Preface

There are several free living nematodes that can cause problems when growing sugar beets, but the problems are mostly limited in geographic spread and in economic importance. That does not hold for the white cyst-nematode, *Heterodera schachtii*. Curious enough this is largely a man made pest problem, related to intensive sugar beet cultivation. It is certainly not a recent phenomenon, but its detection dates back to 1859.

Due to the high cost for transporting the sugar beet harvest, farmers and industry always have tried to concentrate the sugar beet crop around the factories.

Biological methods have always been the most important in reducing the impact of cyst-nematodes. Longer rotations, avoiding other crops hosting the cyst-nematodes, improving soil structure and early drilling were widely practiced. Breeding has contributed in two very different fields. The first is the development of Brassica varieties (e.g. white mustard) which can be cultivated in the winter as green manure and which are able to reduce the population numbers of the cyst-nematodes. The second is the development of resistant or tolerant sugar beet varieties.

The search for resistance in sugar beet started already in the fifties of the twentieth century, and although a gene was found in Beta procumbens, a beet species related to the cultivated sugar beet, this has not yet delivered sugar beet varieties with a large commercial impact. It appeared to be difficult to stabilize the presence of this gene in sugar beet.

The discovery of genes giving tolerance to sugar beets to grow and produce well in contaminated fields was a major break through. These varieties also imply a lower multiplication rate of the cyst-nematodes.

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Acknowledgements

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Introduction

In the mid 19th century, sugar beet growing became more concentrated around factories, rotations became shorter and beet, being an excellent first rotation crop, returned with increasing frequency to the same fields. It was at this time that some growers began to observe substantial, unexplained losses that were referred to as ‘beet fatigue’.

In 1859, in plots situated in Germany, Professor Schacht identified the cause of this fatigue: microscopic worms that lived as parasites on the sugar beet roots.

There are about a hundred species of nematode that are harmful to crops. In sugar beet, the beet cyst-nematodes (*Heterodera schachtii*) are the most damaging. These white worms measure about 1 mm in length, their heads containing a hollow needle or ‘stylet’ which is used to perforate plant cell walls and suck out the contents. The presence of this stylet is a characteristic common to all species of phytoparasitic nematodes. They move through the water in the soil with a swimming motion similar to that of a snake, which is why they are often called ‘eelworms’.

Figure 1. Longitudinal Cross Section of a Nematode (Genus *Heterodera*) showing the head containing the stylet used for feeding. The red and blue areas in the diagram are glands that produce the secretions which are discharged into the host plant cells.
In order to develop, beet cyst-nematodes (*Heterodera schachtii*) must penetrate the roots of the plant. This phase causes the emission of many lateral rootlets and sometimes the development of characteristic dense root hair. They then feed on the plant and transform either into males, which are filamentous and move freely in the soil; or females, which are visible on the roots in the form of little white or brown lemon-shaped dots (‘cysts’).

At present, the nematode *Heterodera schachtii* is one of the main sugar beet crop pests and can cause very significant yield losses.
Geographical distribution

This nematode can be found in Europe, Asia, the Middle East, the United States and Australia. In Europe, it is present in practically all regions where sugar beet is intensively grown. It is frequently found in areas where rotations tend to be short and/or include various other plants that host the parasite.

In **France**, the pest affects some 40,000 to 60,000 hectares of sugar beet although intensity of the infestations varies considerably. In 2008, the Aisne and Oise departments were almost totally affected by the parasite. In the Nord-Pas de Calais, the cyst-nematode is mostly present beside the sea and in Flanders, as well as in the region of Cambrai. It is also found in the Artenay-Toury sector in the Eure-et-Loir department, and Loiret. In Champagne, the parasite is rife around and to the north of Reims. Finally, it is also present in Alsace and to the south of Obernai.

In **Belgium**, it is estimated that approximately 60% of fields are infested. However, only 20% of these fields – that is 12% of land on which sugar beet is grown – are thought to be affected by a degree of infestation in excess of 500 eggs and larvae per 100 g of soil (IRBAB¹, 2005). The contaminated areas appear to be very highly correlated with areas in which sugar factories were located during the 1960s and 1970s.

In **Holland**, the IRS² estimates that *H. schachtii* is present in 42% of beet plots in 2007 (Schneider & Wevers, 2007). However, only 10% of these plots are characterized by a medium to high infestation rate (> 300 eggs-larvae/hectare). Infestation levels are highest in the regions of Zeeuwse islands, West Brabant and the north and south of the Netherlands.

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¹ IRBAB, Institut Royal Belge pour l’Amélioration de la Betterave; KBVIB, Koninklijk Belgisch Instituut tot Verbetering van de Biet (Belgium).
² IRS, Instituut voor Rationele Suikerproductie (The Netherlands).
In **Britain**, *H. Schachtii* poses no significant problem for the time being, except in the very light soils of certain farms. The length of rotation practiced is probably the main factor explaining the low levels of populations encountered. However, in the future, sugar beet production is expected to intensify in East Anglia and nematode outbreaks will probably become increasingly frequent and severe as a consequence.

In **Italy**, all sugar beet growing land in the region of Emilia-Romania is affected. In **Spain**, the Lebrija area of Andalusia is currently suffering a major increase in pressure from cyst-nematodes on autumnal sugar beet crops.
Symptoms

The symptoms of an *H. schachtii* outbreak appear in the form of limited patches in the field which slowly increase in size over the season. They can be observed from June until harvest:

**In foliage**

- Wilting during the hottest hours of the day (a); beyond a certain level of infestation, the leaves no longer have the capacity to recover during the cooler periods and this wilting becomes permanent.
- Yellowing and eventually necrosis of the outer leaves (b)
- Symptoms of magnesium deficiency (particularly where availability is sufficient to meet requirements)

**In the roots**

- The taproot remains undeveloped; in its place root hair sometimes forms (a) on which little white lemon-shaped dots form with a diameter of between 0.5 and 1 mm. These cysts correspond to the female stage of the nematode development cycle (b). Once they have fully developed, they turn brown and detach from the plant.

*Figure 5. Typical Symptoms in Leaves of *H. schachtii* in Sugar Beet (on the left: a; on the right: b) (Source: ITB).*

*Figure 6. Typical Symptoms in the Roots Caused by *H. schachtii* in Sugar Beet (Left: a; Right: b) (Source: ITB)*
Diagnosis

By observation in the field

The IRBAB/KBIVB (BetaConsult) in Belgium and the IRS (BetaKwik) in the Netherlands have together developed a decision-making aid for growers comprising, in particular, a tool for identifying pests and diseases:


Diagnosis of the disease in the root is fairly easy. However, its symptoms could be confused with those of other root rots (pythium, aphonomycosis) or even with damage caused by lightning. In any event, the grower should contact a specialized laboratory if he suspects he has a nematode problem.

The presence of small, white to brown, lemon-shaped cysts on the root hair is the key to a diagnosis. To do this, the sugar beet must be uprooted carefully and, if necessary, a magnifying glass used to observe the roots.

Nonetheless, some confusion is possible:

- Other nematodes also affect sugar beet.
- Wilting and proliferation of root hair are not exclusively symptoms of *H. schachtii*. They also appear, for example, in the case of Rhizomania disease.
- Leaf symptoms can also be confused with those caused by poor soil structure or magnesium deficiency.
Other nematodes affecting sugar beet

Yellow beet cyst-nematode

The genus *Heterodera* comprises approximately 60 species, two of which may develop on sugar beet: the nematode *Heterodera schachtii* and *Heterodera betae* (until recently confused with *Heterodera trifolii*) (Amiri et al. 2002).

At present, *H. betae* causes damage mostly in the Netherlands, Switzerland, Sweden and Germany. Its dispersal remains very limited: in the Netherlands, this parasite is present in only 1% of sugar beet fields (IRS, 2007).

With the exception of the colour of the females, the symptoms caused by *H. betae* closely resemble those caused by the nematode *H. schachtii*. If this parasite attacks the plant at an early stage, it can cause it to totally disappear, this is not the case with *H. schachtii* (IRS, 2007).

Likewise, the *Heterodera betae* life cycle is very similar to that of *Heterodera schachtii*. The main difference is that the larvae only develop into females. In theory, this should result in more rapid multiplication in the plot. In practice, well thought-out rotation is often enough to control the disease. However, growers should be aware that *H. betae’s* host range is very broad: it includes all the white beet cyst-nematode host plants in addition to certain vegetables and adventitious plants.

The beet crown nematode

Characteristic symptoms caused by the beet crown nematode *Ditylenchus dipsaci* are often visible from summer onwards. This parasite causes cracking in the crown where a corky rot sets in (ITB 2008). It is a free-moving nematode that penetrates the upper part of the root of the plants (hypocotyl) and feeds on their parenchyma.

It is found throughout temperate Europe but it only causes major damage in a small area of sugar beet regions. Generally, it has a moderate impact on yield, sugar content and the industrial quality of the sugar beet.

This nematode has a particularly wide host range, making rotational control quite difficult. Sensitive plants (mainly rye, oats and mustard) must be excluded and rotations must be extended.

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(3) As opposed to non-mobile nematodes such as the genus *Heterodera* which attach themselves to the plant.
Root knot nematodes

In temperate regions, sugar beet attacked by these parasites (*Meloidogyne hapla, M. naasi*) is characterized by inhibited development and a tendency to wilt during the hottest hours of the day. The taproot does not develop normally: in its place, numerous lateral roots appear which swell irregularly in certain places forming galls.

Nematodes of the genus *Meloidogyne* have a cycle that is very similar to the root-cyst nematodes of genus *Heterodera*: the larvae penetrate the roots and develop there. However, the females are not directly visible on the roots. They are found inside the galls (swelling on the root) resulting from the proliferation of plant tissue.

*M. hapla* and *M. naasi* are rife mainly in temperate regions on light textured soils. They are often found in northern Europe, Japan or the United States.

These parasites cause minor damage and the stunting caused is often compensated later in the season. In the end, there may sometimes be a lower sugar content. Early attacks can in some cases kill the plant.

The host range of these nematodes is fairly broad but if a non-host plant is introduced in rotation, their population will decline rapidly so the best means of control is to adapt rotation in particular using trap crops in-between.

*Figure 8. Typical Symptoms Caused by the Sugar Beet Root Knot Nematode.*
Testing for nematodes with soil analysis

In addition to field observation, performing specific soil analyses in plots where presence of *H. schachtii* is suspected is highly recommended. On the one hand, these will confirm the presence of *Heterodera schachtii* with certainty. On the other, the extent of the infestation of the plot will be known (expressed as the number of eggs and larvae per 100 grams of soil).

As the nematode has very limited mobility in the soil, its distribution in a field is often very uneven (Olsson, 2005). Consequently, to obtain a reliable soil analysis result that is representative of the field, it is essential to use the following rules when collecting soil samples.

Where to collect soil in the field?

To be representative, samples must be collected from the entire field where an outbreak is suspected (not only from the specific patches where damage has been observed).

How?

Samples should be taken using a soil auger of 20 to 30 cm in length and 1.5 to 2 cm diameter. Approximately 10 samples should be taken per hectare (IRBAB, 2005). They must then be stored in a plastic bag and labelled - stating the origin (field details and ideally GPS position) and the date it was sampled. The samples must be kept in a cold store (approximately 5°C) if they are not sent immediately to the laboratory, this prevents deterioration of the material.

When?

Typically sampling will take place in the spring before sowing sugar beet and when temperatures are relatively low so that the cysts have not had time to hatch. Ideally, samples should be taken regularly to ensure the best assessment of cyst numbers prior to hatching.

Laboratory identification and counting method

Today, the most commonly used technique is the physical counting of larvae and eggs under a microscope. In certain cases, this may be facilitated by chemical hatching and counting on a television screen.

Molecular techniques used to identify and quantify DNA - ELISA/PCR (Cornelis & Hermann, 2003) or semi-quantitative PCR (Fürstenfeld et al., 2005) – have also been developed. More reliable, quicker and cheaper, they are now used routinely particularly by the Bodengezundheitsdienst (Ochsenfurt) in Germany.

(4) Many other species of nematodes are morphologically similar to the white beet cyst-nematode yet harmless to sugar beet: *H. avenae* (oat cyst-nematode), *H. goettingiana* (pea cyst-nematode), *H. carotae* (carrot cyst-nematode), *H. trifoli*. Clearly, when these species are present at the same time in the same field, a simple physical count may be unreliable.
Epidemiology

Cycle

Sugar beet nematodes can survive as cysts for up to five or six years in the soil and in the absence of host plants. The cysts are the shape of small lemons and the size of a pen tip. Each cyst contains eggs and larvae.

In favourable climatic conditions (generally in the spring: between 16 and 28°C, the optimum is 25°C) where the cysts are in contact with root secretions from the host plant, the cyst hatches and the filamentous larvae move through the soil in the direction of the rootlets.

The larvae use their stylet to penetrate the root tissue and then burrow towards the vascular cylinder to establish a feeding site or syncytium. The formation of this site follows injection by the nematode of specific substances into the plant cells, these substances modify the cellular metabolism enabling the nematode to accomplish its entire cycle of development. At the same time, the nematode inhibits sap circulation, this is the stage at which the plant’s growth and development are affected.

The larvae gradually develop into males or females: the head of the latter remains fixed to the feeding site (the syncytium) and their bodies swell until they appear on the root surface where they become easily visible to the naked eye. The males disappear into the soil to fertilize the females. After fertilization, the females die: their white, lemon-shaped bodies gradually transform into brown cysts containing up to 600 eggs (from 100 to 300 on average).

Dispersal and growth factors

The nematode’s capacity for moving through the soil is relatively limited. However, cysts can be dispersed by water (rainfall, run-off, irrigation, etc.) and soil transportation (erosion, earth work, uprooting).

Depending on climatic conditions (rainfall, temperatures, etc.) and the presence of hosts, several cycles of development may take place while the crop grows. In Northern European conditions, this is typically 2 to 3 generations per year; approximately 3 in the Southern European; and approximately 5 in South Californian conditions. On average a cyst takes a little less than 40 days to complete its development cycle.

Optimum multiplication conditions for the sugar beet nematode are:

- A wet spring (but a dry period will also increase visible symptoms on infested plants)
- High soil temperatures
- Light soil types (but the parasite may be present in any type of soil)
Hosts

The sugar beet nematode has a very wide host range:

✔ Some cultivated species (beets, spinach, cabbage and canola/rapeseed)
✔ Some intermediate crop species (white mustard, fodder radish and some leguminous plants)
✔ A multitude of adventitious plants

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*Table 1. The host range of the white, cyst-nematode (Heterodera schachtii) includes some farmed species and many adventitious plants (according to Schlang, Olsson and the ITB).*
Economic importance

The scale of the damage caused by *H. schachtii* depends on the extent of the initial contamination, the date of sowing (an infection of a young plant is more problematic), soil conditions and the climate.

According to the ITB, yield losses can reach 30% of the regional average but more commonly it is 15%. The IRS mentions yield losses of approximately 35%, although this may reach 70% in some cases. The IRBAB believes that the presence of each larva in 100 g of soil may result in the loss of 3 kg of sugar per hectare. Losses are greater during a dry hot year (up to 6 kg of sugar per larvae), although they are also detectable during very low infestations (less than 50 eggs and larvae).

The damage caused by a nematode outbreak in the field consists mainly of:
- Increased soil tare (following formation of root hair)
- Yield losses

The beet sugar content is only slightly modified by an outbreak of beet cyst-nematodes. The industrial quality of beet (extractability, sodium, potassium and amino nitrogen content) is not affected by this pest, so long as foliar regrowth is minimal. *Heterodera schachtii* is also known to increase plants susceptibility to a secondary invasion by diseases such as *Fusarium spp.* (Schneider & Wevers, 2007; Ayola García *et al.*, 2007).
Disease control

No chemical means of control is currently approved in Europe. The use of nematicide (e.g. aldicarb) is banned. Use of 1.3 dichloropropene is not economically viable for sugar beet crops. Currently, protection of sugar beet from cyst-nematodes is based on a combination of two methods of control: agronomic measures and dual Rhizomania-nematode tolerant seeds.

Agronomic measures

- ✔ Widening of the rotation: a minimum of 2 years and ideally 4 between two sugar beet crops
- ✔ Avoid host species during rotation (canola/rapeseed, cabbage, spinach, etc.)
  Canola/rapeseed or any other non-nematicide crucifer must be avoided at all costs in fields contaminated by *H. schachtii*. If canola/rapeseed was the preceding crop, ensure volunteers are routinely destroyed.
- ✔ Sowing a resistant crucifer as an intermediate crop (nematicide green manure)
  Certain cultivars of white mustard (*Sinapsis alba*) and fodder radish (*Raphanus sativus*) show nematode resistance (Smith *et al*., 2004; Westphal & Becker, 2001). For this green manure to effectively reduce the nematode population before sowing sugar beet, the temperature of the soil must be high enough for the cysts to hatch. This will only happen if the cover is sown rapidly in the summer, i.e. if the preceding crop is harvested early (e.g. winter barley). In addition, to encourage the maximum hatching of the larvae, a relatively warm, rainy autumn is also necessary.
- ✔ Improvement of the soil and drainage structure
- ✔ Early sowing

Dual Rhizomania-nematode tolerant seeds

- ✔ Many of the conditions which favour a Nematode infestation - waterlogging, low fertility sites, etc - are also conducive to a build up of Rhizomania. Where nematode presence is demonstrated, it is sensible to choose varieties with both Rhizomania and Nematode tolerance

The application of only one of these measures will not be enough to combat cyst-nematode. In practice, it is important to use an integrated approach by combining the control methods.
Dual Rhizomania-nematode tolerant varieties

Breeding for resistance

At present, there are two main sources of resistance to the white, beet cyst-nematode, *H. schachtii* (Zhang et al., 2008). The first was identified in the 1950s in Beta procumbens. For this source, several resistant genes were detected but the dominant gene, Hs1pro-1, gradually proved to be the main resistance-controlling gene.

Its mode of action is ingenious and combines two mechanisms:

1. By blocking the formation of the feeding sites, it stimulates hatching of the larvae and at the same time, limits of the formation of the feeding cell system (syncitium) and therefore of the development of the nematode.
2. By altering the male:female ratio to favour males it reduces the quantity of eggs and larvae produced per generation.

This source of resistance was introduced by crossing sugar beet with a series of back crosses from elite sugar beet lines, this was assisted by using molecular markers that make it easier to identify the presence of resistance in the plant. The first variety to exploit this source of resistance was probably Nemafort produced by SESVanderHave.

The source of resistance associated with the gene Hs1pro-1 is total. It makes it possible both to significantly reduce the nematode population of a field and to achieve better output than sensitive varieties in infested land. However, performance in healthy land of this first generation of dual Rhizomania-nematode tolerant varieties was significantly lower than those of sensitive varieties.

A second polygenic and recessive source of resistance to beet cyst-nematode was identified some years ago in the wild species of sugar beet *Beta vulgaris* ssp. *maritima*. This resistance is partial as, at most, it stabilises rather than reduces the cyst-nematode population in the field. For this reason, tolerance or partial resistance is the most common term. The first commercial hybrid based on a source of this type to have been proposed in Europe was the variety Pauletta from KWS. Compared with the first generation of dual Rhizomania-nematode tolerant varieties, Pauletta performed better in healthy ground although always to a lesser extent than solely Rhizomania tolerant varieties.

Other sources of resistance exist. Among these, source WB42, discovered at the IRS, has been studied in the greatest detail. Such sources are sometimes based on a few genes, sometimes on a large number. They can be dominant, additive or recessive. Their resistance is partial as they do not prevent multiplication – albeit limited – of the parasite in the field. However, they make it possible to allow economic production from infested land.

SESVanderHave: a strong source of nematode tolerance

In 2008, SESVanderHave registered its first dual Rhizomania-nematode tolerant variety. The variety Bison demonstrated a tolerance to beet nematode together with excellent Rhizomania resistance.
Advantages and disadvantages

In the event of extreme nematode pressure, only the use of dual Rhizomania-nematode tolerant varieties will make it possible to avoid major losses in yield (Gutierrez Sosa, 2007).

However, seeds of this type of variety also have certain disadvantages:

✓ In healthy ground, the yield of these varieties is significantly lower in varieties with Rhizomania tolerance alone: some 6 to 7% according to the ITB and the IRBAB.
✓ The question of durability of nematode resistance requires further research. This is currently being undertaken by the ITB in collaboration with INRA and plant breeders.
✓ In the event of extreme Rhizomania pressure, certain varieties with dual tolerance may have insufficient tolerance to Rhizomania.

This phenomenon was observed during the 2007 campaign to the south of Paris (ITB, 2007) where extreme P type Rhizomania pressure was observed. This resulted in insufficient yield for the dual Rhizomania-nematode variety as shown in the graph below:

![Dual Rhizomania-Nematode Tolerant Varieties](image)

Figure 10. Current Dual Tolerant Rhizomania-Nematode Varieties Sometimes Show Certain Weaknesses in the Case of Extreme Rhizomania Pressure.

✓ High degree of sensitivity to cercospora and oidium species
✓ Tolerant varieties, unlike resistant varieties, do not reduce the population of nematodes in the soil. However, these tolerant varieties do show partial resistance. In comparison with nematode sensitive varieties, tolerant varieties will see an average increase in the nematode population when the initial level is low, medium or high. In sensitive varieties the build up is more rapid.
When should a nematode tolerant variety be sown?

It is very difficult to predict the damage that will be caused by the cyst-nematode solely on the basis of nematode sampling as damage depends not only on nematode pressure but also on the timing of the outbreak and the impact of climatic conditions during the campaign. Because of this variability, threshold recommendations vary for each country.

In France, the ITB relies more on observations in the field rather than nematode sampling alone. It advises sowing a dual Rhizomania-nematode tolerant variety in the event of:

- A record of lower output than the local average
- Symptoms of magnesium deficiency in the foliage
- Wilting at the hottest time of day
- The presence of cysts, if possible confirmed by a sampling and analysis in a laboratory

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Table 2. Threshold of Infestation above which Sowing a Dual Rhizomania-nematode Tolerant Variety is Recommended (Ayala Garcia et al., 2007).

Obs. = as soon as White Beet Cyst-Nematode is observed.

Recent progress made by plant breeders with dual Rhizomania-nematode tolerant varieties has resulted in a marked increase in the use of this type of variety by growers. For example, in France, during 2007 these varieties were used in 9% of the country. In certain regions that were particularly badly affected such as Aisne, up to 30% of varieties were dual nematode/Rhizomania tolerant. In the Netherlands and Belgium, use of these varieties is lower although it is expected to increase in the future.
References


