Chapter 1

Prolog

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General information about *Heterodera schachtii* in sugar beet

The beet cyst nematode *Heterodera schachtii* (Schmidt) is a severe problem for sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) production in Central Europe (Müller, 1999). This nematode is prevalent in many sugar beet growing regions especially at fields where sugar beet is cultivated in a narrow crop rotation. In Germany, approximately 25 % of the area cultivated with sugar beet is infested (Kaemmerer et al., 2014). This number is even higher in Northern Germany (> 80 %, Schlinker, personal communication 2013). Without host plants, *H. schachtii* can outlast within cysts in the soil for years. Juveniles will hatch when the development to second stage juveniles is completed (Fig. 1) and infest the roots of host plants. They penetrate the elongation zone behind the root tip and initiate the transformation of root cells to syncytia (specialized feeding structures). Syncytia concurrently increase with the nematode development to adults (Jung and Wyss, 1999) and may lead to impaired root functioning, which limits crop performance and results in yield losses.

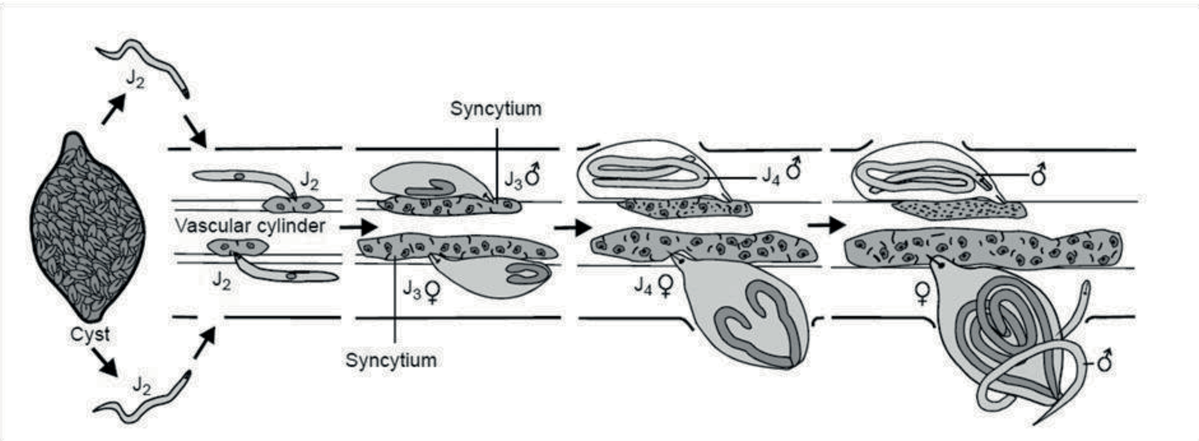

Fig. 1: Life cycle of cyst nematodes. J_2, J_3, J_4 juveniles in the second, third and fourth developmental stages (Jung and Wyss, 1999).

The duration of completing one nematode generation strongly depends on soil temperature. Under German climate conditions, two to three generations of *H. schachtii* per year are possible (Müller, 1979; Krüssel and Warnecke, 2014). Usually, an early infestation of sugar beet with nematodes of the first generation in spring leads to stronger damages to the crop than late infestations of second or third generation nematodes during the growing period (Niere, 2009). Longitudinal growth of the tap root is disturbed and the beet develops additional fibrous roots resulting in the typical "root beard" (Decker, 1969). Heavily infested plants are reduced in growth and develop smaller and brighter leaves. As soon as radiation increases and soil dries, wilting symptoms can be observed. As beet cyst nematodes are heterogeneously distributed over naturally infested fields, stress symptoms in sugar beet infested with *H. schachtii* typically occur in patches (Hbirkou et al., 2011). Sugar yield losses can vary substantially depending on the nematode population density, their distribution in infested fields and the characteristics of the grown sugar beet variety (see box above about terminology). Schlang (1991) reported yield losses of more than 25 % under conditions typical for Central Europe.

Susceptibility, tolerance and resistance of sugar beets to *H. schachtii*

Three types of sugar beet varieties are available which are categorized as susceptible, tolerant or resistant to *H. schachtii*. However, none of them is protected against nematode invasion (Jung and Wyss, 1999). A susceptible plant, i.e., a plant which facilitates nematode reproduction, can be either tolerant with limited growth suppression or intolerant with relatively strong growth suppression in response to nematode infection (Müller, 1998). In this study, the term susceptibility is used equivalent to intolerance. Resistance is defined as the ability of a plant to limit nematode multiplication (Müller, 1989), because syncytia deteriorate in early stages of nematode development. Resistant plants may also carry tolerance.

Different relations between yield decline and initial nematode population (Pi) have been observed previously. In most cases a high Pi resulted in a high yield reduction of intolerant varieties (Griffin, 1988; Heijbroek et al., 2002; Kenter et al., 2014). This yield decline may be traced back to root damages and a reduction of root extension (Gierth, 2005), root functioning and to further physiological effects such as an impeded uptake of nutrients (Trudgill, 1980) and water. Schmitz et al. (2006) found a decline in leaf photosynthesis comparing infested with non-infested susceptible sugar beets in greenhouse experiments. Furthermore, nematode infestation reduced nitrogen availability, which resulted in a reduced leaf chlorophyll content (Schmitz et al, 2006). At field scale, nematodes might additionally cause a delay of canopy development and reduced leaf N status, which decreases radiation use efficiency.

As tolerance reflects the capacity of the plant to withstand nematode damage, physiological knowledge of nematode-host interactions together with an understanding of the relationship between yield loss and nematode infestation is required to understand the physiological mechanisms of tolerance. These mechanisms are usually nonspecific, but compensatory root growth, delayed plant senescence and enhanced water and nutrient uptake are suggested (Trudgill, 1991). Hypothetically, tolerant varieties have a better developed rooting system and thus a better ability to access soil water compared to susceptible varieties. Gierth (2005) for example observed in a greenhouse study that susceptible sugar beets infested with nematodes could not establish sufficient fibrous roots to compensate damage due to early nematode infestation when compared to tolerant plants. Growth of the susceptible variety was impaired accordingly.

Options to control *H. schachtii*

Population dynamics of *H. schachtii* at field scale can be highly variable and depend on various factors such as Pi, soil temperature and soil moisture as well as further unknown parameters (Daub and Westphal, 2012; Niere, 2009). Hatching of juveniles out of cysts occurs in the presence of host plants or to some extent spontaneously (Heyland and Hambüchen, 1991). Nematode reduction under fallow or non-hosts strongly depends on environmental conditions but averages 30 to 40 % per year (Niere, 2009). Therefore, a wide crop rotation including non-hosts together with the control of weed hosts is an option to lower the nematode density.

A common tool to reduce the nematode population is the cultivation of nematode resistant catch crops before sugar beet (Buhre et al., 2014), which are cultivated on approximately 40 % of the German sugar beet cropping area. Two different catch crop species with resistance to *H. schachtii* are available: white mustard (*Sinapis alba L.*) and oilseed radish (*Raphanus sativus* L. spp. *oleiformis* Pers.). In Germany, testing of newly developed catch crop varieties for resistance to plant parasitic nematodes is performed at Julius Kühn Institut in Braunschweig. Nematode population before (Pi_{CC}) and after growing a catch crop variety (Pf_{CC}) is determined in closed containers. Reproduction factors, i.e., the Pf_{CC}/Pi_{CC} ratio, are used to establish the resistance rating of the specific tested variety. A resistance rating of 1 is related to a reproduction factor less than 0.1, a rating of 2 to reproduction factors from 0.1 to 0.3 and of 3 to reproduction factors greater than 0.3 to 0.5. All varieties that exceed the reproduction factor of 0.5 are categorized as susceptible (Bundessortenamt, 2014). Mustard varieties are reported to be less effective in reducing the nematode density compared to oilseed radish varieties even if the resistance rating is the same (Heinrichs, 2011). Nonetheless, compared to oilseed radish, mustard varieties are more often cultivated as catch crops before sugar beet (Buhre et al., 2014). Mustard crop establishment is easier and winter-killing by frost is more likely than for oilseed radish. In contrast, oilseed radish might develop thick taproots that might not be frost-killed and possibly impede sugar beet sowing.

Nematode reduction due to resistant catch crops strongly depends on the development of an adequate catch crop stand with a sufficient rooting density. A prerequisite to establish sufficiently high catch crop biomass is an early sowing date immediately after pre-crop harvest in late July or early August. However, under Northern German climate conditions early sowing can be impaired by delayed harvest of winter wheat which is frequently grown as a sugar beet pre-crop. Under favorable conditions, the nematode reduction by mustard can be up to 70 % (Heinrichs, 2011). But, great differences in the nematode reduction at field scale depending on the mustard variety and environmental conditions occur (Niere, 2009; Smith et al., 2004).

A further option to combat *H. schachtii* is the cultivation of nematode resistant sugar beet varieties. High nematode reductions are possible (Heijbroek et al., 2002), but sugar yield is reported to be substantially lower than that of tolerant sugar beet varieties under nematode infestation (Bundessortenamt, 2013). This yield gap stated by Bundessortenamt (2013) is the reason why sugar beet growers rarely cultivate resistant sugar beet varieties (Märländer et al., 2003). Resistance is proofed by counting developed cysts at single sugar beet plants at Julius Kühn Institut (Bundessortenamt, 2013), but results are not available and a resistance rating comparable to that of catch crops does not exist for sugar beet varieties.

Another possibility to control the nematode population could be the application of nematicides. In the European Union, nematicides are not registered for the use in sugar beet, but in several other countries various pests in different crops are controlled by nematicides (Cabrera et al., 2013). If a resistant catch crop fails to reduce the nematode population, an alternative control strategy comprising the application of nematicides might become necessary.

Challenges in conducting field experiments with *H. schachtii*

In general, observations on population dynamics of *H. schachtii* are highly dependent on Pi. At low Pi, statistical variance of reproduction factors is very high, while at higher Pi (>2000 eggs and juveniles per 100 g soil (E+J)) the variance of estimated reproduction factors is usually smaller (Krüssel and Warnecke, 2014). Thus, statistical differences between treatments – if apparent – are more likely to be detected at high infestation levels. Nonetheless, extremely high infestation is not widespread. In Northern Germany, a monitoring from 2009 to 2013 demonstrated that nematode infestation was present on 84 % of the sugar beet cultivation area, while on 76 % it was below 1000 E+J (Schlinker, personal communication 2013). To account for this, environments should be studied which cover a wide range of Pi to represent typical infestation levels ranging from non-infested to high infestation levels.

In addition, beet cyst nematodes do not only vary from one field to the other but are also heterogeneously distributed within fields. Likewise, stress reactions of sugar beet occur in patterns following the heterogeneous distribution of *H. schachtii* (Hbirkou et al., 2011). This patchy occurrence of nematodes, meaning that high nematode densities can possibly be found next to areas without any infestation, leads to difficulties in designing and analyzing field experiments. A prerequisite for conducting field experiments under conditions of natural nematode infestation is to determine the real nematode density that a sugar beet plant is exposed to. A high variability in evapotranspiration, nutrient uptake (such as nitrogen) and yield of intolerant varieties in response to nematodes at field scale is likely, while tolerant varieties might show less variability. Therefore, all measurements – being either nematode density, sugar yield, evapotranspiration or other parameters related to