invades the roots of its host and produces tan coloured, lemon shaped cysts that are visible on the roots of infected plants (Liu et al., 2009). Infestations result in poor yield (Krusberc et al., 1997) and show symptoms of isolated patches of unhealthy plants that are stunted and pale coloured (Koshy and Swarup 1971).

Based on its morphology *H. zeae* belongs to the *H. schachtii* group of nematodes (Koshy et al., 1970).

5.1.2 Life cycle

The general life cycle of all cyst nematodes follows the same process (Figure 8) and described as follows:

- Second stage juveniles emerge from the cysts at plant emergence when environmental conditions are favourable.
- Juvenile stage 2 nematodes then invade roots and establish a fixed feeding site (called a “syncytium”), and undertake a further three moults before becoming adults (i.e. there are four juvenile stages in total).
- Male syncytium begins to deteriorate at juvenile stage 4 allowing the male to become detached from the host plant.

- Adult vermiform (worm shaped) males mate with lemon-shaped females.
- Females begin to swell and rupture the root cortex as eggs are produced within the female's body. Female's syncytium begins to deteriorate.
- Female nematodes die and the egg filled body of the dead female is referred to as the cyst. Cysts of *Heterodera* spp. change from white to a darker colour after the female's death.
- Eggs hatch within the cyst and first stage juveniles moult and become second stage juveniles within the cyst. The second stage juveniles stay inside the cyst until conditions are suitable for emergence prior to finding a host plant.

The cyst stage allows the juvenile nematodes to survive in the absence of host plants for long periods of time and is the stage of the lifecycle that is most likely to spread to new areas.

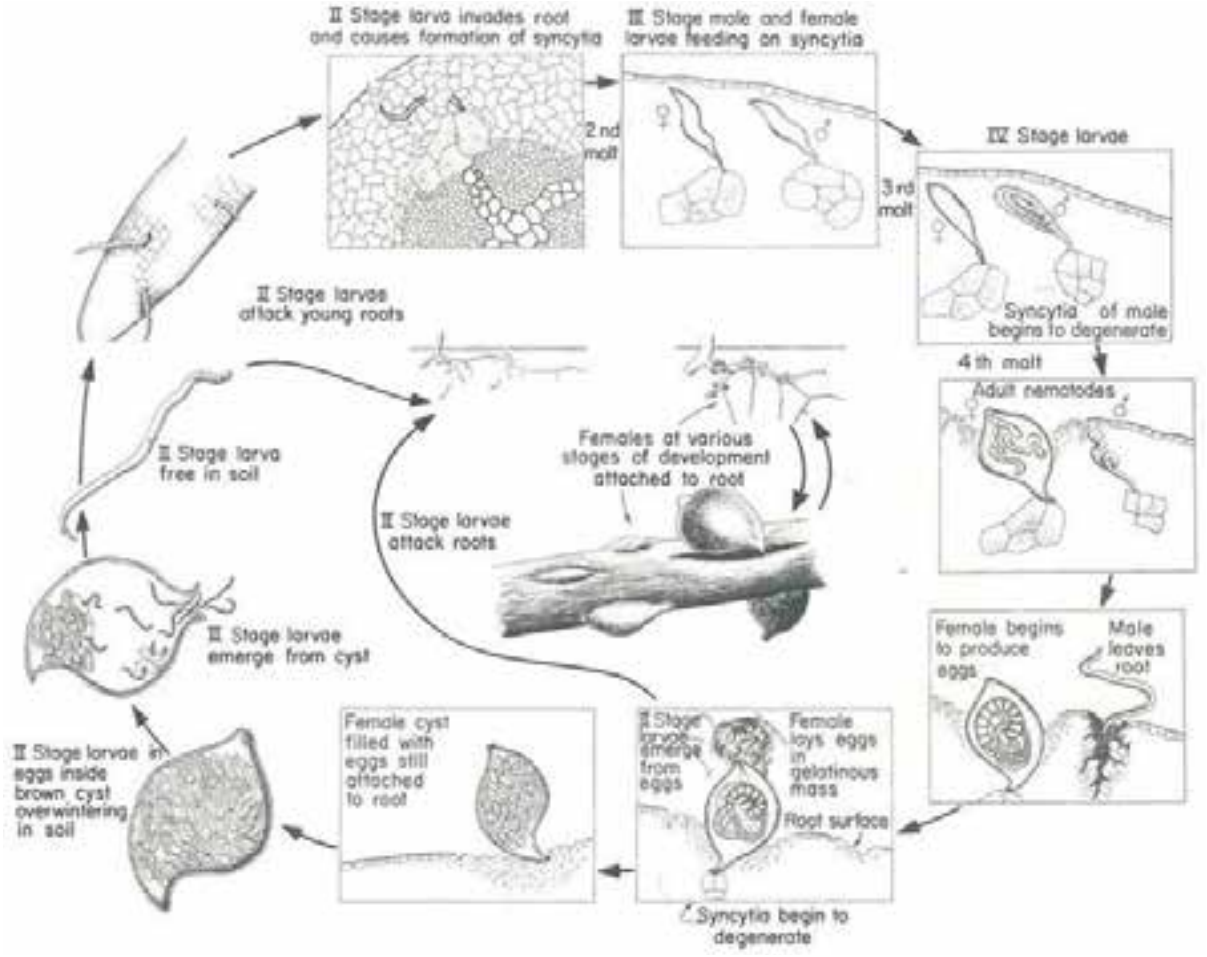

Figure 8. *Life cycle of Heterodera cyst nematodes (reproduced from Agrios, 1978).*

Hutzell and Krusberg (1990) studied the effect of temperature on the development of *H. zeae* raised on maize. They reported that reproduction occurred from 20 to 36°C, with higher number of eggs and faster development at higher temperatures. Reproduction is greatest at 33°C with development from second stage juvenile (J2) to second stage juvenile (J2) (i.e. a complete lifecycle) taking 15-18 days (Hutzell and Krusberg 1990). Eggs did not hatch at 20°C but at 25°C the lifecycle took 42-43 days to complete.

5.1.3 Dispersal

H. zeae, like all *Heterodera* spp. produce cysts. The cysts protect the eggs and developing juveniles from desiccation allowing the nematodes to survive without a host for a significant period of time. The nematode could potentially be spread over long distances as cysts in soil contaminating machinery, footwear, plants or goods. Lehman (1994) suggests that *H. zeae* is likely to have entered the USA on soil brought in by the United States military to test the performance of machinery on different soil types.

Once in a country the nematode can be spread between properties, and within paddocks, by the movement of soil either on machinery, footwear, animals or by water movement. Irrigation also poses a risk of spreading the nematode between regions (Lehman 1994) especially if irrigation water is recirculated and used on multiple paddocks within a single farm. There is also a risk that strong winds could blow the cysts to new areas.

5.1.4 Host range

The Maize cyst nematode predominantly affects maize crops (Koshy et al., 1970; Ringer et al., 1987) however it has also been reported on a large number of other host plants, including grains, fodder and weed species in the Gramineae family. Ringer et al., (1987) screened species from 68 plant families and identified that the nematode affected 42 of the 204 species tested, all of which were in the Gramineae family.

A detailed list of the known hosts of *H. zeae* is given in Table 5. These species could be considered in surveys to determine the spread of the pest following an incursion.

Table 5. *Known hosts of H. zeae*

Common name	Species name	Family	Reference
Western wheatgrass	*Agropyron smithii*	Gramineae	Ringer et al., (1987)
Bent grass	*Agrostis tenuis*	Gramineae	Ringer et al., (1987)
Meadow foxtail	*Alopecurus pratensis*	Gramineae	Ringer et al., (1987)
Sweet vernal grass	*Anthoxanthum odoratum*	Gramineae	Ringer et al., (1987)
Oats	*Avena sativa*	Gramineae	Ringer et al., (1987)
Bamboo	*Bambusa* spp.	Gramineae	Ringer et al., (1987)
Sideoats grama	*Bouteloua curtipendula*	Gramineae	Ringer et al., (1987)
Broadleaf signal grass	*Brachiaria platyphylla*	Gramineae	Ringer et al., (1987)
Buffalo grass	*Buchloe dactyloides*	Gramineae	Ringer et al., (1987)
Feathertop reed grass	*Calamagrostis eipgejos*	Gramineae	Ringer et al., (1987)
Rhodes grass	*Chloris gayana*	Gramineae	Ringer et al., (1987)
Job's tears	*Coix Lachryma-Jobi*	Gramineae	Ringer et al., (1987)

Common name	Species name	Family	Reference
Indian crabgrass	*Digitaria longiflora*	Gramineae	Lauritis et al., (1983)
Jungle rice	*Echinochloa colona*	Gramineae	Lauritis et al., (1983)
Barnyard grass	*Echinochloa crus-galli*	Gramineae	Ringer et al., (1987)
Tall fescue	*Festuca elatior*	Gramineae	Ringer et al., (1987)
Red fescue	*Festuca rubra*	Gramineae	Ringer et al., (1987)
Barley	*Hordeum vulgare*	Gramineae	Koshy et al., (1970); Ismail (2009); Ringer et al., (1987)
Green sprangletop	*Leptochloa dubia*	Gramineae	Ringer et al., (1987)
Perennial ryegrass	*Lolium perenne*	Gramineae	Ringer et al., (1987)
Muhly	*Muhlenbergia Montana*	Gramineae	Ringer et al., (1987)
Rice	*Oryza sativa*	Gramineae	Ismail (2009); Ringer et al., (1987)
Indian rice grass	*Oryzopsis hymenoides*	Gramineae	Ringer et al., (1987)
Giant panic	*Panicum antidotale*	Gramineae	Ringer et al., (1987)
Witchgrass	*Panicum capillare*	Gramineae	Ringer et al., (1987)
Bambatsi panic	*Panicum coloratum*	Gramineae	Ringer et al., (1987)
Fall panicum	*Panicum dichotomiflorum*	Gramineae	Ringer et al., (1987)
Broomcorn; Proso millet	*Panicum miliaceum*	Gramineae	Ringer et al., (1987)
-	*Panicum plenum*	Gramineae	Ringer et al., (1987)
Fountain grass	*Pennisetum setaceum*	Gramineae	Ringer et al., (1987)
Reed canary grass	*Phalaris arundinacea*	Gramineae	Ringer et al., (1987)
Common timothy	*Phleum pratense*	Gramineae	Ringer et al., (1987)
Common reed	*Phragmites australis*	Gramineae	Ringer et al., (1987)
Annual bluegrass	*Poa annua*	Gramineae	Ringer et al., (1987)
Kentucky bluegrass	*Poa pratensis*	Gramineae	Ringer et al., (1987)
Sugar cane	*Saccharum officinarum*	Gramineae	Ismail (2009); Ringer et al., (1987)
Cereal rye	*Secale cereale*	Gramineae	Ringer et al., (1987)
Foxtail millet	*Setaria italica*	Gramineae	Jain (2009)
Sorghum	*Sorghum bicolor*	Gramineae	Ismail (2009); Ringer et al., (1987)
Sudan grass	*Sorghum sudanense*	Gramineae	Lauritis et al., (1983)
Green needle grass	*Stipa viridula*	Gramineae	Ringer et al., (1987)

Common name	Species name	Family	Reference
Eastern gamagrass	*Tripsacum dactyloides*	Gramineae	Ringer et al., (1987)
Wheat	*Triticum aestivum*	Gramineae	Ismail (2009); Ringer et al., (1987)
Liverseed grass	*Urochloa panicoides*	Gramineae	Lauritis et al., (1983)
Chapule	*Zea diploperennis*	Gramineae	Ringer et al., (1987)
Maize	*Zea mays*	Gramineae	Koshy et al., (1970); Ringer et al., (1987)
Teosinte	*Zea mexicana*	Gramineae	Ringer et al., (1987)

The number of cysts reported by Ringer et al., (1987) varied between varieties when multiple varieties were tested. Also when different strains of the nematode were tested on the same host plant the researchers found that not all strains attacked and reproduced on the same host, for example only one of the nematode strains tested affected rice.

5.1.5 Current geographic distribution

This nematode occurs in the Northern Hemisphere in countries with warm climates. It has been reported in Asia, Africa, North America and Europe (see Table 6).

Table 6. *Countries where H. zeae is known to occur*

Country	Reference
Egypt	Aboul-Eid and Ghorab (1981)
India	Koshy et al., (1970); Abdollahi (2009); Sharma et al., (2009)
Pakistan	Maqbool (1981); Abdollahi (2009)
Portugal	Correia et al., (2005)
Thailand	Abdollahi (2009)
USA	Abdollahi (2009); Ringer et al., (1987)

5.1.6 Potential geographic distribution in Australia

As described in Table 6 this nematode is found in warm parts of the Northern Hemisphere. As climatic conditions between Australia and the pest's current distribution are similar, the nematode may be a problem in parts of Australia especially for the maize growing regions with soils temperatures above 25°C. Hutzell and Krusberg (1990) described the effects of temperature on the reproduction of the Maize cyst nematode and found that the optimal temperature for reproduction was 33°C, and that eggs didn't hatch when kept at 20°C, but did hatch at temperatures above 25°C.

5.1.7 Symptoms

H. zeae causes a number of symptoms on its host plants. These include:

- Isolated patches of unhealthy plants that are stunted and pale coloured (Koshy and Swarup 1971).
- Reduced yield (Krusberc et al., 1997).
- Presence of tan coloured, lemon shaped cysts on the host plant's roots (Liu et al., 2009).

5.1.8 Diagnostic information

No National Diagnostic Protocol has been developed or endorsed for *H. zeae* or any other exotic nematode that affects the grains industry.

For diagnostic facilities and advisory services that can be utilised in the event of an incursion see Section 10.2 Appendix 2.

5.1.8.1 MORPHOLOGICAL AND PHYSIOLOGICAL DIAGNOSIS

General background on morphological diagnosis of Heterodera nematodes

There are a number of cyst nematodes that infect cereals and grasses in the cyst nematode complex. Of these, only one species, *H. avenae* has been recorded in Australia, and in Australia this species is believed to be made up of only race, Ha13. This identification may need to be confirmed by taxonomists overseas and by molecular techniques. Molecular tests have been developed in Australia for *H. avenae* and sequence data is available at CSIRO Plant Industries, Canberra.

As the cyst structure is usually the most readily available life stage, taxonomic characters are often related to the cyst. Morphologically, cyst nematodes are identified by assessing differences in the vulval cone structure of the adult cyst. In the *Heterodera* genus, the cuticle in the area surrounding the vulva (of the mature cyst) ruptures and is termed the fenestra. The fenestra is used as a diagnostic feature.

In some cyst nematode species, the cuticle thickens at the end of the vagina and forms an underbridge, as is the case for *H. zaea*.

Measurements of juvenile nematodes can also be a diagnostic feature including the lateral lines, stylet length and the length of the hyaline (clear) part of the tail.

Samples of suspected exotic nematodes would require identification by a nematode taxonomist, as species identification based on morphology requires expertise for the preparation and measurement of specimens and diagnostic structures.

Identification of H. zeae

H. zeae can be identified by its four bullae (finger-like projections) on the cyst cone arranged in the shape of the letter "X". Cysts are lemon shaped with a "neck" and light-brown to tan in colour (Correia et al., 2005). A thin underbridge is present in *H. zaea* (Figure 9). Abdollahi (2009) reported between 0 and 14 secondary bullae in the specimens he examined. The fenestra is used as a diagnostic feature and in *H. zeae* the fenestra is described as being ambifenestrate (Koshy et al., 1970).

The morphology of the second stage juvenile and size of distinctive features can also aid in diagnosis. Sharma et al., (2009) studied this species from locations in India but found a degree of variation in the size of various features.

Table 7 provides a summary of the key morphological features of this nematode.

Table 7. *Key morphological features of H. zeae*

Structure	Size (µm)	Reference
Female - length (including neck)	459 to 796	Golden and Mulvey (1983)
Female - width (including neck)	275 to 515	Golden and Mulvey (1983)
Female – stylet	22.4 to 25.8	Golden and Mulvey (1983)
Male[4] – length	640.8 to 993.6 (average 806.9)	Hutzell (1984)
Male – width	23.2 to 43.2 (average 27.8)	Hutzell (1984)
Male – stylet length	24 to 24.8 (average 24.2)	Hutzell (1984)
Cyst – length (including neck)	454 to 785	Golden and Mulvey (1983)
Cyst – width	255 to 551	Golden and Mulvey (1983)
Cyst – neck length	66.5 to 96.3	Correia et al., (2005)
Cyst – neck width	53.5 to 61.5	Correia et al., (2005)
Fenestral - length	35 to 45	Golden and Mulvey (1983)
Fenestral - width	16 to 34	Golden and Mulvey (1983)
Vulval slit - length	29 to 42	Golden and Mulvey (1983)
Vulval bridge - width	6 to 16	Sharma et al., (2009)
Underbridge - length	30 to 41	Golden and Mulvey (1983)
Underbridge - width	7.6 to 12	Golden and Mulvey (1983)
Underbridge - depth	29 to 38	Golden and Mulvey (1983)
Second stage juvenile body - length	340 to 451	Sharma et al., (2009)
Second stage juvenile body -width	15 to 27	Sharma et al., (2009)
Second stage juvenile stylet - length	19 to 25	Sharma et al., (2009)
Second stage juvenile tail - length	35 to 66	Sharma et al., (2009)
Second stage juvenile hyaline tail terminus - length	18 to 30	Sharma et al., (2009)

[4] Note males rarely encountered in soil samples (Hutzell 1984).

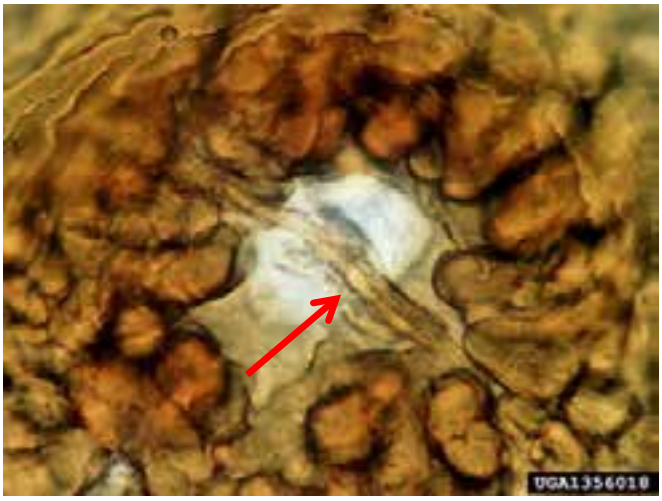

Figure 9. *Vulval cone of H. zeae, note underbridge (arrow). Source: Jonathan D. Eisenback Organization: Virginia Polytechnic Institute and State University, Bugwood.org*

5.1.8.2 PCR

Polymerase Chain Reaction (PCR) is a rapid, specific, and sensitive test that can be used to detect and diagnose this nematode. Szalanski et al., (1997) describe using Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR RFLP) to identify cyst nematodes, including *H. zeae*. The paper suggests that these methods can be used to identify this nematode, however no commercial tests are currently available to detect this pest.

5.1.9 Pest risk analysis – Maize cyst nematode

Potential or impact	Rating
Entry potential	Medium
Establishment potential	High
Spread potential	Medium
Economic impact	High
Overall risk	Medium

5.1.9.1 ENTRY POTENTIAL

Rating: Medium

H. zeae could enter the country as cysts in soil adhering to machinery, vehicles, equipment, containers, seeds, clothing and footwear or as a contaminant of other goods. Alternatively the nematode could enter the country on the roots of live plants. Given the increase in international travel and movement of goods there is a potential of *H. zeae* entering Australia.

The entry potential of *H. zeae* is considered to be **Medium**.

5.1.9.2 ESTABLISHMENT POTENTIAL

Rating: High

H. zeae has a wide host range (see Table 5) occurring in countries with a warm climate (see Table 6). The nematode requires temperatures above 25°C to successfully reproduce (Hutzell and Krusberg 1990). The nematode is adaptable and could therefore establish in Australia in the warmer climates. These areas have a number of susceptible hosts, including maize.

The establishment potential of *H. zeae* in Australia is considered to be **High**.

5.1.9.3 SPREAD POTENTIAL

Rating: Medium

H. zeae is spread by the movement of cysts. These can be spread in soil carried between areas on machinery, equipment, footwear or on live plants (e.g. seedlings). The cysts could also be spread between paddocks with irrigation water, especially if the irrigation water is used across multiple paddocks (Lehman 1994). Strong winds could also potentially blow the cysts between areas.

The spread potential of *H. zeae* is considered to be **Medium**.

5.1.9.4 ECONOMIC IMPACT

Rating: High

H. zeae is responsible for significant crop losses overseas. Krusberc et al., (1997) reported losses of between 21 and 73% in inoculated maize grown in pots. If such losses were to occur in Australia than an incursion of *H. zeae* in Australia would be expected to have a significant economic impact on maize producers, impacts may also extend to sweet corn and silage producers.

The economic impact of *H. zeae* on the grains industry is considered to be **High**.

5.1.9.5 OVERALL RISK

Rating: Medium

Based on the individual ratings above, the combined overall risk of *H. zeae* is considered to be **Medium**.

5.2 Pest Details – Soybean Cyst Nematode (*H. glycines*)

Common name:	Soybean cyst nematode
Scientific name:	*Heterodera glycines* Ichinohe, 1952
Synonyms:	Sudden death syndrome; yellow dwarf disease in soybean
Taxonomic position:	Kingdom: Animalia
	Phylum: Nematoda
	Class: Secernentea
	Family: Heteroderidea
	Genus: *Heterodera*

The information from this plan has been primarily obtained from documents as cited in the reference section, as well as a Pest Risk Review (Plant Health Australia 2005b).

5.2.1 Background

H. glycines is considered to be the most damaging pest of soybeans in the world (Wrather et al., 1997; Koenning et al., 1999) and has been associated with significant crop losses. Wrather et al., (1997) calculated that losses in the top ten soybean producing countries (USA, Brazil, China, Argentina, India, Canada, Paraguay, Indonesia, Italy, Bolivia) due to *H. glycines* as being ~15 million tonnes and a cost of $3.31 Billion USD (note: not all of the ten countries have the nematode). However yield losses in the United States vary between regions and range from 0-15% (Koenning et al., 1999), while other US papers suggest yield losses of between 16 and 32% (Young 1996).

There are at least 16 races of *H. glycines* (Riggs and Schmitt 1988) with this nematode belonging to the *H. schachtii* group (EPPO 2008).

The nematode invades the roots of its host and develops into a white then dark brown coloured, lemon shaped cyst that are visible on the roots of infected plants (Ravichandra 2010). These together with crop symptoms like isolated patches of unhealthy plants that are stunted and pale coloured are caused by the parasite attacking the plant's roots (Ichinohe 1988).

Soybean cyst nematodes can survive without access to a host for up to 11 years (Riggs and Niblach 1993).

5.2.2 Life cycle

See Section 5.1.2 and Figure 8 for the general life cycle for cyst nematodes.

The timing of *H. glycines* life cycle is dependent on temperature. Alston and Schmitt (1988) studied this nematode and found that below 20°C eggs did not hatch and that when temperatures reached 36°C the eggs died. The researchers found that the optimum temperature for emergence from the cyst was 24°C and that the lifecycle was shortest at 31°C taking only 18 days to complete.

5.2.3 Dispersal

H. glycines, like all cyst nematodes produce cysts. These cysts protect the eggs from desiccation and in turn assist with the nematodes ability to be spread over long distances. The nematode could potentially be spread over long distances as cysts in soil contaminating machinery, footwear, plants or other goods.

Once in a country the nematode could be spread between properties, and within paddocks, by soil movement either on machinery, footwear, animals or by water movement. Irrigation also poses a risk of spreading the nematode between regions (Lehman 1994) especially if irrigation water is recirculated and used on multiple paddocks within a single farm. There is also a suggestion that cysts of *H. glycines* can be spread over long distances in the digestive tracts of birds, as viable cysts of *H. glycines* have been detected in the faeces of Brown headed cowbird (*Molothrus ater*), Grackle (*Quiscalus quiscula*), and Starling (*Sturnis vulgaris*) after feeding with seed contaminated with cysts (Epps 1971). Strong winds could also blow the cysts to new areas.

5.2.4 Host range

The Soybean cyst nematode predominantly affects soybean crops (Koenning et al., 1999; Wrather et al., 1997, however it has also been reported on a number of other host plants in several dicot plant families.

See Table 8 for a detailed list of the known hosts of *H. glycines*.

Table 8. *Known hosts of H. glycines*

Common name	Species name	Family	Reference
Buda pea	*Aeschynomene indica*	Fabaceae	Rajan and Lal (2005)
Virginia jointvetch	*Aeschynomene virginica*	Fabaceae	Riggs (1977)
Beetroot	*Beta vulgaris*	Chenopodiaceae	Rajan and Lal (2005)
Small flowered bittercress	*Cardamine parviflora*	Brassicaceae	Johnson et al., (2008)
Pigeon pea	*Cajanus cajan*	Fabaceae	Rajan and Lal (2005); Valle et al., (1996)
Shepherd's-purse	*Capsella bursa-pastoris*	Brassicaceae	Venkatesh et al., (2000)
Chickweed	*Cerastium vulgatum*	Caryophyllaceae	Noel (1993)
Sunn hemp	*Crotalaria juncea*	Fabaceae	Valle et al., (1996)
Geranium	*Geranium* sp.	Geraniaceae	EPPO (2008)
Soybean	*Glycine max*	Fabaceae	Ichinohe (1952); Koenning et al., (1999); Wrather et al., (1997); Venkatesh et al., (2000)
Wild soybean	*Glycine soja* (syn. *G. ussuriensis*)	Fabaceae	Ichinohe (1955)

Common name	Species name	Family	Reference
Japanese clover	*Kummerowia striata*	Fabaceae	Rajan and Lal (2005)
Korean lespedeza; Korean clover	*Kummerowia stipulacea* (syn. *Lepedeza stipulaceae*)	Fabaceae	Skotland (1956)
Henbit deadnettle	*Lamium amplexicaule*	Lamiaceae	Epps and Chambers (1958); Venkatesh et al., (2000); Riggs (1977)
Purple deadnettle	*Lamium purpureum*	Lamiaceae	Venkatesh et al., (2000)
Chinese bushclover	*Lespedeza cuneata*	Fabaceae	Rajan and Lal (2005)
Common lespedeza	*Lespedeza striata*	Fabaceae	Riggs (1977)
Old field toadflax	*Linaria canadensis*	Plantaginaceae	Riggs (1977)
Lupin	*Lupinus* sp.	Fabaceae	Rajan and Lal (2005)
White lupin	*Lupinus albus*	Fabaceae	Epps and Chambers (1958)
Poppy	*Papaver* sp.	Papaveraceae	EPPO (2008)
Foxglove penstemon	*Penstemon digitalis*	Plantaginaceae	Riggs (1977)
Adzuki bean	*Phaseolus angularis* (syn. *Vigna angularis*)	Fabaceae	Ichinohe (1952); Rajan and Lal (2005); Valle et al., (1996)
Mung bean	*Phaseolus aureus*	Fabaceae	Noel (1993)
Common bean	*Phaseolus vulgaris*	Fabaceae	Ichinohe (1952); Melton et al., (1986); Poromarto et al., (2010)
Field pea	*Pisum sativum*	Fabaceae	Rajan and Lal (2005); Noel (1993)
Coffeeweed	*Sesbania herbacea* (syn. *S. exaltata*)	Fabaceae	Epps and Chambers (1958); Rajan and Lal (2005); Riggs (1977)
Common chickweed	*Stellaria media*	Caryophyllaceae	Rajan and Lal (2005)
Field pennycress	*Thlaspi arvense*	Brassicaceae	Venkatesh et al., (2000)
Common mullerin	*Verbascum thapsus*	Scrophulariaceae	Rajan and Lal (2005)
Mat bean	*Vigna aconitifolia*	Fabaceae	Rajan and Lal (2005)
Black gram; black lentil	*Vigna mungo*	Fabaceae	Rajan and Lal (2005)
Mung bean	*Vigna radiata*	Fabaceae	Rajan and Lal (2005); Valle et al., (1996)
Cowpea	*Vigna sinensis*	Fabaceae	Diab (1968)

Common name	Species name	Family	Reference
Common vetch	*Vicia sativa*	Fabaceae	Skotland (1956)
Hairy vetch	*Vicia villosa*	Fabaceae	Schmitt and Riggs (1991); Rajan and Lal (2005)

5.2.5 Current geographic distribution

H. glycines occurs in most soybean producing countries. It has been reported in Asia, Africa, South America and North America (see Table 9). It does not occur in Australia.

Table 9. *Countries where H. glycines is known to occur*

Country	Continent	Reference
Argentina	South America	Lax et al., (2004)
Brazil	South America	Evans and Rowe (1998); Subbotin et al., (2001); Wrather et al., (1997)
Canada	North America	Subbotin et al., (2001); Wrather et al., (1997)
China	Asia	Evans and Rowe (1998); Subbotin et al., (2001); Riggs and Schmitt (1988)
Colombia	South America	Subbotin et al., (2001); Evans and Rowe (1998); Gomez and Medina (1983)
Egypt	Africa	Subbotin et al., (2001)
India	Asia	Kaushal et al., (2002)
Indonesia	Asia	Evans and Rowe (1998); Subbotin et al., (2001); Riggs and Schmitt (1988)
Iran	Asia	Maafi et al., (1999)
Italy	Europe	Manachini (2000)
Japan	Asia	Evans and Rowe (1998); Ichinohe (1988); Subbotin et al., (2001)
Korea	Asia	Evans and Rowe (1998); Subbotin et al., (2001)
Paraguay	South America	Centurion et al., (2004)
Puerto Rico	South America	Smith and Chavarria-Carvajal (1999)
Russia	Europe/Asia	Evans and Rowe (1998); Subbotin et al., (2001)
USA	North America	Evans and Rowe (1998); Niblack et al., (1993); Riggs and Schmitt (1988)

5.2.6 Potential geographic distribution in Australia

As described in Table 9 this nematode has a wide geographic distribution and occurs in most soybean producing countries and could therefore potentially survive in Australia. The Soybean cyst

nematode requires temperatures of >20°C for the eggs to hatch (Alston and Schmitt 1988) suggesting the conditions experienced in most Australian soybean growing regions (i.e. northern NSW and Queensland) would be suited for the establishment and reproduction of this pest, for at least part of the year.

5.2.7 Symptoms

Above ground and below ground symptoms of *H. glycines* include:

- The presence of white to dark brown coloured, lemon shaped cysts on the roots of infected plants (Ravichandra 2010, Figure 10).
- Isolated round patches of unhealthy plants that are stunted, pale green coloured and often chlorotic plants (Figure 11) occurring approximately 50+ days after planting (Ichinohe 1988).
- Poorly developed roots (Manachini 2000).
- Fewer pods per plant, fewer seeds per pod and lighter seed weights (Hartman et al., 1995).
- Poor yield and reduced *Bradyrhizobium japonicum* (a soybean rhizobium bacteria) nodulation (Noel 1993).

Stunting symptoms, reduced numbers of pods and reduced yield were also reported by Poromarto et al., (2010) on common bean (*P. vulgaris*) plants infected by *H. glycines*.

Figure 10. *Cysts (some arrowed) on soybean roots. Source: Elizabeth Bush, Virginia Polytechnic Institute and State University, Bugwood.org*

Figure 11. *Stunting and leaf discolouration caused by H. glycines is visible in the two rows on the left of the photograph compared to healthy plants on the right. Source: Erik Stromberg Virginia Polytechnic Institute and State University, Bugwood.org*

5.2.8 Diagnostic information

Currently there is not an endorsed National Diagnostic Protocol for *H. glycines*. However EPPO (2008) has produced a diagnostic protocol for this species.

For diagnostic facilities and advisory services that can be utilised in the event of an incursion see Section 10.2 Appendix 2.

5.2.8.1 MORPHOLOGICAL AND PHYSIOLOGICAL DIAGNOSIS

H. glycines is difficult to identify and requires expert skills to confirm its presence. The Soybean cyst nematode can be identified using the following features:

Females are white when young becoming cream-yellow with age. Cysts are lemon shaped (see Table 10 and Figure 12) and white to dark brown in colour depending on maturity (Ravichandra 2010). The cysts have a well-defined neck (Faghihi et al., 1986). They have a well-developed underbridge (Figure 13), ambifenestrate (Zheng et al., 2009) and well developed brown coloured bullae (Ravichandra 2010).

The morphology of the second stage juvenile and size of distinctive features can also aid in diagnosis, as approximately half the tail of *H. glycines* juveniles are hyaline (Ravichandra 2010). See Table 10 for key morphological traits.

Table 10. *Key morphological traits for identification of H. glycines*

Structure	Size (µm)	Reference
Male – stylet length	25.7 to 27.4	Faghihi et al., (1986)
Cyst - length	717.6 to 764.3	Faghihi et al., (1986)
Cyst - width	434.2 to 440.3	Faghihi et al., (1986)
Cyst – neck length	71.3 to 80.1	Faghihi et al., (1986)
Fenestral - length	41 to 52 µm	Zheng et al., (2009)
Fenestral - width	33 to 48 µm	Zheng et al., (2009)
Vulval slit - length	47 to 55 µm	Zheng et al., (2009)
Underbridge - length	79 to 94 48.7 to 150	Zheng et al., (2009) Centurion et al., (2004)
Underbridge - width	7.4 to 28	Centurion et al., (2004)
Second stage juvenile body - length	373 to 490 (average 439.6)	Ravichandra (2010)
Second stage juvenile body - width	22 to 24 (average 23)	Ravichandra (2010)
Second stage juvenile stylet - length	22 to 25	Bridge and Starr (2007)
Second stage juvenile tail - length	42 to 59.4 (average 50.4)	Ravichandra (2010)
Second stage juvenile hyaline tail terminus - length	20 to 33 (average 26.6)	Ravichandra (2010)

Figure 12. *Lemon shaped cyst of H. glycines containing eggs. Note well developed cone (circle) and neck (arrow). Source: Agroscope FAL Reckenholz Archive, Swiss Federal Research Station for Agroecology and Agriculture, Bugwood.org*

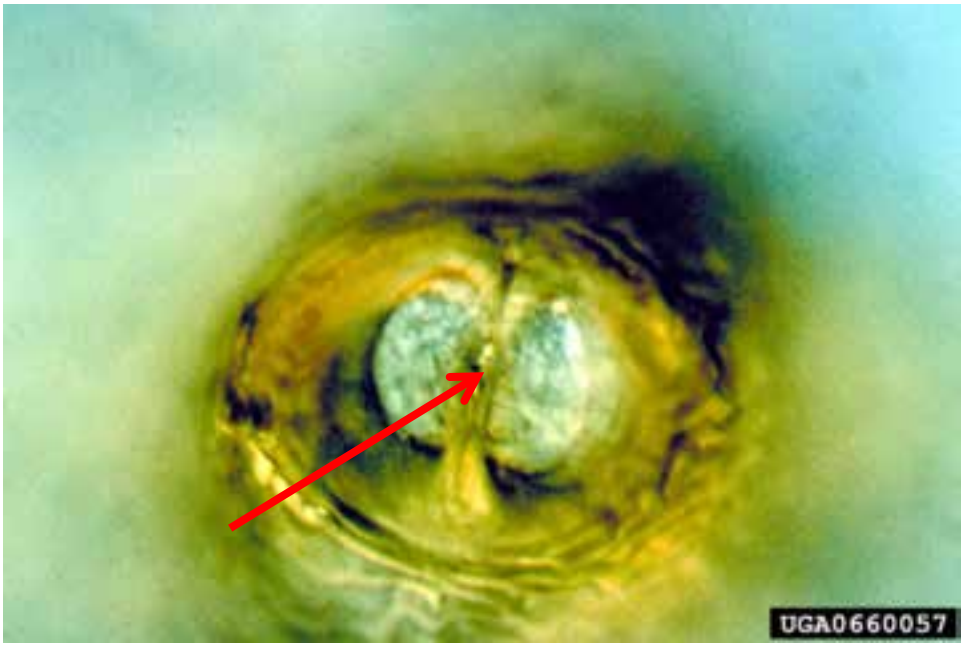

Figure 13. *Vulval cone H. glycines, note underbridge (arrow). Source: Agroscope FAL Reckenholz Archive, Swiss Federal Research Station for Agroecology and Agriculture, Bugwood.org*

5.2.8.2 PCR

Szalanski et al., (1997) use PCR RFLP to identify cyst nematodes, including *H. glycines*. Duplex PCR has also been used to identify the *H. glycines* juveniles and cysts (Subbotin et al., 2001).

Currently there are no commercial tests available to detect *H. glycines*.

5.2.9 Pest risk analysis – Soybean cyst nematode

Potential or impact	Rating
Entry potential	Medium
Establishment potential	Medium
Spread potential	Medium
Economic impact	High
Overall risk	Medium

5.2.9.1 ENTRY POTENTIAL

Rating: Medium

H. glycines could enter the country as cysts in soil adhering to machinery, vehicles, equipment, containers, seeds and footwear. Alternatively the nematode could enter the country on the roots of live plants. Given the increase in international travel and movement of goods there is a potential of *H. glycines* entering Australia. The entry potential of *H. glycines* is considered to be **Medium**.

5.2.9.2 ESTABLISHMENT POTENTIAL

Rating: Medium

H. glycines has a wide host range (see Table 8) and is found in countries with a moderate climate (see Table 9) where it requires temperatures above 20°C for its eggs to hatch (Alston and Schmitt 1988). With these requirements the nematode could become established in most soybean growing areas (northern NSW and Queensland). If the nematode should enter Australia it would have access to potential host plants including soybeans and some fodder species. The establishment potential of *H. glycines* is considered to be **Medium.**

5.2.9.3 SPREAD POTENTIAL

Rating: Medium

H. glycines is spread by the movement of cysts in soil which could be spread between areas on machinery, equipment, footwear or on live plants (e.g. vegetable seedlings, potted plants etc.). The cysts could also be spread between paddocks with irrigation water, especially if the irrigation water is used across multiple paddocks (Lehman 1994). Strong winds could potentially blow the cysts between areas. The cysts could also be spread between areas by birds, as Epps (1971) found that *H. glycines* cysts could survive passing through the digestive system of birds in the USA. The spread potential of *H. glycines* is considered to be **Medium**.

5.2.9.4 ECONOMIC IMPACT

Rating: High

H. glycines is considered the world's worst soybean pest (Wrather et al., 1997) causing soybean yield losses of ~15 million tonnes and in 1994 at a cost to the top ten soybean producing countries of $3.31 billion USD. Yield losses in the United States vary between regions and range from 0-15% (Koenning et al., 1999), while other reports show losses up to 32% (Young 1996). The economic impact of *H. glycines* to the grains industry is considered to be **High.**

5.2.9.5 OVERALL RISK

Rating: Medium

Based on the individual ratings above, the combined overall risk of *H. glycines* is considered to be **Medium**.

5.3 Pest Details –Chickpea Cyst Nematode (*H. ciceri*)

Common name:	Chickpea cyst nematode
Scientific name:	*Heterodera ciceri* Volvas, Greco and Di Vito, 1985
Synonyms:	
Taxonomic position:	Kingdom: Animalia
	Phylum: Nematoda
	Class: Secernentea
	Family: Heteroderidea
	Genus: *Heterodera*

The information from this plan has been primarily obtained from documents as cited in the reference section.

5.3.1 Background

H. ciceri is a major pest of chickpea and also affects lentils (Greco et al., 1986). The nematode was first detected in Syria and based on its morphology it belongs to the *H. schachtii* group (Vovlas et al., 1985). It also occurs in other countries in western Asia (Di Vito et al., 2001). The Chickpea cyst nematode has been associated with yield losses of up to 20% overseas (Evans and Rowe 1998). This species does not occur in Australia.

The nematode invades the roots of its host and females become lemon shaped cysts that are white in colour changing to brown as they mature, these cysts are visible on the roots of infected plants (Kaloshian et al., 1986). Typical symptoms include: patches of stunted yellowing plants with few flowers and few pods (Greco et al., 1992a).

5.3.2 Life cycle

See Section 5.1.2 and Figure 8 for the general life cycle for cyst nematodes.

Kaloshian et al., (1986) examined the effects of different temperatures on nematodes development identifying that the minimum temperature required by the nematode to develop and reproduce was 10°C. At 20°C cysts were observed 38 days after sowing, suggesting rapid reproduction is possible under some conditions. The highest number of eggs was produced when the nematodes were kept at 25°C.

5.3.3 Dispersal

H. ciceri produce cysts, which protect the eggs from desiccation and in turn assist with the nematodes ability to be spread over long distances. The nematode could potentially be spread over long distances as cysts in soil contaminating machinery, footwear, plants or goods entering the country.

Once in a country the nematode can be spread between properties, and within paddocks, by soil movement either on machinery, footwear, animals or by water movement. Irrigation also poses a risk of spreading the nematode between regions (Lehman 1994) especially if irrigation water is recirculated and used on multiple paddocks. There is also a risk that strong winds could blow the cysts to new areas.

5.3.4 Host range

The Chickpea Cyst Nematode predominantly affects chickpeas and lentils (Greco et al., 1986); however it has also been reported on a number of other host plants from two plant families (Fabaceae and Caryophyllaceae), including grain legumes, fodder species and ornamental plants.

Di Vito et al., (2001) showed that nematodes from different locations (i.e. different genotypes) were able to reproduce on different plants, for example only Turkish and Syrian strains of the nematode reproduced well on lucerne and annual medics, while the strains from Jordon and Lebanon did not reproduce on these plants, implying there are different levels of pathogenicity between the different populations/strains of *H. ciceri*.

See Table 11 for a detailed list of the known hosts of Chickpea cyst nematode.

Table 11. *Known hosts of H. ciceri*

Common name	Species name	Family	Reference
Chick pea	*Cicer arietinum*	Fabaceae	Greco et al., (1986); Greco et al., (1992a); Vovlas et al., (1985); Di Vito et al., (2001); Singh et al., (1989)
-	*Cicer bijugum*	Fabaceae	Di Vito et al., (2001); Singh et al., (1989)
-	*Cicer chorassanicum*	Fabaceae	Singh et al., (1989)
-	*Cicer cuneatum*	Fabaceae	Singh et al., (1989)
-	*Cicer echinospermum*	Fabaceae	Singh et al., (1989)
-	*Cicer judaicum*	Fabaceae	Singh et al., (1989)
-	*Cicer pinnatifidum*	Fabaceae	Di Vito et al., (2001); Singh et al., (1989)
Wild chickpea	*Cicer reticulatum*	Fabaceae	Di Vito et al., (2001); Singh et al., (1989)
-	*Cicer yamashitae*	Fabaceae	Singh et al., (1989)
Carnation [5]	*Dianthus caryophyllus*	Caryophyllaceae	Vovlas et al., (1985); Greco et al., (1986); Di Vito et al., (2001)
Grass pea	*Lathyrus sativus*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Greco et al., (1992a); Di Vito et al., (2001)

[5] Reported as a very poor host (Greco et al., 1986). Di Vito et al., (2001) also reported very low numbers of female nematodes on this species.

Common name	Species name	Family	Reference
Lentil	*Lens culinaris*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Greco et al., (1992a); Di Vito et al., (2001)
Lupin[6]	*Lupinus albus*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Di Vito et al., (2001)
Annual medic[7]	*Medicago rigidula*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986)
Lucerne[6]	*Medicago sativa*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Di Vito et al., (2001)
Field pea	*Pisum sativum*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Greco et al., (1992a);
Common bean[6,8]	*Phaseolus vulgaris*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986) Di Vito et al., (2001)
Annual medic[9]	*Medicago rigidula*	Fabaceae	Di Vito et al., (2001)
Crimson clover[10]	*Trifolium incarnatum*	Fabaceae	Greco et al., (1986); Di Vito et al., (2001)
Red clover[11]	*Trifolium pratense*	Fabaceae	Greco et al., (1986)
Faba bean[11]	*Vicia faba*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Di Vito et al., (2001)
Vetch[11]	*Vicia sativa*	Fabaceae	Vovlas et al., (1985); Greco et al., (1986); Di Vito et al., (2001)
Cowpea	*Vigna unguiculata*	Fabaceae	Vovlas et al., (1985)

[6] Reported as a poor host (Greco et al., 1986).

[7] Reported as a very poor host (Greco et al., 1986).

[8] Only some of the populations of Chickpea cyst nematode (Turkish and Syrian) reproduced on these plants (Di Vito et al., 2001).

[9] Only some of the populations of *H. ciceri* (Turkish and Syrian) reproduced on these plants (Di Vito et al., 2001).

[10] Reported as a very poor host (Greco et al., 1986). Di Vito et al., (2001) also reported low numbers of female nematodes on this species and some strains of the nematode reproduce better than others.

[11] Reported as a very poor host (Greco et al., 1986). Di Vito et al., (2001) also reported very low numbers of female nematodes on this species.

5.3.5 Current geographic distribution

This nematode occurs in four countries in western Asia (see Table 12).

Table 12. *Countries where H. ciceri is known to occur*

Country	Reference
Syria	Vovlas et al., (1985); Di Vito et al., (2001)
Jordon	Di Vito et al., (2001)
Lebanon	Di Vito et al., (2001)
Turkey	Di Vito et al., (1994); Di Vito et al., (2001)

5.3.6 Potential geographic distribution in Australia

The Chickpea cyst nematode requires a minimum temperature of 10°C to develop and is currently present in west Asian countries with Mediterranean climates (Kaloshian et al., 1986). With parts of the Australian grain belt having a Mediterranean climate the potential geographic distribution in Australia would include the main chickpea producing areas of Queensland and New South Wales.

5.3.7 Symptoms

H. ciceri causes a number of symptoms on its host plants. These include:

- Lemon shaped cysts (that are white when immature and change to a brown colour as they mature) on the roots of infected plants (Kaloshian et al., 1986).
- Isolated patches of stunted, yellowing plants with few flowers and few pods (Greco et al., 1992a).
- Poor yield of chickpeas if nematode population exceeds ~1.15 eggs/cm^3 of soil, or 2.5 eggs/cm^3 of soil for lentils (Greco et al., 1988).
- The roots of infected plants often lack nodules (*Rhizobium* spp.) (Greco et al., 1992b).

Greco et al., (1992a) described the symptoms on lentils (*Lens culinaris*), field peas (*Pisum sativum*) and grass peas (*Lathyrus sativus*) as similar, but less severe, than those observed on chickpeas (*Cicer arietinum*).

5.3.8 Diagnostic information

Currently there is not an endorsed National Diagnostic Protocol for *H. ciceri*.

For diagnostic facilities and advisory services that can be utilised in the event of an incursion see Section 10.2 Appendix 2.

5.3.8.1 MORPHOLOGICAL AND PHYSIOLOGICAL DIAGNOSIS

H. ciceri can be diagnosed based on its morphology and the physical size of some key morphological features. Males are vermiform (worm like). Females are lemon-shaped with well defined “neck” and white to yellow-brown in colour, depending on maturity (Vovlas et al., 1985). The cysts of *H. ciceri* are a white colour when young becoming a brown colour as they mature (Kaloshian et al., 1986). Cysts are lemon shaped with defined necks and have a well-developed underbridge, ambifenestrate, the semifenestrae are semicircular and sub-equal in size. There are numerous dark brown bullae around the vulval cone (Vovlas et al., 1985). See Table 13 for details on the size of key morphological features on Chickpea cyst nematode.

Table 13. *Morphology of H. ciceri (from: Vovlas et al., 1985)*

Structure	Size (µm)
Male – length	1235-1488 (average 1308)
Male – width	29-30
Male – stylet	28-30 (average 29)
Female – length	550-950 (average 773)
Female – width	300-520 (average 451)
Female – stylet	29-31 (average 30)
Cyst – length	570-930 (average 796)
Cyst – width	350-550 (average 452)
Cyst - neck	115
Eggs – length	123-143 (average 134)
Eggs –width	48-53 (average 50)
Fenestral - length	32-52 (average 40)
Fenestral - width	20-37 (average 27)
Vulval slit - length	43-60 (average 50)
Underbridge - length	115-160 (average 125)
Second stage juvenile body - length	440-585 (average 525)
Second stage juvenile body - width	19-22 (average 21)
Second stage juvenile stylet - length	27-30 (average 28.6)
Second stage juvenile tail- length	53-72 (average 60)
Second stage juvenile hyaline tail terminus - length	31-42 (average 36)

5.3.8.2 PCR

The evolutionary history of the *Heterodera* nematode complex (including *H. ciceri*) have been studied using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR–RFLP) and Internal Transcribed Spacer region of the ribosomal DNA (ITSrDNA) sequences (Madani et al., 2004), techniques that can also be used for molecular diagnosis.

Currently there are no commercial PCR tests available for the diagnosis of this nematode.

5.3.9 Pest risk analysis – Chickpea cyst nematode

Potential or impact	Rating
Entry potential	Medium
Establishment potential	High
Spread potential	Medium
Economic impact	High
Overall risk	Medium

5.3.9.1 ENTRY POTENTIAL

Rating: Medium

H. ciceri could enter the country as cysts in soil adhering to machinery, vehicles, equipment, containers, seeds and footwear. Alternatively the nematode could enter the country on the roots of live plants. Given the increase in international travel and movement of goods there is a potential of *H. ciceri* entering Australia. The entry potential of *H. ciceri* can be considered to be **Medium**.

5.3.9.2 ESTABLISHMENT POTENTIAL

Rating: High

H. ciceri is able to infect and reproduce on a number of host plants (chickpeas, lentils, lucerne and lupins) in the Fabaceae family (see Table 11) which are widely planted throughout the grain belt.

H. ciceri requires temperatures above 10^{o}C to successfully reproduce (Kaloshian et al., 1986) and is currently found in countries with a Mediterranean climate (see Table 12). Australia has suitable climatic conditions for the reproduction of this nematode. The establishment potential of *H. ciceri* is considered to be **High**.

5.3.9.3 SPREAD POTENTIAL

Rating: Medium

H. ciceri is able to be spread by the movement of cysts in soil which can be spread between areas on machinery, equipment, footwear or on live plants (e.g. potted plants, seedlings, etc.). The cysts could also be spread between paddocks with irrigation water, especially if the irrigation water is used across

multiple paddocks (Lehman 1994), alternatively strong winds could potentially blow the cysts between areas. The spread potential of *H. ciceri* is considered to be **Medium**.

5.3.9.4 ECONOMIC IMPACT

Rating: High

H. ciceri has been associated with chickpea yield losses of up to 20% (Evans and Rowe 1998) and therefore would have the potential to impact on the grain legume industries if it was to establish in Australia. The economic impact of *H. ciceri* is considered to be **High.**

5.3.9.5 OVERALL RISK

Rating: Medium

Based on the individual ratings above, the combined overall risk of *H. ciceri* is considered to be **Medium**.

6 Pest management

6.1 Response checklist

The following checklist (Table 14) provides a summary of the generic requirements to be identified and implemented within a Response Plan for an incursion of an exotic nematode into Australia.

Table 14. *Checklist of requirements to be identified in a Response Plan*

Checklist item	Further information
Destruction methods for plant material, soil and disposable items	Section 7.1.1, 7.1.2
Disposal procedures	Section 7.1.5
Quarantine restrictions and movement controls	Section 7.3
Decontamination and property clean up procedures	Section 7.5
Diagnostic protocols and laboratories	Sections 5.1.8, 5.2.8, 5.3.8 and 10.2
Trace back and trace forward procedures	Section 7.6
Protocols for delimiting, intensive and ongoing surveillance	Section 6.2
Zoning	Section 7.4
Reporting and communication strategy	Section 10.3

A range of specifically designed procedures for the emergency response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia 2013).

6.2 Surveys and epidemiology studies

Information provided in Sections 6.2.1 to 6.2.3 provides a framework for the development of early detection and delimiting surveys for nematodes.

As nematodes are spread by soil it is important that personnel avoid moving infested soil between paddocks and properties. Footwear, tools, equipment and vehicles should be thoroughly washed of soil and plant debris and then sanitised with a registered disinfectant. Extra precautions should be taken when working areas known to be infested by nematodes.

6.2.1 Technical information for planning surveys

When developing surveys for presence and/or distribution of exotic nematodes, the following characteristics provide the basic biological knowledge that informs the survey strategy:

- Plant material may be asymptomatic, or may not display obvious symptoms at all growth stages.
- Host species in Australia are likely to be numerous and widely dispersed and may be present within farm paddocks, as well as home gardens, landscape plantings, nurseries and as weeds.
- The risk of nematode movement on seed, machinery, equipment, clothing and footwear contaminated with soil is significant.
- Irrigation water could potentially spread the nematode between paddocks.
- There is a risk that the wind and birds could also spread the cyst nematodes between areas.
- Production areas and significant proportions of Australia may have favourable climatic conditions for the nematodes spread and establishment.

6.2.2 Surveys for early detection of an incursion

Points to consider in effectively monitoring nematode populations are:

- Ensure that the laboratory diagnostician has expertise in this form of diagnosis.
- Initial surveys should concentrate on symptomatic plants (i.e. plants showing stunting, discolouration and yellowing, see Sections 5.1.7, 5.2.7 and 5.3.7 for species specific details).
- If nematodes are detected, or suspected, samples of the infected plants, and soil samples from the paddock should be collected for diagnosis. See Section 10.2 for details on diagnostic facilities.

Points to consider in monitoring infected areas are:

- The host range of the nematode must be determined and hosts grouped into risk categories for transmission and expression of the pest (high, medium and low).
- Conditions under which transmission, amplification and expression of the pest occurs must be determined to assess the likelihood of detection and reporting through general surveillance and to assist with the development of protocols for targeted surveillance.
- Potential pathways for distribution of nematode infected material must be determined. Often this will be caused by the movement of soil on vehicles and equipment.
- Depending on the nematode, host species in Australia are likely to be numerous and widely dispersed and may be present within farms, nurseries, home gardens, landscape plantings or as weeds.
- Nematologist expertise will be required to determine diagnostic protocols and sampling requirements.

General points to consider in effective surveillance for the presence of nematodes, such as the three *Heterodera* species described in this contingency plan, are noted in Ravichandra (2010) and Bridge and Starr (2007). Many of these points can also be applied to surveillance of other nematodes. Ravichandra (2010) provides some additional information on sampling and extraction techniques.

General points to consider when carrying out surveys for nematodes include:

- The likelihood of detecting nematodes if they are present depends on many factors including soil type, crop and cultivar, time of year, vertical distribution of nematodes in the soil, number of samples collected, size of samples, etc.
- Nematodes are typically distributed in non-uniform, non-random patterns in fields (i.e. they form aggregated clusters that are distributed irregularly across fields). Numerous smaller samples provide a higher chance of detection: composite samples of at least 20 cores minimise variation and optimise time and effort.
- Narrow cores of 2.5 cm width are sufficient for soil sampling.
- Systematic sampling such as zig-zag patterns can be used within a field to obtain sub-samples for a composite sample.
- Samples should be collected from a depth of 10-30 cm (i.e. within the root zone).
- Samples of host plant roots or surrounding soil should be collected to obtain cysts (such as those created by *Heterodera* spp.) or galls (such as those created by *Meloidogyne* spp.).
- Samples must be processed to extract nematodes and/or cysts from soil and debris.
- Specimens must be prepared for identification using molecular or morphological techniques.

6.2.3 Delimiting surveys in the event of an incursion

In the event of an incursion, delimiting surveys are essential to inform the decision-making process. Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas of poor growth or discolouration. The normal procedure is to collect symptomatic plants (and their roots) and to test them to confirm the presence of the nematode. If confirmed, root and soil samples taken at random from the same crop should be tested to enable an estimate of the pest incidence. Surrounding crops should then be surveyed. The extent of the survey beyond the initial infected crop should be guided by the test results from surrounding crops.

As nematodes can be spread in soil contaminating seed, machinery, equipment, clothing and footwear trace-back of seed, personnel and equipment may help calculate the number of properties to be tested. If the equipment etc. has been moved between sites, delimiting surveys should be conducted at each site.

When establishing delimiting surveys the following should be considered:

- The size of the survey area (Figure 14) will depend on the size of the infected area and the severity of the infection, as well as potential movement of plant (particularly root) material and soil during the period prior to detection. Delimiting surveys should comprise local surveys around the area of initial detection concentrating on areas of poor crop growth.
- Nematodes can be spread by the movement of irrigation water, soil (on equipment, seed, vehicles and clothing), the wind, or animals (e.g. birds, livestock, etc.).
- All potential host species of the nematode (see Sections 5.1.4, 5.2.4 and 5.3.4 for hosts of the three example species), and soil samples from the areas around them, should be surveyed, with particular attention to the species in which the nematode was initially detected.
- In addition to inspection of possible host plants, material should be collected for diagnostic purposes (refer to Section 6.2.4).
- If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may be helpful.

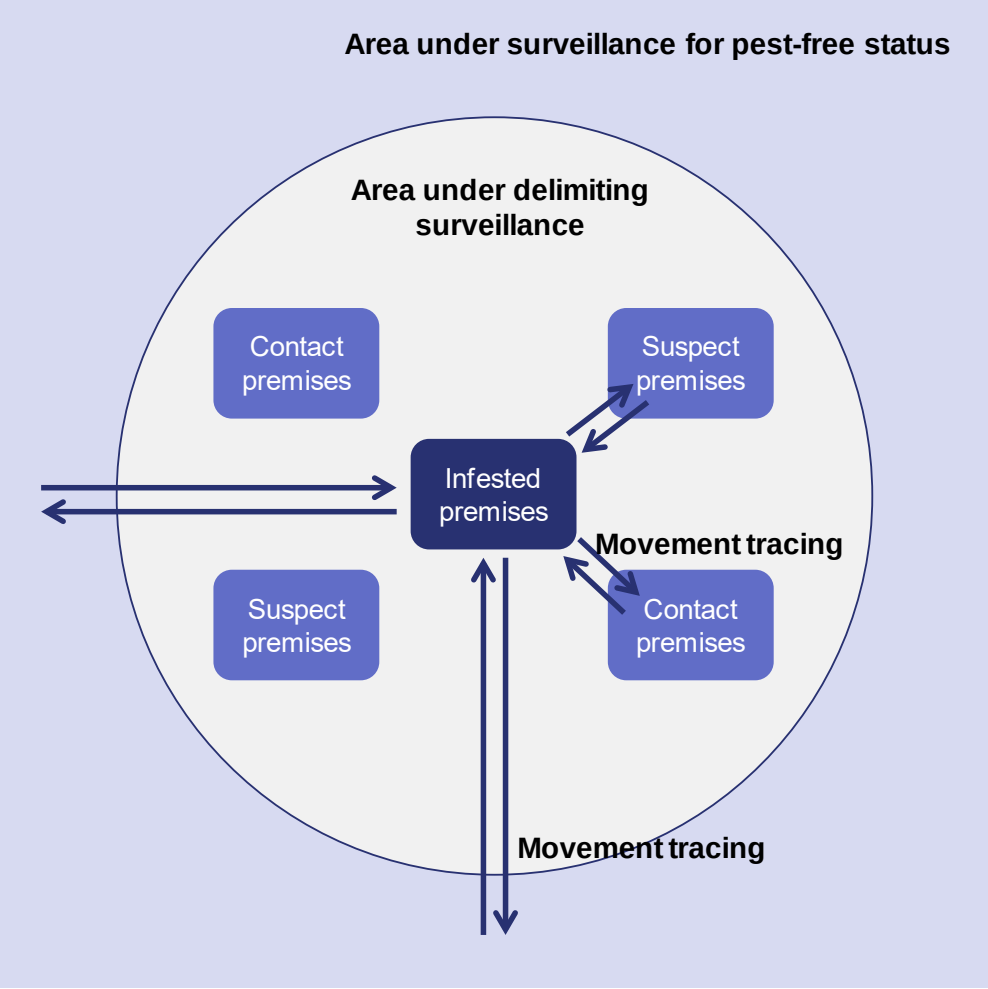

Figure 14. *Diagram of a delimiting survey showing surveillance activities from the infected premises*

6.2.4 Collection and treatment of samples

Protocols for the collection, transport and diagnosis of suspect Emergency Plant Pests (EPPs) must follow PLANTPLAN (Plant Health Australia 2013). Details are provided in the Standard Operating Procedure (SOP) for *Collection and transport of EPPs* available as a supporting document of PLANTPLAN (Plant Health Australia, 2013) (**www.planthealthaustralia.com.au/wp-content/uploads/2013/12/SOP-Collection-and-transport-of-EPPs.pdf**). Any personnel collecting samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis.

All sample containers should be clearly labelled with the name, address and contact phone number of both the sending and receiving officers. In addition containers should be clearly labelled in accordance with the requirements of PLANTPLAN (Plant Health Australia, 2013). Containers should be carefully sealed to prevent loss, contamination or tampering of samples. Additional labelling includes the identification of plant species/parts affected, location of site of sample collection in the paddock (preferably with a GPS reading) as well as symptoms and an image if available.

Refer to PLANTPLAN for packing instructions.

6.2.4.1 COLLECTION OF SPECIMENS

SAMPLING PROCEDURES

Samples should be collected from soil around the host plant. If soil is sampled, cores of soil (including root material) should be taken from within the root zone (10-30 cm below the soil surface) from numerous sites across a field to form a composite sample of approx. 2 kg of soil (Bridge and Starr 2007).

Sampling for mature, dark coloured *Heterodera* cysts should be carried out close to harvest or over the summer period. If other nematodes are to be collected than it is important to understand their biology with for example the Needle nematodes (*Longidorus* spp.) often move deeper into the soil as the season progresses (Ravichandra 2010).

Seek advice from a nematode specialist on the appropriate sampling protocols.

NUMBER OF SPECIMENS TO BE COLLECTED

A composite sample of around 20 cores over a 5 hectare area is recommended (Bridge and Starr 2007). Record the location, preferably as GPS co-ordinates, or alternatively, a map reference or distance and direction from a suitable landmark. If the land is privately owned, record the owner's details including contact telephone numbers.

HOW TO COLLECT AND SEND SOIL/ROOT SAMPLES

If plant roots are sampled, samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well-preserved state. An esky with ice packs or portable fridge should be carried when sampling crops. Samples need to be delivered to the appropriate laboratory immediately and should be transported in cool, moist conditions that avoid extremes of temperature (freezing or above 35°C). If samples are not processed within a day or two, they should be kept in a refrigerator. Samples should be considered perishable in 3-4 days (Bridge and Starr 2007).

If soil is sampled, dry soil can be taken (cysts are resistant to desiccation and very hardy).

EXTRACTION OF NEMATODES FROM SOIL

Once soil or root samples have been collected the nematodes must then be extracted for identification. Nematodes can be extracted from soil samples in a number of ways.

Bridge and Starr (2007) describe the use of Baermann-type methods for the extraction of active nematodes from soil where a thin layer of soil or plant samples are placed onto a screen covered with tissue paper that is suspended over a container holding water. The soil/plant material is covered with water and left for 24-48 hours during which time the nematodes move into the water and collected.

Inactive nematodes can also be extracted from samples by sieving a solution of water and soil through a 2 mm sieve then through progressively finer sieves until the nematodes are collected. Most nematodes will be collected on 90-53 µm sieves (Bridge and Starr 2007).

As mature (dead) nematode cysts of *Heterodera* spp. float when dry they can also be extracted from dry soil. A Fenwick can is used for flotation extraction. Alternatively a simple flotation extraction method comprises placing a sample of air dried soil (200 – 500 g) in a larger container. A strong jet of water is used to separate light organic matter fraction (containing cysts) to the surface without flowing over the container. Soil is allowed to settle for 30 seconds then the water containing the organic matter and cysts is poured through two nested sieves (250 µm on top and 150 µm on bottom). The

process is repeated at least twice. Material on the sieves is gently rinsed to the bottom of the sieve and then washed into a beaker. Organic matter in the beaker is concentrated onto filter paper or fine mesh material and examined for dry cysts.

White (immature) nematode cysts can also be observed by direct examination of plant roots however care must be taken as cysts can easily be dislodged as roots are washed free of soil.

6.2.5 Epidemiological study

There are many factors that affect the development of exotic nematodes in fields. These include: the presence of virulent strains in the environment, susceptibility of the crop varieties, climatic conditions, irrigated or non-irrigated crops and interactions with other micro-organisms. Population densities are also important as nematode symptoms may not be apparent when low populations occur.

The number of infected plants within a crop will depend on the source and amount of primary inoculum available and whether environmental conditions have been favourable for the nematode to spread from initial foci.

Sampling of crops within a district will be based upon the origins of the initial suspect sample(s). Factors to consider will be:

- Whether contaminated soil has moved from the infected paddock on vehicles or machinery.
- Whether dry cysts could have blown between paddocks.
- The proximity of other susceptible crops to the initial infected crop, both in the current growing season and previous seasons.
- It is likely that by the time an infestation of a new species of nematode has been detected, the infestation will have been present for some time.
- The proximity of other susceptible plants to the initial infestation source, including both the current and previous growing seasons. This will include crops on the infected property and those on neighbouring properties. Alternative hosts should also be considered, including weeds, fodder and garden plants.
- Machinery or vehicles that have been into the infested area or in close proximity to the infestation source.
- The extent of human movement into and around the infested area. A possible link to recent overseas travel or visitors from other regions or the recent importation of plant material, machinery or goods from other regions should also be considered.
- The temperature and environmental conditions. Temperature and environmental conditions affect the severity and spread of the nematode and therefore need to be taken into account.

6.2.6 Models of spread potential

No models of spread potential have been developed for the three cyst nematodes used as examples in this contingency plan. Nematodes are primarily spread by the movement of soil adhering to clothing, footwear, vehicles, equipment and machinery. It is also possible for nematodes to be spread within and between crops by the movement of water and the wind (cysts can potentially be blown in soil). These pathways would need to be considered when examining the spread potential of exotic nematodes.

6.2.7 Pest Free Area guidelines

The establishment and maintenance of pest free areas (PFAs) would be a resource-intensive process. Prior to development of a PFA consideration should be given to alternative methods (e.g. treatments or enclosed quarantine) that achieve an equivalent biosecurity outcome to a PFA.

Determination of PFAs should be completed in accordance with the International Standards for Phytosanitary Measures (ISPMs) 8 and 10 (IPPC 1998a, 1999).

Additional information is provided by the IPPC (1995) in Requirements for the Establishment of Pest Free Areas. This standard describes the requirements for the establishment and use of PFAs as a risk management option for phytosanitary certification of plants and plant products. Establishment and maintenance of a PFA can vary according to the biology of the pest, pest survival potential, means of dispersal, availability of host plants, restrictions on movement of produce, as well as PFA characteristics (size, degree of isolation and ecological conditions)

In the event of an incursion, specific guidelines for surveys and monitoring will be provided by the Consultative Committee on Emergency Plant Pests (CCEPP). General points to consider are:

- Design of a statistical delimiting survey for symptoms on host plants (see Section 6.2 for points to consider in the design).
- Plant sampling should be based on the rates given in 6.2.4.1 and taken at random with in the crop.
- Preliminary diagnosis can be based on plant symptoms and nematode morphology.
- PCR methods for confirmation of the nematodes identity.
- Surveys should also consider alternative host plants (see Sections 5.1.4, 5.2.4 and 5.3.4 for host plants of the three example species) and not be limited to the primary infected host.
- Information (including absence of the pest) should be recorded.

6.3 Availability of control methods

Once introduced and established to an area nematodes can survive for extended periods even in the absence of host plants. Containment procedures to retard the spread of the nematode are required to minimise the impact on the industry and improve the probability of eradication success.

The following outlines some general information for the control of exotic nematodes.

6.3.1 General procedures for control

Control of nematodes is likely to be largely reliant on the use of crop rotations, chemicals and reducing the spread of the nematode between areas by controlling the movement of people and machinery. Specific control measures will be determined by a CCEPP, however, general procedures include:

- Keep traffic out of affected areas and minimise movement in adjacent areas.
- Adopt best-practice property hygiene procedures to retard the spread of plant material (particularly roots) and soil containing nematodes between paddocks and adjacent properties.

- After surveys are completed, and permission has been obtained from the Chief Plant Health Manager or the CCEPP, destruction of the infested plant material, is an effective control.
- Avoid including host plants in crop rotations. For example if *H. zeae* was found in maize other susceptible Gramineae crops should be removed from the crop rotation for a suitable period of time (to be determined by the survival ability of the nematode in the absence of host plants).
- On-going surveillance of infected areas to ensure the pest is eradicated.

6.3.2 Control of infected areas

6.3.2.1 CONTROL OF INFECTED AREAS

If a large area is infected, kill any surviving plants in the area, preferably with herbicides (note herbicides have to be registered by the APVMA for the purpose), treat the site with an appropriate nematicide (if available) and remove the crop debris. Do not plough the paddock as this could spread the nematode to non-infected parts of the paddock. Once the dead plants have broken down, sow an alternative non-host crop or pasture to prevent erosion (aerial or broadcast seeding may be an option to minimise the risk of spreading nematodes across the paddock on machinery).

Particular care must be taken to minimise the transfer of cysts, plant material or soil from the area and surveys of the surrounding area must continue for some time to ensure that the eradication regime was successful.

All equipment used on the site should be thoroughly cleaned down, with products such as a farm degreaser or a 1% bleach solution and washed down with a pressure cleaner on the affected farm. The clean down procedure should be carried out on a hard surface or preferably a designated wash-down area to avoid mud being recollected from the affected site onto the machine.

Host plants should not be planted for a number of years to give the best possible chance of eradication success.

6.3.3 Weed management

Weeds may serve as alternate hosts of nematodes. Shepard's purse (*Capsella bursa-pastoris*.) and Common mullerin (*Verbascum thapsus*) are hosts of *H. glycines* and are weeds in some parts of Australia. If weed species are found to be potential hosts of the nematode local weed populations may need to be controlled, using a suitable herbicide. Special attention should be paid to weeds along fence lines and road sides adjacent to infected areas.

6.3.4 Chemical control

The nematicides Aldicarb and Dibromochloropropane have reduced levels of *Heterodera* nematodes and therefore improved cereal yields in *H. avenae* infested paddocks in southern Australia (Rovira et al., 1981). There are currently a limited number of chemicals that are labelled for the control of plant parasitic nematodes (see Table 4). In the event of an incursion nematicides may have a role in an eradication program.

Table 15 details the chemicals that have been used to control *H. zeae, H. glycines* and *H. ciceri* overseas. However any chemical used in Australia as part of a control or eradication program must be approved for that use by the APVMA.

Table 15. *Chemicals used to control Heterodera nematodes overseas*

Chemical	Reference	Is the chemical registered for use in Australia
Heterodera zeae		
Aldicarb	Sethi and Srivastava (1986)	No
Carbofuran	Kaul and Sethi (1987)	Yes
Fensulfothion	Sethi and Srivastava (1986)	No
Oxamyl	Kaul and Sethi (1987)	Yes – used on banana, capsicum and tomato.
Phenamiphos	Kaul and Sethi (1987)	No
Heterodera glycines		
Aldicarb[12]	Noel (1990); Noel (1993); Smith et al., (1991)	No
Carbofuran	Noel (1993)	Yes
Ethoprop	Noel (1993)	No
Fenamiphos	Noel (1993)	Yes – used on banana, carrot, parsnip, crucifers, ginger, ornamentals, pineapple, potato, strawberry, tomato, sugarcane, turf.
Thuringiensin[13]	Noel (1990)	No
Heterodera ciceri		
Aldicarb	Di Vito et al., (1991)	No

6.3.5 Cultural Control

Cultural controls can be used to control nematodes. Effective cultural controls include:

- Crop rotations. Crop rotations that do not include susceptible host plants can be a viable option, during eradication efforts or if the nematode's inoculum is only short lived. Saxena et al., (1992) found that when *H. ciceri* host plants were not included in the crop rotation for 2-3 years, the crop yield when they were planted again improved as the *H. ciceri* population declined in the absence of host plants. Similarly Noel (1993) suggests that a crop rotation that only includes soybean once every three to four years can be an effective management option

[12] Noel (1993) suggests that this chemical doesn't provide yield benefits over resistant soybean cultivars.

[13] Noel (1990) found that although this chemical helped to control the nematode it was less effective than aldicarb. Also when a resistant soybean variety was used in the experiment it out yielded the plants treated with this chemical.

for soybean producers to protect their crops from *H. glycines*. Crop rotations were also useful for controlling *H. zeae* in Egypt (Ismail 2009).

- Weed control to remove volunteer plants and weed hosts can also help reduce nematode populations.
- The use of resistant varieties is another cultural control. Noel (1993) reported that resistant varieties of soybean can produce better yields than conventional crop varieties treated with nematicides to control *H. glycines*. Information on resistant varieties is given in Section 6.3.6.
- Solarisation of the site, by covering with polyethylene film, for 6-8 weeks was effective at controlling *H. ciceri* and *Pratylenchus thornei* (Thorne's root lesion nematode) in Syria (Di Vito et al., 1991) however the expense of the exercise will limit its use.

6.3.6 Host-Plant Resistance

The use of resistant host plants is a widely used method of controlling plant pests and offers a low cost way of dealing with pest nematodes. However it should be remembered that there are different strains of the pest so a variety that is resistant to a strain in one country may not be resistant to the strain occurring in another.

6.3.6.1 HETERODERA ZEAE

Host plant resistance can be used to help produce a crop. Hashmi et al., (1993) tested 23 maize cultivars and reported varying levels of *H. zeae* parasitism. Some degree of resistance exists with the mean number of cysts and females produced per pot ranging from 30 on the least susceptible variety to 8,183 on the most susceptible variety.

6.3.6.2 HETERODERA GLYCINES

Resistance to *H. glycines* is often used and is well researched. Chen et al., (2001) reported resistant cultivars achieved higher yields and supported lower nematode populations compared to susceptible cultivars. In a similar study Koenning (2004) described some resistant cultivars of soybean but the varieties tested were not resistant to all races of the nematode.

Rao-Arelli (1994) described the genetics behind resistance in four soybean varieties that are resistant to *H. glycines* race 3. Multiple genes were involved in creating the resistance and the genes involved varied between the cultivars as the resistance in two cultivars was gained by one dominant and one recessive gene, another variety used two recessive genes, while the fourth used two dominant genes and one recessive gene.

6.3.6.3 HETERODERA CICERI

Few resistant cultivars of chickpea exist. However researchers are screening resistance from wild *Cicer* species. Di Vito et al., (1996) screened thousands of lines of chickpea and its wild relatives for resistance to *H. ciceri* finding only a small number of *C. bijugum*, *C. pinnatifidum*, and *C. reticulatum* lines that displayed resistance to the nematode and could act as gene sources for developing resistance in chickpeas (*C. arietinum*). The researchers crossed a resistant *C. reticulatum* line with high yielding, blight resistant chickpeas (*C. arietinum*) finding F_2 and F_3 plants from this cross that displayed resistance to the nematode.

Recently such crosses have resulted in the development of more productive *H. ciceri* resistant chickpea lines, such as ILC 10765 and ILC 10766 which were derived from crosses between *C. reticulatum* and *C. arietinum* (Malhotra et al., 2002). Since then other chickpea lines have also been developed such as FLIP 2005-8C and FLIP 2005-9C which are derived from *C. reticulatum* but have better agronomic traits than earlier crosses (Malhotra et al., 2008). This work suggests that it is possible to breed plant resistance to the nematode as a cost effective way of managing the pest, and that some resistant varieties currently exist overseas.

6.3.7 Biological control

Recently a number of papers have been published that identify potential organisms that could control these nematodes. Mostly these are nematophagous fungi.

A brief list of the organisms that may be useful for the biological control of the three cyst nematodes is given in Table 16.

Table 16. *Antagonistic organisms associated with H. zeae, H. glycines and H. ciceri*

Name of antagonistic organism	Life form of antagonistic organism	Nematode stage affected	Reference
Heterodera zeae			
Paecilomyces nostocoides	Fungi	Cysts	Dunn (1983)
Heterodera glycines			
Chalara hyaline	Fungi	Cysts	Morgan-Jones et al., (1984)
Comamonas acidovorans	Bacteria	Eggs and Juvenile stage 2	Tian et al., (2000)
Exophiala pisciphila	Fungi	Egg	Gintis et al., (1983)
Flavobacterium johnsoniae	Bacteria	Eggs and Juvenile stage 2	Tian et al., (2000)
Fusarium solani	Fungi	Egg	Gintis et al., (1983)
Fusarium oxysporum	Fungi	Egg	Gintis et al., (1983)
Methylobacterium zatmanii	Bacteria	Eggs and Juvenile stage 2	Tian et al., (2000)
Paecilomyces lilacinus	Fungi	Egg	Gintis et al., (1983)
Paecilomyces variotii	Fungi	Egg	Gintis et al., (1983)
Paraphoma radicina	Fungi	Cyst	Gintis et al., (1983)
Phoma terrestris	Fungi	Egg	Gintis et al., (1983)

Name of antagonistic organism	Life form of antagonistic organism	Nematode stage affected	Reference
Phytophthora cinnamonmi	Fungi	Egg	Gintis et al., (1983)
Scytalidium fulvum	Fungi	Cysts and eggs	Gintis et al., (1983)
Scytalidium fulvum	Fungi	Cysts	Morgan-Jones et al., (1984)
Streptomyces cyaneus chartreusis	Bacteria	Eggs and Juvenile stage 2	Tian et al., (2000)
Streptomyces spp.	Bacteria	Eggs and Juvenile stage 2	Tian et al., (2000)
Verticillium lecanii	Fungi	Cysts	Meyer and Meyer (1996)
Heterodera ciceri			
No species reported	N/A	N/A	N/A

7 Course of action – eradication methods

Additional information is provided by the IPPC (1998b) in Guidelines for Pest Eradication Programmes. This standard describes the components of a pest eradication programme which can lead to the establishment or re-establishment of pest absence in an area. A pest eradication programme may be developed as an emergency measure to prevent establishment and/or spread of a pest following its recent entry (re-establish a pest free area) or a measure to eliminate an established pest (establish a pest free area). The eradication process involves three main activities: surveillance, containment, and treatment and/or control measures.

7.1 Destruction strategy

7.1.1 Destruction protocols

- No plant material should be removed from the infested area unless part of the disposal procedure.
- Disposable equipment, infested plant material or soil should be disposed of by autoclaving, high temperature incineration or deep burial.
- Any equipment removed from the site for disposal should be double-bagged.
- All vehicles and farm machinery that enter the infected field should be thoroughly washed, preferably using a detergent, farm degreaser or a 1% (available chlorine) bleach solution.

7.1.2 Decontamination protocols

If decontamination procedures are required, machinery, equipment and vehicles in contact with infected plant material or soil or present within the Quarantine Area, should be washed to remove soil and plant material (especially roots) using high pressure water or scrubbing with products such as a farm degreaser or a 1% bleach solution in a designated wash down area. General guidelines for wash down areas are as follows:

- Located away from crops or sensitive vegetation.
- Readily accessible with clear signage.
- Access to fresh water and power.
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting).
- Gently sloped to drain effluent away.
- Effluent must not enter water courses or water bodies.
- Allow adequate space to move larger vehicles.
- Away from hazards such as power lines.
- Waste water, soil or plant residues should be contained.
- Disposable overalls and rubber boots should be worn when handling infected soil or plant material in the field. Footwear and clothes in contact with infected soil or plant material should be disinfected at the site or double-bagged to remove for cleaning.
- Skin and hair in contact with infested plant material or soil should be washed.

In the event of an incursion of an exotic nematode, additional or modified procedures may be required for the destruction of the pest. Any sterilisation procedure must be approved for use in the endorsed Response Plan.

For further information, refer to *Disinfection and decontamination* guidelines available as a supporting document of PLANTPLAN (Plant Health Australia, 2013) (**www.planthealthaustralia.com.au/wp-content/uploads/2013/12/Guidelines-Disinfection-and-decontamination.pdf**).

7.1.3 Priorities

- Confirm the presence of the pest/pathogen
- Limit movement of people and prevent movement of vehicles and equipment through affected areas.
- Stop the movement of any plant material, soil or machinery that could be carrying nematodes or cysts from the infected area.
- Determine the strategy for the eradication/decontamination of infected host material.
- Determine the extent of the infestation through survey and plant material trace back and trace forward which would be assessed on a case by case basis and included within the response plan.

7.1.4 Plants, by-products and waste processing

- Any soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial.
- Plant debris (especially roots) from the destruction zone must be carefully handled and transported.
- Infested areas or paddocks should remain free of susceptible host plants (including crops, weeds, alternative hosts and volunteer plants) (see Sections 5.1.4, 5.2.4 and 5.3.4) until the area has been shown to be free from nematodes. The exact period of time that the infested area should remain free of host plants will be determined by the survival ability of the nematode. For example as *H. glycines* can survive as cysts in the soil without a host for up to 11 years (Riggs and Niblach 1993) a paddock found to be infested with *H. glycines* may have to remain free of host plants for 11+ years if eradication is to be successful.

7.1.5 Disposal issues

- Particular care must be taken to minimise the transfer of infected plant material (especially roots) and soil from the infected area.

7.2 Containment strategies

For some exotic pest incursions where eradication is considered impractical, containment of the nematode may be attempted to prevent or minimise its spread and impact on other areas. The decision to eradicate or contain the nematode will be made by the National Management Group based on scientific and economic advice.

7.3 Quarantine and movement controls

Consult PLANTPLAN (Plant Health Australia 2013) for administrative details and procedures.

7.3.1 Quarantine priorities

- Plant material (especially plant roots) and soil at the site of infestation to be subject to movement restrictions as such material could potentially spread the nematode to new areas.
- It is less likely plant products such as hay and grain will be a source of movement of cysts, however assessment should be made of plant products to ensure potential contamination of soil containing cysts does not occur.
- Machinery, equipment, vehicles and disposable equipment in contact with infested plant material or soil, or present in close proximity to the site of infestation to be subject to movement restrictions.

7.3.2 Movement controls

If Restricted or Quarantine Areas are practical, movement of equipment or machinery should be restricted and movement into the area only occurs by permit. The industry affected will need to be informed of the location and extent of the pest occurrence.

Movement of people, vehicles and machinery, from and to affected farms, must be controlled to ensure that infected soil or plant debris (especially root material) is not moved off-farm on clothing, footwear, vehicles or machinery. This can be achieved through the following; however specific measures must be endorsed in the Response Plan:

- Signage to indicate quarantine area and restricted movement into and within these zones.
- Fenced, barricaded or locked entry to quarantine areas.
- Movement of equipment, machinery, plant material or soil by permit only. Therefore, all non-essential operations in the area or on the property should cease.
- Where no dwellings are located within these areas, strong movement controls should be enforced.
- Where dwellings and places of business are included within the Restricted and Control Areas movement restrictions are more difficult to enforce, however limitation of contact with infested plants should be enforced.
- Clothing and footwear worn at the infected site should either be double-bagged prior to removal for decontamination or should not leave the farm until thoroughly disinfected, washed and cleaned.
- Residents should be advised on measures to minimise the inadvertent transport of nematodes and cysts from the infested area to unaffected areas.
- Plant material or plant products, including seed, must not be removed from the site unless part of an approved disposal procedure.
- All machinery and equipment should be thoroughly cleaned down with a high pressure cleaner (see Section 7.1.2) or scrubbed with products such as a farm degreaser or a 1% bleach (available chlorine) solution, prior to leaving the affected area. Machinery should be inspected for the presence of soil and plant debris and if found must be treated in an appropriate manner. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine. When using high pressure water, care should be taken to contain all plant material and mud dislodged during the cleaning process.

7.4 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, biology of the pest, climatic conditions and the proximity of the infested property to other infested properties. This will be determined by the National Management Group during the production of the Response Plan. Further information on quarantine zones in an Emergency Plant Pest (EPP) incursion can be found in Section 4.1.4 of PLANTPLAN (Plant Health Australia 2013). These zones are outlined below and in Figure 15.

7.4.1 Establishing Quarantine Zones

Delimiting surveillance will inform the establishment of quarantine zones and identify the Restricted Area(s) (RA), Control Area (CA) and Pest Free Area (PFA). The size of each quarantine zone will be determined by a number of factors including location of the incursion, climatic conditions, pest biology and proximity of an Infected Premises (IP) to other IPs.

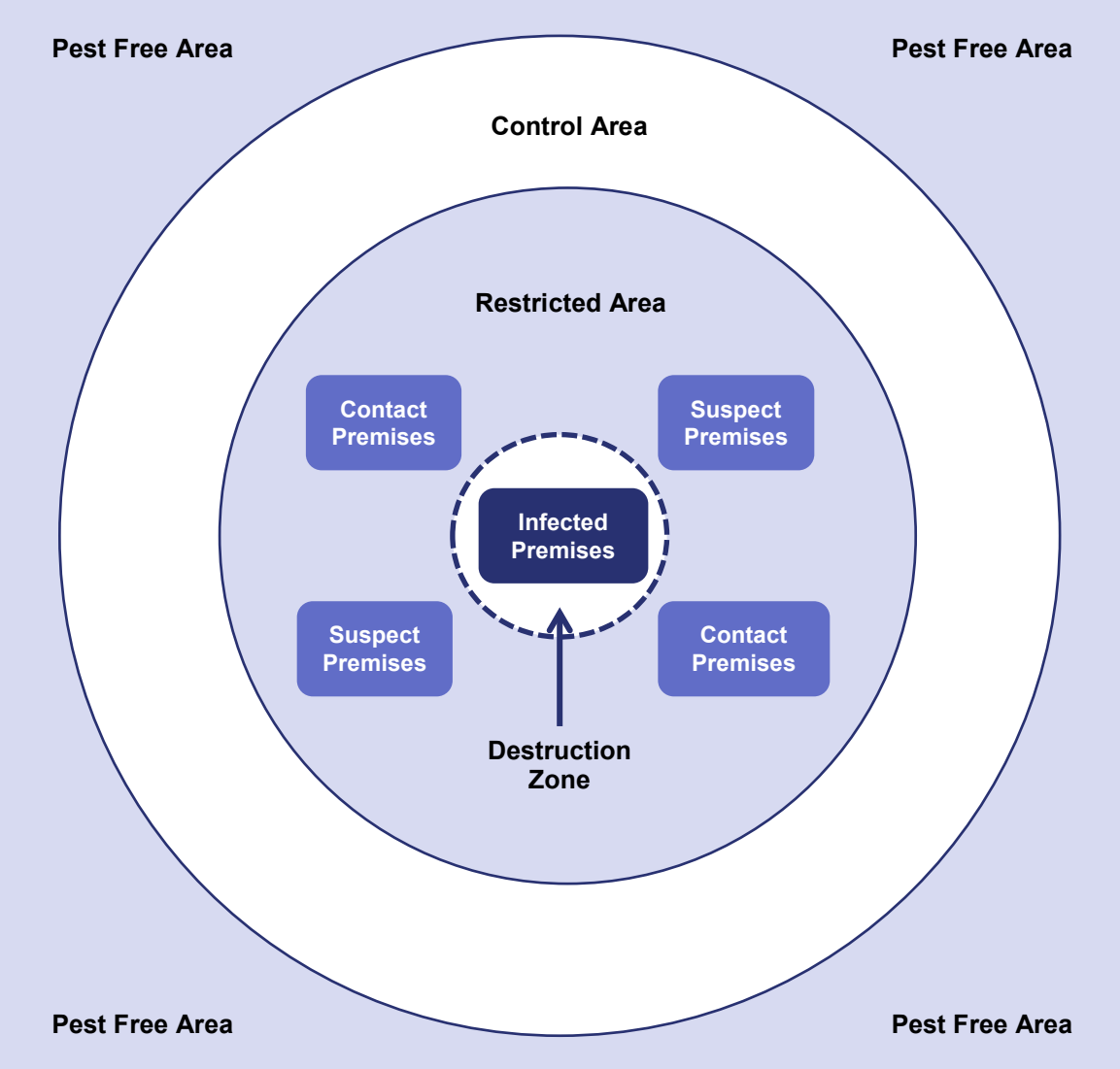

Figure 15. *Schematic diagram of quarantine zones used during an EPP incursion (not drawn to scale)*

7.4.2 Destruction Zone

The size of the Destruction Zone (i.e. zone in which the pest and all host material is destroyed) will depend on, distribution of the pest (as determined by delimiting surveys), ability of the pest to spread, factors which may contribute to the pest spreading and the time of season.

All host plants should be destroyed after the level of infection has been established. The delimiting survey will determine whether or not neighbouring host crops are infected and need to be destroyed. If spread is likely to have occurred prior to detection, the Destruction Zone may include contiguous areas that have been in contact with, or are associated with the same management practices as, the infected area Particular care needs to be taken to ensure that plant material and soil are not moved into surrounding areas that are not showing symptoms of the pest. Where possible, destruction should take place in dry conditions to limit mud being spread within the field on boots and protective clothing.

7.4.3 Restricted Area

Data collected from surveys and tracing (trace back and trace forward) will be used to define the RA, which comprises all properties where the pest has been confirmed (Infected Premises or IP), properties which have come into direct or indirect contact with an IP or infected plants (Contact Premises or CP) and properties which may have been exposed to the pest (Suspect Premises or SP). The RA will be subject to intense surveillance and movement control, with movement out of the RA to be prohibited and movement into the RA to occur by permit only.

7.4.4 Control Area

A CA is established around a RA to control the movement of susceptible hosts and other regulated materials until the extent of the incursion is determined. There may be multiple RAs within one CA. When the extent of the EPP Incident has been confidently defined, the RA and CA boundaries and movement controls may need to be modified, and where possible reduced in size commensurate with appropriate controls.

Additional zones can be utilised as required for operational purposes.

7.5 Decontamination and farm clean up

Decontaminant practices are aimed at eliminating the nematode thus preventing its spread to other areas.

7.5.1 Decontamination procedures

General guidelines for decontamination and clean up:

- Keep traffic out of affected area and minimise it in adjacent areas.
- Adopt best-practice property hygiene procedures to retard the spread of the nematode between fields and adjacent properties.
- Machinery, equipment and vehicles in contact with infested or infected plant material or soil present within the Quarantine Zone, should be washed to remove soil and plant material using high pressure water or scrubbing with products such as a degreaser or a bleach solution in a designated wash down area as described in Section 7.1.2.
- Only recommended materials are to be used when conducting decontamination procedures, and should be applied according to the product label.

- Infested plant material should be disposed of by autoclaving, high temperature (enclosed) incineration or deep burial.

For further information, refer to *Disinfection and decontamination* guidelines available as a supporting document of PLANTPLAN (Plant Health Australia, 2013) (**www.planthealthaustralia.com.au/wp-content/uploads/2013/12/Guidelines-Disinfection-and-decontamination.pdf**).

7.5.2 General safety precautions

For any chemicals used in the decontamination, follow all safety procedures listed within each MSDS.

7.6 Surveillance and tracing

7.6.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Initial surveillance priorities include the following:

- Surveying all host growing properties and businesses in the pest quarantine area.
- Surveying all properties and businesses identified in trace-forward or trace-back analysis as being at risk.
- Surveying all host growing properties and businesses that are reliant on trade with interstate or international markets which may be sensitive to the presence of the nematode.
- Surveying other host growing properties (including suburban gardens, etc. if applicable).

7.6.2 Survey regions

Establish survey regions around the surveillance priorities identified above. These regions will be generated based on the zoning requirements (see Section 7.4), and prioritised based on their potential likelihood to currently have or receive an incursion of this pest. Surveillance activities within these regions will either allow for the area to be declared pest free and maintain market access requirements or establish the impact and spread of the incursion to allow for effective control and containment measures to be carried out. Detailed information regarding surveys for exotic nematodes have been outlined elsewhere in this plan (refer to Section 6.2).

Steps outlined in Table 17 form a basis for a survey plan. Although categorised in stages, some stages may be undertaken concurrently based on available skill sets, resources and priorities.

Table 17. *Phases to be covered in a survey plan*

Phase 1	• Identify properties that fall within the buffer zone around the infected premise. • Complete preliminary surveillance to determine ownership, property details, production dynamics and tracings information (this may be an ongoing action).
Phase 2	• Preliminary survey of host crops on properties in buffer zone establishing points of pest detection.
Phase 3	• Surveillance of an intensive nature, to support control and containment activities around points of pest detection.
Phase 4	• Surveillance of contact premises. A contact premise is a property containing susceptible host plants, which are known to have been in direct or indirect contact with an infected premises or infected plants. Contact premises may be determined through tracking movement of materials from the property that may provide a viable pathway for spread of the nematode. Pathways to be considered are: o Items of equipment and machinery which have been shared between properties including bins, containers, irrigation lines, vehicles and equipment. o The producer and retailer of infected material, if this is suspected to be the source of the outbreak. o Labour and other personnel that have moved from infected, contact and suspect premises to unaffected properties (other growers, tradesmen, visitors, salesmen, crop scouts, harvesters and possibly beekeepers). o Movement of plant material (especially roots) and soil from controlled and restricted areas. o Storm and rain events that result in air or water-borne dispersal of the nematode during these weather events.
Phase 5	• Surveillance of farms, gardens and public land where plants known to be hosts of the nematode are being grown.
Phase 6	• Agreed area freedom maintenance, post-control and containment.

7.6.3 Post-eradication surveillance

The period of pest freedom sufficient to indicate that eradication of the pest has been achieved will be determined by a number of factors, including growth conditions, the previous level of infection, the control measures applied and the pest's biology.

Specific methods to confirm the eradication of nematodes may include:

- Establishment of sentinel plants at the site of infection.
- Maintain good sanitation and hygiene practices throughout the year.
- Monitoring of plants for signs of the pest.
- If symptoms are detected, samples are to be collected and stored and plants destroyed
- Alternate non-host crops should be grown on the site and any self-sown plants sprayed out with an appropriate herbicide
- Surveys comprising soil/root sampling should be undertaken for a defined period after eradication has been achieved (or as endorsed by a CCEPP). Note the biology of the nematode will dictate the minimum number of years that surveys need to be undertaken for, if long lived the surveys will need to continue for a longer period of time.

8 Technical debrief and analysis for stand down

Refer to PLANTPLAN (Plant Health Australia 2013) for further details. The emergency response is considered to be ended when either:

- Eradication has been deemed successful by the lead agency, with agreement by the Consultative Committee on Emergency Plant Pests and the Domestic Quarantine and Market Access Working Group
- Eradication has been deemed impractical and procedures for long-term management of the pest risk have been implemented

A final report should be completed by the lead agency and the handling of the incident reviewed.

Eradication will be deemed impractical if, at any stage, the results of the delimiting surveys lead to a decision to move to containment/control.

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Department of Agriculture, Fisheries and Forestry (DAFF) Manual of Importing Country Requirements (MICoR) database **http://www.daff.gov.au/micor/plants**

Pest and Disease Image Library (PaDIL) **www.padil.gov.au/**

10 Appendices

10.1 Appendix 1: Standard diagnostic protocols

For a range of specifically designed procedures for the emergency response to a pest incursion refer to Plant Health Australia's PLANTPLAN (**www.planthealthaustralia.com.au/plantplan**).

10.2 Appendix 2: Resources and facilities

Formal diagnostic services for plant pests in Australia are delivered through a network of facilities located in every state and territory. These services are provided by a range of agencies, including state and territory governments, the Australian Government, commercial and private diagnostic laboratories, museums, CSIRO and universities. A current listing of these facilities can be found at **www.npbdn.net.au/resource-hub/directories/laboratory-directory**.

The national network is supported by the Subcommittee on Plant Health Diagnostic Standards (SPHDS), which was established to improve the quality and reliability of plant pest diagnostics in Australia. SPHDS also manages the production of National Diagnostic Protocols.

For more information on the diagnostic services, or to identify an appropriate facility to undertake specific pest diagnostic services, refer to www.npbdn.net.au or contact the SPHDS Executive Officer on **SPHDS@daff.gov.au**.

10.3 Appendix 3: Communications strategy

A general Communications Strategy is provided in Section 4.1.5 of PLANTPLAN (Plant Health Australia, 2013).

10.4 Appendix 4: Market access impacts

Within the Department of Agriculture, Fisheries and Forestry (DAFF) Manual of Importing Country Requirements (MICoR) database (**http://www.daff.gov.au/micor/plants/**) export of some material may require an additional declaration regarding freedom from the nematode. Should exotic nematodes be detected or become established in Australia, some countries may require specific declaration. Latest information can be found within MICoR, using a search for the specific species.

The DAFF MICoR database was searched in August 2013 for current trade restrictions relating to the three nematodes used as examples in this contingency plan. *H. glycines* has some trade restrictions associated with it these are summarised in Table 18. No trade restrictions were found on MICoR for *H. ciceri* and *H. zeae.*

Table 18. *Trade restrictions associated with H. glycines, as identified on the MICoR database*

Country	Commodity	Restrictions
Canada	*Adianthum* spp. – nursery stock - plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Alocasia* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Alpinia* spp. – nursery stock - plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Asplenium* spp. – nursery stock - plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Blechnium* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Kenya	*Brassica napus* seed – Grains / Seeds - Sowing	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Cordyline* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Cyathea* spp. – Nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Kenya	*Desmodium* spp. seed – Grains / Seeds - Sowing	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Dichorisandra* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Diospyros* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Drosera* spp. –nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Etlingera* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.

Country	Commodity	Restrictions
Canada	*Freycinetia* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Hedychium* spp.– nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Heliamphor*a spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Heliconia* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Hemerocallis* spp. – nursery stock – bulbs and tubers	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Hibiscus* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Hoya* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Huperzia* spp.– nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Iris* spp. – nursery stock – bulbs and tubers	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Ukraine	*Iris* spp.– nursery stock – bulbs/tubers	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Lavandula* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Myrmecodia* spp.– nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Nepenthes* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	Orchidaceae – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.

Country	Commodity	Restrictions
Canada	*Paeonia* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Paulownia* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Phlebodium* spp. – nursery stock – plants	An import permit and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required. A phytosanitary certificate is not required
Canada	*Pinguicula* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Pyrostegia* spp.– Nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Rosa* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Sarracenia* spp. – nursery stock - plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Strongylodon* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Stynsepalum* spp.– nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Tacca* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Thelypteris* spp. – nursery stock – plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Utricularia* spp. – nursery stock –plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.
Canada	*Zephyranthes* spp. – nursery stock - plants	An import permit, phytosanitary certificate and a declaration that Soybean cyst nematode (*H. glycines*) is known not to occur in Australia are required.