41 Integrated nematode management of *Ditylenchus dipsaci* in onion: A nematode in a world all on its own

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Introduction

Stem and bulb nematode, *Ditylenchus dipsaci*, is one of the few plant parasitic nematode species infesting above-ground plant parts. This is likely the reason why already in 1857 it was described from seed heads of teasel (*Dipsacus fullonum*), being one of the earlier records of plant parasitic nematodes. It can be transmitted through infested planting material and seed, survive longer periods in the soil, has a broad host range and a relatively short life cycle and high multiplication rate. Further, it is able to cause substantial post-harvest losses in stored onions, making it a difficult nematode to manage.

Economic importance

Until very recently, in the Netherlands stem nematodes were a quarantine pest in onion and lucerne seed, bulb onions and ornamental bulb crops. The quarantine status implied an obligation to try to contain and prevent further spread of stem nematodes. This meant that when stem nematodes were found in the harvested crop, special measures had to be taken to control the nematode in the field. Because of the quarantine status and the consequences that followed from an observed field infestation, farmers in general were hesitant to speak openly about the problem. This made it more difficult to get insight into and manage the problem with stem nematodes. At the end of 2019, the status was changed to regulated non-quarantine pest (RNQP) (EU, 2019). The aim with a RNQP is to prevent economic damage of specific crops. For the cultivation of onions, it is required that at the time of inspection no visual symptoms of stem nematodes should be found, or that infested plants immediately are removed and stem nematodes are not found in a representative sample, or adequate physical or chemical measures were taken and nematodes are not found in a representative sample of the plant material (EU, 2019).

A single bulb onion infested by stem nematodes can be a source of rot to the entire healthy bulb onion lot at storage (Fig. 41.1). Therefore, when stem nematodes are found in an onion lot it is strongly advised to immediately sell it for consumption.

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Host range

Stem nematodes have a very wide host range, including more than 500 plant species and consist of different races that vary in their host range (Botjes and Ritzema Bos, 1905; Sturhan and Brzeski, 1991). Some of the races have a very narrow host range, whereas others are polyphagous. The 'onion race' is known to be polyphagous and can infest onion as well as oats, potato, maize, sugar beet, *Phaseolus* and *Vicia* bean, pea and carrot. Different populations of the 'onion race' have been shown to vary somewhat in host range. Other stem nematode races like rye, daffodil and tulip races are also able to infest onion. Others like the red clover and hyacinth races are not known to infest onion. Besides known hosts of arable crops, many weeds are maintenance hosts to stem nematodes during winter and fallow periods.

Fig. 41.1. A sliced onion bulb infected by stem nematode: typical symptoms of spongy and slimy rottenness. Photograph courtesy of Wageningen University & Research, Field Crops.

Distribution

In the Netherlands, problems with stem nematodes in onions are known from the main onion growing regions in the north and south-west of the Netherlands, but also in the polder area that has been reclaimed from the sea. The south-west of the country originally was an area where *Phaseolus* beans and peas (*Pisum*) were grown to be dried for human consumption. As these crops are very good hosts to stem nematodes, problems were widespread.

Symptoms of damage

Onions that are infested with stem nematodes have twisted and deformed leaves that are brittle and have a bluish colour (Fig. 41.2). The bulbs are often spongy and cracked. In cases of severe infestation, plants die. Especially when the weather is cold and humid, the spread of the infestation is rapid and patches of affected and dead plants quickly increase in size. In such conditions, plant growth is slow and therefore plants are often retarded and unable to survive (Fig. 41.3).

Biology and life cycle

Stem nematodes are known to reproduce sexually. All juvenile and adult stages of the nematode can infect a host plant, although the fourth juvenile stage (J4) is the main infective stage. The life cycle is temperature dependent and takes approximately 20 days to complete at

Fig. 41.2. A plot of onions infected with stem nematodes: typical symptoms of winding and excessive tillering. Photograph courtesy of Wageningen University & Research, Field Crops.

Fig. 41.3. Hot spots of stem nematode infestation foci in onion fields: typical symptoms of retardation and sparse growth pattern. Photograph courtesy of Wageningen University & Research, Field Crops.

15°C. Every female may lay 200–500 eggs. As a result an infestation starting with lower initial population densities can quickly increase to large numbers. The J4 may enter a survival stage ('dauer larva') that is able to withstand dry conditions for a long time. Dauer larvae have been found to be able to become active and infective after more than 20 years. This is the reason why an infestation may be very persistent. It is unknown what conditions are favourable for longer time persistence in the soil.

Generally, all Dutch river and marine clays soils appear to be infested by stem nematodes (Seinhorst, 1956a). In the south-eastern part of the Netherlands, onion bloat caused by stem nematodes is only associated with and spreads easily on sandy and loam soils along the river. In the south-western part it was found that onion bloat is persistent on all heavy clay soils (>30% clay particles), but on light (<30% clay particles) and sandy soils only when onions are grown more frequently, i.e. more than once in 3 or 4 years.

Soil conditions influence the activity of stem nematodes, which are found to be more active (mobile) in clay and loamy soils than in sandy soils (Seinhorst, 1950). Activity of stem nematodes also depends on soil conditions such as soil moisture, temperature and aeration. Moisture equivalent is critical for nematode activity, with moisture equivalent being the percentage of water that a soil can retain in opposition to a centrifugal force 1000 × gravity. Lower soil temperatures (5–10°C) are more favourable for the activity of stem nematodes. A temperature of 20°C does not directly impact the nematodes themselves but activates soil factors that are unfavourable for stem nematodes (Seinhorst, 1950). These factors are suppressed by partial sterilization, which indicates a biological origin. The unfavourable soil condition can also be transferred through addition of a soil extract. At 36°C the activity of stem nematodes stops. Some variation in the optimum temperature has been reported for different races.

Interactions with other nematodes and pathogens

Interactions with other nematodes and pathogens on onion are not known.

Recommended integrated nematode management (INM)

The INM approach that is practiced in the Netherlands is based on prevention, inventory, crop rotation, inundation (flooding creating anaerobic conditions) and, as the last option, the use of chemical nematicides. The majority of growers and advisers who have infestations follow some of the recommendations outlined below.

Prevention, hygiene and field inventory

It is very difficult to control stem nematodes when they are present in a field, so prevention is very important. It starts with the use of certified planting material and seed that is free of stem nematodes. Stem nematodes can be easily transported by seed, planting material, residues like straw and hay and contaminated soil. Thus, a high level of hygiene on the farm, for example cleaning machinery before moving from one field to the other, avoids the spread of nematodes among fields. In both sandy and clay soil, weeds are a source of infection and a maintenance host for stem nematodes and should be managed. Recognition of an infestation in some crops and weeds that do not show any symptoms is difficult. Regular field observations and removing plants with visible symptoms limits further spread of the nematodes. Considering the persistence of dauer larvae, the history of onion bloat incidence in a field may give information about the risk of reoccurrence of stem nematodes even after years of cropping non-hosts. Previous history of failure of onions due to stem nematodes also may help to locate and narrow the sampling unit at the time of sampling to determine the central population density of the infestation foci and to decide on future management based on a decision support system (DSS). Localizing infestation foci using geographic information systems can further be developed to manage the damage of stem nematodes in the future.

Crop rotation

A DSS for management of plant parasitic nematodes is available in the Netherlands. The web-based DSS 'Aaltjesschema' (in Dutch) and 'Best-4Soil' (now available in 22 languages, www.Best4Soil.eu/database, accessed 2 January 2021) help farmers and extension service in selecting the most ideal cropping frequency and order, including the use of green manure crops, to manage population densities below the damage threshold. The websites contain both information on the rate of multiplication of the nematodes on the crop and sensitivity of the crop to damage, as well as additional background information. However, development of stem nematode safe crop rotations is not yet possible due to the occurrence of races and insufficient information on host plant specificity. Knowledge about the host status for stem nematode races can help selecting crops that can safely grow on a certain infestation. Hosts and non-hosts differ in their influence on the degree of stem nematode infestation of the soil (Seinhorst 1957). Seasonal fluctuation of stem nematodes is also affected by soil type. Generally, population densities of stem nematodes decline in the winter in both clay and sandy soils. The rate of decline is much faster in light and sandy soils as compared to that of heavy clay soils (>30% clay particles) (Seinhorst, 1957). In a heavy clay soil in winter, a population declines to densities of <100 stem nematodes/500 g soil, whereas in sandy soils they may decline to <5 stem nematodes/500 g soil. The damage threshold for onions is 0–10 stem nematodes/500 g soil, thus crop rotation is not effective for heavy soils whereas in lighter soils onions can be grown once in 3 to 4 years.

Inundation and anaerobic soil disinfestation (ASD)

In the cultivation of ornamental bulbs and onions, inundation is now widely used as a method to control stem nematodes. The soil infected with stem nematodes must be inundated slowly and remain inundated for a period of 14 weeks, with a soil temperature exceeding 16°C. This means that in the temperate climate in the Netherlands, inundation can only be applied in summer, preferably the latest at the beginning of July. The method is most effective when the water does not leak, indicated by the amount of water that must be added over time. Control below the detection level is possible in lighter soils, but in heavy clay a small proportion of the stem nematodes may remain unaffected. On certain fields that are not suitable for application of inundation, either due to slope, a low groundwater table or lack of access to large amounts of water, ASD may be an alternative. An amount of 40 tonnes/ha of fresh, easily degradable organic material (e.g. grass) is incorporated into the soil to a depth of 40 cm. The soil is irrigated with 15–20 mm water, then covered with virtually impermeable film and left for at least 6 weeks at a soil temperature of at least 16°C. Inundation is more effective than ASD.

Additional measures

As a last option when other control measures fail, non-fumigant nematicides can be used, although the number of allowed nematicides is nowadays limited. The target with chemical nematicides is always to bring down population densities below the damage threshold (fumigants) and/or to postpone the moment of infection (non-fumigants).

Optimization of nematode management

The management of stem nematodes in onion can be optimized by the following:

- Starting with clean plant material requires a method of disinfection that controls the nematodes while leaving the plant material untouched. Ornamental bulbs are heated in a water bath, but the prevalent temperatures that are used seem to be ineffective. For bulb onions this procedure has not been adopted.
- A detailed understanding of the soil properties related to stem nematode distribution might help in the management of stem nematodes.
- The distribution of stem nematodes in the field is highly variable, it is difficult to determine a clear infestation focus and the pattern of spatial distribution is unknown. This information is needed to calculate the detection probability using a standard method of sampling. Developing a standard method of sampling might help to optimize management by early detection of infestations and applying site specific methods of control.

  One of the main objectives of sampling is to estimate initial population densities for advisory DSS in the management of soil dwelling plant parasitic nematodes (Seinhorst, 1988; Been and Schomaker, 2006). So far there is no specific sampling method for stem nematodes. To develop a sampling method, including the bulk sample size needed for estimating nematode density per unit area with a certain degree of precision, understanding the horizontal and vertical spatial distribution of stem nematodes is important. It is known that 80–100% are found in the upper 20 cm of the topsoil. The horizontal distribution of stem nematodes in onions both in clay and sandy soil is mostly roundish and irregular, as active spread by the nematodes is predominant.

  Using a standard method, the detection probability in clay soil for central population densities of 10 and 100 stem nematodes/500 g soil is 23% and 90%, respectively. In sandy soil where the activity of the stem nematodes is lower, the probability of detection is <2% for a central population of 15 stem nematodes/1000 g soil. Considering the low damage threshold and irregular distribution, field monitoring and records of the cropping history are necessary and may lower the sample size that is required to detect the infestation foci. However, even when no stem nematodes are found in the soil, damage might still be observed in the onions (Seinhorst, 1956b). This illustrates the need for the development of reliable methods of sampling and extraction that increase the probability of detecting low levels of infestation.

- DSS for management of plant parasitic nematodes like Aaltjesschema and NemaDecide depend on studies of population dynamics and damage threshold parameters in both sandy and clay soils. The available studies on host status are limited and mostly unsuitable to be utilized for DSS. New studies are required with a full range of nematode densities under controlled conditions to estimate the host status of onion cultivars and other crops that are attacked by the onion race of *D. dipsaci*. This will help to optimize the management of stem nematodes in planned rotations, at least in sandy soils. Results can be applied to field conditions based on estimating initial population densities using an established accurate sampling method. This might further be coupled with geographic information systems to provide scenarios of management options using several crops with known host status and related damage.
- Improvement of the detection method of stem nematodes in soil is needed. At present, stem nematodes are extracted from soil using Oostenbrink and Seinhorst elutriator followed by Baermann tray. When dauer larvae do not become active during the extraction period, they are missed. Further, the very low damage threshold calls for a high level of detection that needs improvement.
- Rapid molecular diagnostic tools to differentiate races of stem nematodes is fundamental to decide on available relevant control measures related to the specific race using a DSS.
- Finally, it is necessary to strengthen the relationship between plant protection service, extension services and farmers, which helps to get accurate insight into infestation of fields with stem nematodes and implement necessary control measures.

Future research requirements

A sampling method needs to be developed based on new sampling data and estimation of the coefficient of aggregation of *Ditylenchus dipsaci*. It is important to upgrade and calibrate techniques used in estimating densities and the activity of stem nematodes. This might help understanding the reasons for an increased activity of stem nematodes in partially sterilized soils, which may be related to the specific niche of stem nematodes in the soil. Further, the chemical nature of the soil extract from soil that inhibits stem nematode activity needs to be elucidated. This requires detailed soil analysis using gas chromatography to identify and quantify the chemicals involved. The information obtained could be the first step to understand why specific infested spots in a field remain static for many years without spreading within and between fields. Moreover, it also helps to understand soil conditions affecting the mortality of stem nematodes.

Breeding for resistance against stem nematodes has been successful in lucerne and in red and white clover, but also in oats and rye. As several stem nematode races can infest onion, breeding for resistance needs to be targeted at a range of possible infective races to be effective as a management tool.

Outlook: anticipating future developments:

In general, problems with stem nematodes are most severe when the spring is cool and moist. Climate change in the Netherlands is predicted to result in increasing temperatures, with less frequent rainfall and a higher incidence of heavy showers. It is difficult to predict how this will affect the incidence of problems with stem nematodes. In practice, it has been found that after a warm and dry spring with a low stem nematode incidence, problems still arose after the first rainfall in summer.

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