INTERACTIONS BETWEEN THE BEET CYST NEMATODE, *HETERODERA SCHACHTII* (SCHMIDT), SUGAR BEET AND BRASSICA TRAP CROPS

By

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Abstract

The beet cyst nematode (BCN), *Heterodera schachtii* (Schmidt), is a commonly occurring and widespread pest of sugar beet (*Beta vulgaris* ssp. *vulgaris*) which is found in all regions of the world where sugar beet is grown. The nematode feeds on the fibrous roots of suitable host plants, restricting their growth through impeded water and nutrient uptake and can reduce yield by up to 50%. BCN infestations require careful long term management to limit their spread and reduce yield losses.

This Thesis outlines research which aimed to develop a greater understanding of how varieties of sugar beet interact with BCN and also investigate the use of brassica trap crops for BCN population control. To achieve these aims, a series of glasshouse, micro-plot and field experiments were conducted to understand more about how sugar beet interact with the nematode. Resistant sugar beet varieties were shown to be most effective at population management. However, the use of these resistant varieties is hampered by poorer yield potentials. This research project showed that yield increases of resistant varieties can be obtained by establishing higher plant population densities.

Varieties of sugar beet which are declared tolerant to BCN infestations have also been evaluated and appear to be partially resistant to BCN. This trait also explains their ability to yield well in infested fields due to reduced feeding and removal of nutrients from the sugar beet. The tolerant varieties exhibited physiological resilience to infestation in relation to chlorophyll content, with initial reductions in chlorophyll in response to nematode infestation but then later a recovery, while in susceptible varieties the reduction in chlorophyll content persisted. The greater understanding of these varieties, and methods to assess them, will

enable them to be used to their full potential to reduce yield losses as a consequence of BCN infestations in sugar beet and other host crops.

The use of BCN resistant brassica trap crops has also been tested in field experiments. BCN population reductions were obtained from some brassica varieties, a class one radish (*Raphanus sativus*) and a class two mustard (*Sinapis alba*), but not all trap crops caused significant population reductions. This response is likely related to different hatching stimuli produced by root leachates from the different trap crop varieties and was further investigated using laboratory bioassays. Some varieties of trap crop showed distinct peaks in their ability to stimulate BCN hatch, whereas, other varieties had a more sustained impact on hatch. This stresses the importance in timing of sowing of trap crops to optimise their ability to control BCN infestations. However, further research is required to understand whether, with the use of BCN tolerant varieties of sugar beet, whether the use of brassica trap crops is justifiable for BCN control alone.

As a whole, the findings contained within this PhD thesis will help to develop future control strategies to manage BCN infestations and protect yields of sugar beet.

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List of Abbreviations

Abbreviation	Full Form
°C	Degree Celsius
µl	microlitre
µm	micrometre
AHDB	Agriculture and Horticulture Development Board
Amax	Maximum assimilation
Amino N	Amino Nitrogen
ANOVA	Analysis of Variance
BBRO	British Beet Research Organisation
BCN	Beet Cyst Nematode
BSPB	British Society of Plant Breeders
BTS	Betaseed
CER	Controlled Environment Room
cm	centimetre
CO_2	Carbon Dioxide
DAS	Days After Sowing
Di H_2O	Deionised Water
DNA	Deoxyribonucleic Acid
EPPO	European and Mediterranean Plant Protection Organisation
et al.	et alia
FAO	Food and Agriculture Organisation of the United Nations
Fig	Figure
g	gram
GDD	Growing Degree Days
GPS	Global Positioning System
GSL	Glucosinolate
ha	Hectare
HR	Hatching Rate
ICUMSA	The International Commission for Uniform Methods of Sugar Analysis

IP	Inflection Point
IPM	Integrated Pest Management
ISC	Initial Syncytial Cell
ITC	Isothiocyanate
iWUE	Instantaneous Water Use Efficiency
J2	Second Stage Juvenile
K	Potassium
l	litre
LSD	Least Significant Difference
m	metre
M	Mustard
MAFF	Ministry of Agriculture, Food and Fisheries
mg	milligram
mm	millimetre
ml	millilitre
n	number
N	Nitrogen
Na	Sodium
NDVI	Normalised Difference Vegetation Index
Ø	Diameter
PCN	Potato Cyst Nematodes
PCR	Polymerase Chain Reaction
Pf	Final Population
Pi	Initial Population
PPD	Plant Population Density
R	Radish
R^2	Coefficient of Determination
Rf	Reproductive Factor
RL	Recommended List
RLS	Root Leachate Solution
RTK	Real-time Kinematic

SB LT	Light tolerant sugar beet
SB R	Resistant Sugar beet
SB S	Susceptible Sugar beet
SB T	Tolerant Sugar beet
SCN	Soybean Cyst Nematode
SEM	Standard Error of the Mean
SPAD	Soil Plant Analysis Development
STR	Strube
SVH	SES Vander Have
SYN	Syngenta
TM	Trade mark
UK	United Kingdom
USA	United States of America
WUE	Water Use Efficiency

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Joint Authorship Statement

The published works contained within this thesis were jointly authored and submitted for publication in suitable scientific journals. The breakdown for authorship is as follows:

A. Wright – Lead Author who undertook the experimentation, data analysis and drafting of all manuscripts

J. Bussell – Assisted with curve fitting the canopy data from the field experiments detailed in chapter two

M. Stevens – Assisted in experimental design, formulation of hypotheses and provided feedback for all manuscripts

M. Back – Assisted with techniques relating to work with cyst nematodes, formulating of hypotheses, feedback on all manuscripts and is final author for chapter five

D. Sparkes – Lead all project areas, oversaw all experimental designs and the conductance of the experiments. Assisted A. Wright with the data analysis for all chapters and is final author for chapters two, three and four

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Chapter 1

General introduction & literature review

1. Introduction

Nematodes cause significant yield losses to crop yields every year across a wide range of cultivated crop species worldwide. Crop yield losses to all nematodes were recently predicted to exceed $80 billion annually, although due to low level, undetectable, infestations this figure could actually be far greater (Nicol et al., 2011). Nematodes have evolved over the past billion years to inhabit almost every climate on earth and it is estimated that there may be nearly one million different species, despite only 15,000 species having been classified so far (World Conservation Monitoring Centre, 1992). Nematodes therefore have become specialised and infect most plant species and can be particularly harmful to our major crop species.

One species of particular economic importance is the beet cyst nematode (BCN), *Heterodera schachtii* (Schmidt, 1871). It infests a wide range of plant species with the majority being from the *Brassicaceae, Betoidae* and *Amaranthaceae* families. The name of the nematode relates to the crop it causes the most significant damage to, sugar beet (*Beta vulgaris* ssp. *vulgaris*), where yield losses can be up to 40% (Blok et al. 2018). In Europe alone it has been estimated to cause sugar beet yield losses in excess of €90 million per annum (Müller, 1999), and losses in the UK may be as high as £3.8 million each year (Wright et al., 2017).

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is one of two crops cultivated globally for the production of sugar in the form of sucrose. Sugar beet dominates temperate cropping for

sugar production whereas sugar cane (*Saccharum officinarum*) is cultivated in tropical and sub-tropical regions. These account for 20% and 80% of world white sugar production respectively (FAO, 2009). Sugar beet is a spring planted crop which develops a large leafy canopy which intercepts light, produces sucrose and stores it in a large conical root, which when harvested usually exceeds one kilogram in weight. This root contains almost 20% sucrose when harvested and harvest usually occurs in the autumn and winter (Draycott, 2006).

The UK produced 9,430,000 tonnes of sugar beet in 2014 from 116 000 ha of land which was refined into 1.4 million tonnes of sugar, accounting for 62% of the UK's sugar demand. (British Sugar, 2018; FAOSTAT, 2014). Beet production is centred in Eastern England and the East Midlands around the four processing factories in Suffolk, Norfolk and Nottinghamshire.

Beet cyst nematode is found in all factory areas in the UK. An integrated approach is required to control the pest whilst optimising yields. This thesis describes a series of experiments undertaken to understand more about the interactions between BCN, modern sugar beet varieties and the options for control using resistant *Brassica* varieties.

1.1 Distribution, classification and historical importance of beet cyst nematode

1.1.1 Distribution of BCN

Beet cyst nematode can be found in over 40 countries and is present on every continent on earth (excluding Antarctica), in both tropical and temperate regions (Moens et al., 2018). Prior to the commercial cultivation of sugar beet, BCN is believed to have sustained itself on the wild relative of sugar beet, sea beet (*Beta maritima*) and other species such as goosefoots

(*Chenopodium L.*) and it is likely that the epicentre for the evolution of BCN was within Europe, along the Mediterranean coast (Evans and Rowe, 1998).

Beet cyst nematode can be found all along the coast of Northern Europe on sea beet and is usually distributed in climates where temperatures do not regularly exceed 28°C whereas *Heterodera betae* (the yellow beet cyst nematode) prefers warmer climates of more southern parts of Europe (Gracianne et al., 2014). BCN can be found all along the northern European coastline, between Spain and Denmark. These coastal populations can then spread to fields growing suitable host species. Historically, within the United Kingdom, infestations were found all over the country in isolated patches. However, due to the contraction of the area where sugar beet is grown it now only poses a serious threat to crops grown in the East of England and is largely concentrated in the fens and southern Norfolk (Cogman and Morris, 2009). This is because BCN favours sandy, loamy and organic soil where the nematodes can move freely (Turner and Subbotin, 2013) from the cyst to the host plant, where the nematode invades and feeds on the root before reproducing and dying.

1.1.2 Classification of beet cyst nematode

The understanding of the evolution and phylogeny of cyst nematodes has developed greatly over the last 70 years. In the 1950s twelve species were recognised (Evans and Rowe, 1998), however, with the advent of molecular genetic techniques in recent years, at least 85 species are now recognised within the genus *Heterodera* (Handoo and Subbotin, 2018). This genus is grouped within the family Heteroderidae and the sub family Heteroderinae. Also within this sub-family are the other major cyst nematode genuses, namely *Globodera* and *Punctodera* and there are now 121 recognised cyst nematode species (Handoo and Subbotin, 2018).

It is well established that cyst nematodes are amongst the most specialised types of plant parasitic nematodes (Moens et al., 2018) and usually each species will have a range of specific host species which they require to be able to successfully invade, establish a feeding site and eventually reproduce. As illustrated in Table 1, BCN has a diverse range of plant hosts.

1.1.3 Historical importance of BCN

Beet cyst nematode was first identified by botanist Hermann Schacht in 1859 to be the cause of widespread 'beet fatigue' in fields of sugar beet in Germany and was also the first cyst nematode to be described and documented (Müller, 1999). These fields were supporting regular, if not continuous, beet production, which led to intense build-up of BCN populations in the soil and severe damage to the crops. This resulted in regular crop failure and the closure of 24 sugar beet factories in Germany during the 1870s (Müller, 1999).

BCN was not discovered in the UK until 1934 and due to the risk to British sugar beet crops was legally controlled by the Beet Cyst Eelworm Orders between 1944 – 1977 (Cogman and Morris, 2009). The orders made it mandatory for sugar beet growers to enforce long rotations (5 or more years) between host species on infested fields. This time allows for natural decline of populations to less damaging levels, since around 50% of the eggs inside a cyst will hatch each year (Jones, 1956). As the orders have since been revoked, and rotational requirements removed from growers' contracts in 1988 (Cogman and Morris, 2009), sugar beet growers must now be proactive in managing their infested fields when growing host species.

Recent surveys by British Sugar and the British Beet Research Organisation (BBRO) have estimated that approximately 6% of the crop is infested by BCN each year (M. Stevens Pers. Comm.) and currently just under 7% of the crop is now planted with BCN tolerant varieties (Fig 1) (See 1.2.3). This is an area of the market which is showing continual growth since the first tolerant variety 'Fiorenza KWS' was approved in 2009 for cultivation in 2010 (Kerr, 2009) and for the 2019 season six BCN tolerant varieties are now listed, some of which show greater yield potential than some of the conventional 'elite' BCN susceptible varieties (BBRO, 2018).

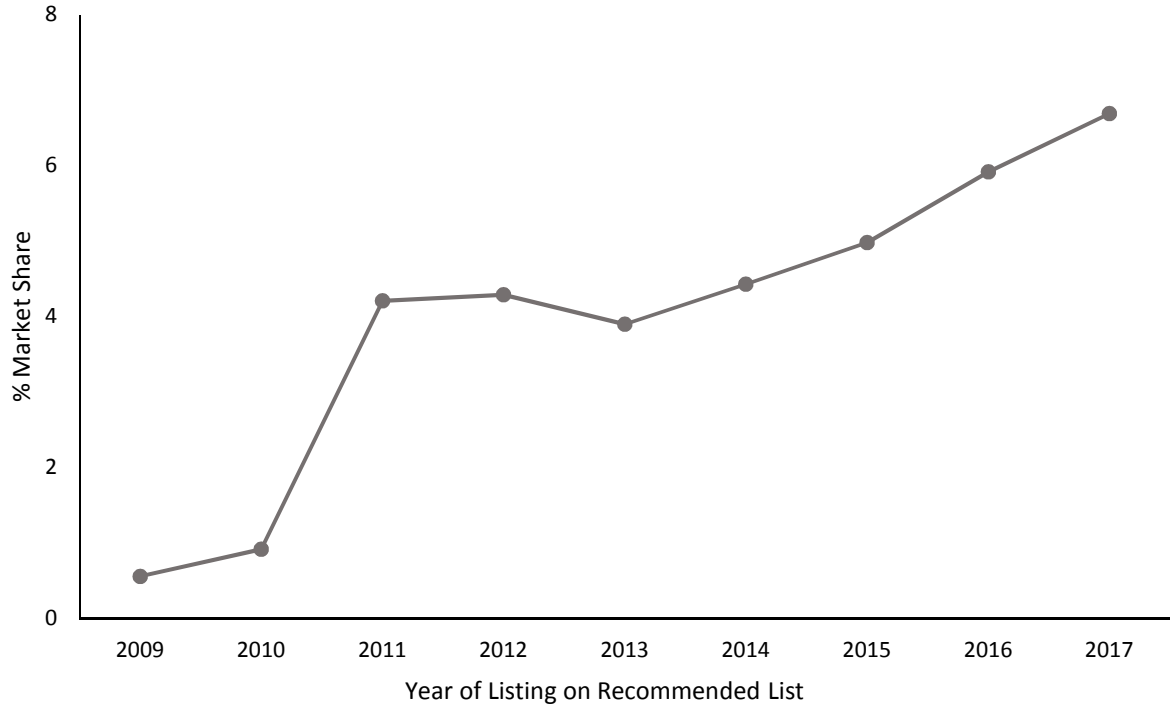


Figure 1 – UK Market share of BCN tolerant varieties since their introduction in 2009 until 2017 (Data courtesy of M. Culloden Pers. Comm., British Sugar, 2017)

2. BCN Biology

2.1 BCN lifecycle.

Cyst nematodes spend their entire life in the soil (as an egg or juvenile inside a cyst, or when migrating from a cyst to the root of a plant) or roots of a host plant, from which they feed. As females complete their life cycle eggs grow and develop inside the body. When the female dies, her body becomes a cyst to protect the progeny within. The cyst dries out and, via the process of cutinisation, becomes tanned. The cyst is then able to protect the eggs until a suitable host crop is grown in the vicinity of the cyst. Host crops release exudates from their roots that stimulate egg hatching from the cyst or the eggs may respond to other stimuli (Turner and Subbotin, 2013; Zheng and Ferris, 1991)

Cyst nematode eggs develop into juvenile nematodes (Fig 2) whilst still inside the cyst. Only when they reach the second juvenile stage (J2) do they hatch out of the egg (Fig 3). Hatching occurs as the nematode uses its stylet, located at the anterior end of its body (Fig 2), to break through the egg shell. The nematode then escapes the cyst by exiting via natural openings, when it is said to have 'emerged'(Sharma and Sharma, 1998). The juveniles move through the soil and are attracted to the host crop roots via chemical exudates from the roots (Fig 3). For this to occur, the soil must be moist to allow for the movement of the nematode, since nematodes are aquatic organisms which move through the thin films of water surrounding soil particles (Neher, 2010). Once the juveniles have migrated through the soil, they break through into the root of a host plant. To do this they use their stylets to cut a slit in the cell walls of the root cells and migrate toward the pericycle (Turner and Subbotin, 2013).

At the pericycle the nematode uses their stylet to break through the cell wall. The nematode produces enzymes, which promote cell wall breakdown and gives rise to a feeding site known as a syncytium (Turner and Subbotin, 2013). The juvenile must be able to maintain the feeding site for a prolonged time to enable its own survival as they are obligate feeders and therefore must obtain all of their nutrition from the host plant. After approximately seven days the juvenile will sexually differentiate, a process driven by abundance of food. Females require 29 times more nutrition than males (Müller et al., 1981) and so if the host is susceptible, and the juvenile has been able to induce the development of a large syncytium which provides ample nutrition, most nematodes develop into females, with a few males usually mating with multiple females. However, should this syncytium not be able to supply enough nutrition for a female to develop the juvenile will usually become a male (Moens et al., 2018). The juveniles then moult into stage three juveniles (J3) and at this moult the genital primordia develop. During the J3 stage the female begins to develop a globular shape due to rapid ovary growth. When the next moult occurs, the nematodes enter the J4 stage. The female ruptures the root cortex and becomes exposed to the soil (Fig 3). Her vulva becomes formed and gives access to the developing reproductive system, ready for fertilisation by male nematodes (Turner and Subbotin, 2013).

Males emerge from the root after a moult from the J4 stage. They cease feeding at this point and become free living in the soil. As a result, they only have a short period in which to reproduce before they run out of sustenance and then die. The males are attracted to the females by sex pheromones released into the rhizosphere. The eggs inside the females become fertilised and develop from eggs to J2 juveniles before the female dies. Her body then undergoes transformation into a cyst as aforementioned (Fig 3). The cyst will eventually

become detached from the host plant and the J2s in the cyst will lie dormant in the soil until a suitable host once again grows in the vicinity or another stimulus causes them to hatch (Turner and Subbotin, 2013; Zheng and Ferris, 1991). If the female is feeding from a very suitable host, and therefore can obtain high levels of nutrition, a gelatinous matrix, often referred to as an egg sac, is often produced outside of the developing cyst (Koenning and Sipes, 1998; Moens et al., 2018). Inside the egg sac eggs are laid which then can hatch very quickly once suitably developed. This allows for rapid recolonization of the host plant with a new generation of BCN. BCN requires a base temperature of 10^{o}C before it will become active and the lifecycle requires a minimum of 300^{o}C days above the base temperature to complete (Dewar and Cooke, 2006). Juveniles within a cyst respond to hatch stimuli. Some juveniles respond to the presence of water and others will respond to root exudates from host plants (Zheng and Ferris, 1991). As a result, populations of BCN decline annually in the absence of a suitable host, reducing approximately 50% each year (Jones, 1956). The optimum temperature for *H. schachtii* development is around 25^{o}C (Smith et al., 2004; Turner and Subbotin, 2013) in addition, BCN is not subject to a diapause (Müller and Rumpenhorst, 2000). As a result, multiple generations can occur in a single cropping year, as long as the appropriate conditions exist. Typically two or three generations complete each year in northern Europe (Dewar and Cooke, 2006) but up to as many as five generations may complete in warmer sugar beet production areas such as California (Roberts and Thomason, 1981). In the future, higher temperatures due to climate change may bring about increased risk from BCN to host crop species, alongside other pest and disease pressures also (Vandenbossche, 2016).

2.2 The syncytium

The establishment and maintenance of the syncytium (plural syncytia) is crucial for survival of the nematode. All nutrition is obtained from this single site, which when fully established becomes a major nutrient sink for the plant.

Syncytial development is both constructive and destructive. Establishment of the syncytium begins when the nematode selects its initial syncytial cell (ISC) and enters the sedentary stage of the lifecycle. A nematode can only induce the formation of one syncytium, so it must be maintained throughout the time the nematode needs to feed from it. Enzymes are secreted into the ISC via the nematode's stylet to manipulate functionality and physiology of the syncytium, through changes in the expression profiles of thousands of genes (Moens et al., 2018). Cell wall degradation occurs and neighbouring protoplasts fuse to increase the syncytium's size. Syncytia may develop to contain what were previously hundreds of individual cells.

Syncytia are unique plant cells and are characterised by many abnormal features such as small vacuoles, multiple enlarged nuclei and cytoplasm which is more dense than a normal plant cell (Kyndt et al., 2013). Female nematodes may extract up to four times the contents of the syncytium in a single day over many feeding cycles. Syncytia are flexible, having the ability to cope with the extreme turgor pressures generated from the high concentration of sugars and other nutrients being manufactured and extracted from the host plant.

Whilst the nematode feeds it is not entirely safe from its host. Plants have various mechanisms to prevent BCN from reaching maturity. Free radicals, such as hydrogen

peroxide, can be produced by the plant in an attempt to kill the nematode (Gheysen and Jones, 2013). If the plant detects the occurrence of the syncytium it can also enact the hypersensitive response to kill the cells off and thus cut off the supply of nutrients to the plant. However, not every species is able to do this and therefore two distinct types of hosts exist (i) susceptible (those unable to stop syncytia development) and (ii) resistant hosts (those able to prevent or reduce syncytia formation or development)(Moens et al., 2018).

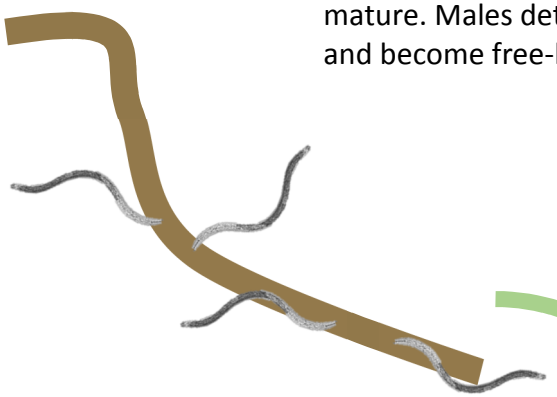


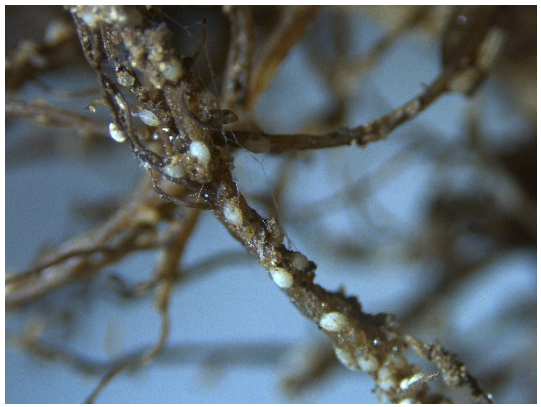
Figure 2 – Second stage *H. schachtii* juvenile

Second stage juveniles (J2s) hatch from eggs and emerge from cyst. They then move through the soil, following chemical cues from roots

J2s invade roots of a host plant and each establish a syncytium. Juveniles feed and go through three moults until they are mature. Males detach from root and become free-living.







Females develop into cysts which contain up to 600 eggs and juveniles.

The cyst will protect the J2s for up to 10 years

(Image shows cyst crushed open to release eggs and juveniles inside)



Females swell, rupture the root and expose their vulval cones to the rhizosphere. Males detect sex pheromones and mate with females.

Figure 3 – Lifecycle of *Heterodera schachtii*, the beet cyst nematode (not to scale)

2.3 Host plants of beet cyst nematode

With cyst nematodes being obligate parasites they must find and infiltrate the roots of a suitable host in order to feed and survive. Host species for BCN are varied and host crops exist in many different plant families (*Amaranthaceae, Caryophyllaceae, Brassicaceae, Fabaceae* & *Polygonaceae*). Host suitability also varies between species and the most suitable species can be found within the families of *Amaranthaceae* sub families *Chenopodiaceae and Betoidae* and also the *Brassicaceae* (Table 1).

In crop production systems, both crop species and weeds can act as hosts for BCN. These host crops can fall into three distinct categories dictated by the suitability of the plant to host the nematode and to establish and maintain its syncytia (Wyss et al., 1984) and how it subsequently yields.

Susceptible hosts

Susceptible hosts include conventional varieties of sugar beet and oilseed rape. Susceptible varieties of these crops do not inhibit the syncytia of BCN, which means that juveniles reach maturity and reproduce, typically resulting in yield losses of 30-40% (Blok et al., 2018).

Resistant hosts

These species/varieties offer control of the nematode by acting as a host but then cause breakdown of the syncytia prior to maturity. Therefore, there is no or very limited negative effect on the host and the nematode does not complete its lifecycle. Populations of the nematodes in the soil will therefore see a net reduction when a resistant variety is grown. Resistance is the counterpart to susceptibility and is not associated with yield. A resistant

plant's significantly reduced ability to support reproduction of BCN must be confirmed versus a known susceptible variety which supports high reproduction levels (Perry and Moens, 2013).

In a susceptible host, the syncytia can be maintained to provide the nematode with sufficient nutrition for the full length of its development within the root. However, in resistant plants the syncytia will degrade a few days after infiltration by the nematode. Females require approximately 29 times more nutrition from the plant in order to develop successfully and therefore their survival is critically related to successful syncytia development (Müller et al., 1981). Wyss et al. (1984) found identical cell changes occurred in both susceptible and resistant varieties of radish when BCN initially infiltrated a root. The nematode is still able to break through the root cells until it finds a suitable host cell. It then initiates syncytia formation within 42 hours of invasion. In a suitable, non-resistant host the syncytia will form and allow the nematode to feed and develop.

In a resistant host the syncytia breaks down (Müller and Rumpenhorst, 2000), usually within a few days. The plant cells surrounding the syncytium die due to the hypersensitive response to the nematode and females in turn starve and die. Males may still be able to obtain sufficient sustenance (due to their lower nutrition requirement) from the failing syncytia but when they enter the free living J4 stage they have no, or very few, females to mate with (Wyss et al., 1984) and populations are reduced.

Tolerant hosts

Some varieties are considered tolerant of nematode attack and are able to produce a yield that is equivalent to a susceptible crop grown in the absence of BCN. However, they do not have any means of preventing reproduction of the nematode. Hence, the yield is unaffected but the nematode population Increases. Tolerance is a trait related to yield and not the reproduction of BCN on the host (Perry and Moens, 2013). A tolerant variety might be able to yield very well in the presence of BCN and may also cause a population build-up greater than a susceptible variety.

2.3.1 Resistant sugar beet

Since the mid-1990s, resistant varieties, which include the BCN resistance gene $HS1^{pro-1}$ from *Patellifolia procumbens* have been bred into sugar beet to reduce BCN populations (Cai 1997, Muller 1999, Stevanto et al. 2015). Whilst these can actively reduce populations, genetic drag has introduced characteristics to the beet which make them less favourable to grow and they do not yield as well as susceptible varieties. Resistant BCN varieties have never been listed for cultivation in the UK but are, or have been, grown in The Netherlands, Germany and France (Blake, 2011; Müller, 1999). These varieties are proven to be resistant to BCN during official variety testing conducted by the German Federal Plant Cultivar Office (Bundessortenamt, 2014)

2.3.2 Tolerant sugar beet

As previously mentioned, varieties tolerant to BCN were introduced to the UK market in 2009 and had been available in European markets a few years before (Blok et al., 2018). These have no reported resistance and the tolerance is declared by the breeders when the varieties are

sent for variety testing and have shown yield maintenance when grown in the presence of BCN, compared to susceptible varieties (Blok et al., 2018). Currently, little is known about the origin of the tolerance and performance when infested, but is believed to originate from *Beta vulgaris* ssp. *maritima*, and genes such as *HsBvm-1* infer quantitative (partial) resistance to BCN to these variety types (Lange and De Bock, 1994). Hauer et al. (2016) found that tolerant varieties did yield better than susceptible varieties but did not suppress population build-up of BCN across a wide range of infested sites in Germany. Findings from other recent studies have also shown reduced population build-up on these varieties, but that these differences are not always consistent between varieties and breeders (Reuther et al., 2017).

To be consistent with commercial terminology used about variety types, throughout this thesis varieties considered to have major gene resistance to BCN will be called resistant varieties. Likewise, varieties which may be partially or quantitatively resistant to BCN, will be referred to as Tolerant to BCN. This is because of their ability to maintain yield in BCN infested sites and reflects the manner in which these varieties are developed and tested.

2.3.3 Other host crop species of BCN

In addition to sugar beet there are many other reported BCN hosts (Table 1). These are important to consider if they are being grown on infested fields in a rotation which also features sugar beet. For example, cultivation of oilseed rape (*Brassica napus*) or high value brassica vegetable crops (*Brassica oleracea*) may be subjected to yield losses and exacerbate BCN populations in fields with infestation and then result in higher BCN population pressures during the cultivation of the next sugar beet crop.

Some species, such as radish (*Raphanus sativus*) and mustard (*Sinapis alba*) which are now becoming popular to grow as a cover crop between two commercial crops may also allow for BCN populations to increase if not managed responsibly, especially if BCN susceptible varieties are chosen for cultivation.

Table 1 – List of known common host plant species of *Heterodera schachtii* adapted from BBRO BCN Technical Guide (2009)

PLANT FAMILY & SPECIES	COMMON NAME	SUSCEPTIBILITY	PLANT FAMILY & SPECIES	COMMON NAME	SUSCEPTIBILITY
Caryophyllaceae			***Brassicaceae***		
Agrostemma githago	Corncockle	+	*Raphanus raphanistrum spp. raphanistrum*	Wild Radish	++
Dianthus caryophyllus	Wild Carnation/Clove Pink	++	*Raphanus sativus*	Fodder Radish	(+)++
Saponaria officinalis	Soapwort/Wild Sweet William	++	*Brassica rapa ssp. chinensis*	Chinese Cabbage	+++
Stellaria media	Common Chickweed	++	*Brassica napus ssp. oleifera*	Oilseed Rape	+++
Amaranthaceae			*Brassica. oleracea ssp.*	All cabbage species	+++
Amaranthus caudatus	Love lies bleeding	++	*Camelina sativa*	Gold of Pleasure	+++
Amaranthus retroflexus	Common Amaranth	++	*Capsella bursa-pastoris*	Shepherd's Purse	+++
Amaranthus hybridus	Green Pigweed	+++	*Lepidium sativum*	Garden Cress	+++
Chenopodium album	Fat Hen	+	*Sinapis alba*	White Mustard	+++
Atriplex patula	Common Orache	++	*Thlaspi arvense*	Field Pennycress	+++
Chenopodium hybridum	Sowbane/Maple-Leaved Goosefoot	++	***Fabaceae***		
Chenopodium quinoa	Quinoa	++	*Trifolium resupinatum*	Reversed Clover	(+)+
Spinacia oleracea	Spinach	++	*Lathyrus sativus*	Indian Pea	++
Atriplex hortensis	Garden Orache	+++	*Vicia villosa*	Fodder Vetch	++
Blitum bonus-henricus	Good King Henry	+++	***Polygonaceae***		
Beta vulgaris ssp. vulgaris	Garden Beetroot	++	*Fagopyrum esculentum*	Buckwheat	+
Beta vulgaris ssp. vulgaris	Sugar Beet	+++	*Fagopyrum tataricum*	Green Buckwheat	+
Beta vulgaris ssp. vulgaris	Fodder Beet	+++	*Persicaria lapathifolia*	Pale Persicaria	++
Beta vulgaris ssp. cicla	Chard	+++	*Rumex acetosella*	Sheep's Sorrel	++

* Number of + denotes susceptibility with most susceptible species listed with +++ and least susceptible +

2.4 BCN hatch responses

It is imperative that BCN juveniles find a suitable host, otherwise the juvenile cannot establish a syncytium and complete its lifecycle, ultimately producing a new generation. Cysts might lay dormant and dehydrated as this ensures survival and also allows for easy distribution of the cysts to a new area of soil (Perry and Curtis, 2013). Alternatively, juveniles may readily hatch and infect the same plant as their parents and complete another generation in the same year (Cooke, 1993; Müller and Rumpenhorst, 2000).

Whereas some species of cyst nematode, such as the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*, mainly respond to hatching factors released into the rhizosphere from potato root exudates (Perry, 2002), *Heterodera schachtii* has evolved a range of hatching responses to maximise the chance of BCN juveniles successfully reproducing. Zheng and Ferris (1991) found four distinct types of juvenile hatch response:

- Host mediated obligate quiescence where BCN encysted eggs need cues from a host plant to affect their development (Turner and Subbotin, 2013). This also allows for BCN to remain viable in the soil until a host is available, therefore promoting *H. schachtii* persistence in the soil (Perry, 2002).
- Host mediated diapause where the development of BCN encysted eggs is arrested until specific requirements are met. This allows unfavourable environmental conditions to be avoided (Turner and Subbotin, 2013).
- Time mediated diapause where encysted eggs have their development arrested for a certain amount of time (Turner and Subbotin, 2013) and the passage of time is the only factor to influence hatch (Zheng and Ferris, 1991).

- Non-dormant eggs that hatch rapidly in water which then allow for rapid re-infestation of a suitable host (Zheng and Ferris, 1991).

A BCN cyst will contain juveniles with all states of hatch response, ensuring that some of the progeny will successfully complete a life cycle in the coming 5 to 10 years so long as a host species grows in the vicinity of the cyst.

2.5 Diagnosis of beet cyst nematode infestations

Beet cyst nematode is easiest to diagnose when a crop of sugar beet is being grown in an infested field. The crop will display above ground symptoms of infestation (See section 1.3) whilst some of the plants can be uprooted to inspect the lateral roots. The nematodes are easiest to spot when they have freshly emerged from the root cortex; immature females appear white and will still be attached to the roots at this point (Figure 2). In the UK, they are likely to be present on the roots of infested sugar beet crops between late May and September.

Soil sampling is commonly used to determine if a field is infested. Cores of soil are usually taken on a grid pattern across a set area of land. In the UK, British Sugar protocols for field sampling for BCN state that 100 cores are taken from a 4 Ha area of a field (Cogman and Morris, 2009). Cores are usually taken using a 'cheese-sampler' type corer (Southey, 1986) down to a set depth of soil, often between 15 and 30 cm. From this bulk sample a sub sample is obtained after thorough mixing of the soil. This sub sample is then processed to extract the cysts from the soil. Various methods exist with which to do this, such as the Fenwick Can (Fenwick, 1940), Trudgill Tower (Trudgill et al., 1972), Wye Washer (Winfield et al., 1987) or

centrifuge techniques (IRS, Pers. Comm. 2015). All of these methods separate the organic fraction of the soil, which contains the cysts, from the mineral part. The organic fraction which is recovered is then termed 'the float'. From 'the float' the cysts are then counted under a stereomicroscope and the float must be kept moist to prevent death of the eggs and juveniles inside the cysts since *Heterodera* cysts cannot tolerate drying (Shepard, 1970).

The presence of *Heterodera* can be confirmed by the characteristic lemon shaped cysts, but species determination requires taking measurements of juveniles or mounting the vulval cone of the cyst for assessment of the perineal region (Hooper, 1986). More recently PCR based techniques have been developed to confirm the species and may in the future offer more rapid identification of infestations (Amiri et al., 2002; Gamel et al., 2017).

3. Sugar beet infestation by beet cyst nematode

Typical symptoms of sugar beet infested with BCN are root bearding (Müller, 1999) and retarded growth (Schmitz et al., 2006). Root bearding is caused by excessive lateral root proliferation, adjacent to the syncytium, initiated by the nematode attacking the plant. Once established, the syncytium halts the meristematic activity of the root tip and the elongation zone of the root is also shortened. The result of this is that root hairs will grow directly at the root tip (Müller, 1999) (Fig 4). The tap root of the plant will also often stop growing and instead the plant starts producing multiple side roots resulting in a fanged appearance.



Figure 4 – Image of two sugar beet plants grown in infested fields. The plant on the left is showing typical BCN infestation symptoms such as the excessive proliferation of lateral roots and the smaller storage root. The plant on the right shows more typical growth of a sugar beet plant.

These symptoms make the beet very small and difficult to harvest and the soil tare (the amount of soil retained by the root after harvest) increases as soil is retained by the roots. The root system is also inefficient and unable to support normal growth. The nematodes remove nutrients that the plant would otherwise be using to grow and develop and also the bearded root system cannot provide enough water for the leaves to transpire sufficiently and therefore cannot photosynthesize effectively.

A glasshouse study on *Heterodera glycines* (soybean cyst nematode) found a reduction in photosynthesis and chlorophyll content in soybean, *Glycine max*. The soybean suffered from the ill effects, even at low levels of infestation (Asmus and Ferraz, 2002). As *H. glycines* and *H. schachtii* are closely related species (Radice et al., 1988) it is possible that photosynthesis and chlorophyll content of sugar beet will be reduced during BCN infestation. A reduction in carbon dioxide assimilation in the leaves of infested sugar beet (Schmitz et al., 2006) supports this assumption. Using this finding Schmitz et al. (2006) found that they could detect (in laboratory conditions) the early onset of infestation, prior to visible symptoms appearing, using chlorophyll fluorescence.

Infestation with BCN also limits the supply of nitrogen to the plant, which reduces growth of the crop. Infestation can be spotted due to the nitrogen deficiency as the colour of older leaves prematurely change from green to yellow (Jaggard and Qi, 2006) and also suffer from visible wilting (Cooke, 1987). Petiole extension and reduced leaf size are also common symptoms of BCN infestation (Cooke, 1987). The reduction of the uptake of nitrogen, which can exceed over 5kg per hectare per day in a healthy crop (Armstrong et al., 1986), explains how BCN can impact the final crop yield in the UK by up to 37% on mineral soils (Cooke, 1984), but less so on organic soils (Cooke, 1991). However, newer varieties of beet have been introduced and losses are now often reported to be much higher at 60% on susceptible varieties (BBRO, 2009) and an up to date, accurate quantification of the actual level of loss due to BCN would be useful to assess the threat to the crop in the UK.

It is also important that BCN is identified correctly as the plant symptoms could be confused with other diseases and pests of sugar beet. This is particularly true in relation to rhizomania

caused by *Beet necrotic yellow vein virus* (transmitted by the soil borne plasmodiophorid protozoa *Polymyxa betae*) (Stevens et al., 2006). Since 2013 all sugar beet varieties grown in the UK, including BCN tolerant varieties, have been partially resistant to the common strains of rhizomania due to introgression of the 'Holly' gene (Kerr, 2012; Stevens et al., 2006). This has reduced the incidence of rhizomania almost completely and consequently the chances of confusing rhizomania and BCN infestations. However, the symptoms of both conditions should be well understood in case a crop becomes infested with either BCN or the AYPR strain of rhizomania, which is able to overcome the holly gene resistance.

4. Control of beet cyst nematode

Due to the persistence of nematode juveniles within the cysts in the soil, BCN can be difficult to control. Whilst there are many options which may be useful, few produce reliable and cost-effective means of control. Some options, such as the use of nematicides, are no longer available for BCN control in Europe and are therefore not discussed.

4.1 Crop rotation

Encysted eggs containing BCN J2s may survive for over ten years in the soil (Jones, 1956; Moriarty, 1966). However, the majority will hatch in the first five years. Every year, due to the hatch responses (See 1.2.4) approximately 50% of the remaining contents in a cyst will hatch. This means that after five years a cyst, which initially contained 300 juveniles will contain fewer than 10 juveniles. Five years without a host grown in the field is usually sufficient to hold populations at non damaging levels (Koch and Gray, 1997). The government control orders from 1944 – 1977 enforced a five year break between hosts (Cotten et al., 1992).

Unfortunately, due to the modern nature of beet production this is not always viable (Müller, 1999) and other control options are desired.

4.2 Sugar beet variety selection

Prior to the development of resistant and tolerant sugar beet varieties, all were susceptible to BCN and suffered yield damage as a result. As previously mentioned (see 1.2.3) resistant and tolerant beet varieties now exist. Resistant varieties have not ever been used in the UK commercially, although they have been trialled (Blake, 2011). However, in Europe they are used, although not widely, to control BCN. More widely favoured by growers in the UK and Europe are tolerant varieties due to their greater yield potentials. This is unfortunate because resistance is an active form of control and the syncytium is destroyed by the hypersensitive response but resistant varieties, with lower yield potentials cannot be justified for widespread cultivation (Märländer et al., 2003). They should be considered as part of an integrated pest management (IPM) strategy though, and may still be useful for growers with very high BCN populations or those who wish to grow other host species in their rotations.

As of the 2019 BBRO recommended list (BBRO, 2018), tolerant varieties yield as highly as the susceptible varieties in field trials. However, evidence to suggest that tolerant varieties can control populations of BCN has not been found. Instead, they have been found to build up populations less severely than susceptible varieties (Hauer et al., 2016; Reuther et al., 2017), and therefore allow fields infested to be planted with sugar beet and yield successfully. Therefore, tolerant varieties should be considered as a management option for BCN rather than a method of control.

4.3 Brassica intercrops

Certain brassica species, namely white mustard and radish, have been developed for the management of BCN. Some brassica species are used as a nematode trap crop and others have been selected for their high concentrations of glucosinolates, which can be used in a process known as biofumigation.

4.3.1 Trap cropping

Trap crops are non-commercial intercrops that are planted to stimulate juvenile hatch and have been researched since the late 19th century (Müller, 1999). A typical example is oilseed rape (*Brassica napus*) where volunteers left in the field after harvest can be used to reduce populations of BCN. At the time when this technique was initially hypothesised, it was not considered to be a practical method of control, but with development of technology an online decision making tool is now available to advise German growers when to destroy oilseed rape volunteers to ensure BCN control (LIZ, 2016)

It is also important to consider how a trap crop fits into an arable rotation. Cooke (1984) defined three criteria that a commercially viable trap crop must meet:

1. Egg hatch must be stimulated
2. The nematode should not be able to reproduce (or there should be a very low level of reproduction)
3. The crop must be a sensible addition to the cropping system and also economically viable.

BCN resistant brassicas, used to lower BCN populations, have been available to use in Europe for over 20 years (Müller, 1999) and are typically varieties of mustard (*Sinapis alba*) and radish (*Raphanus sativus*). German farmers possess recommended lists for different trap crops (Bundessortenamt, 2014) and the varieties are tested for their resistance against BCN in a bioassay prior to being listed. The bioassay is conducted under controlled conditions using plants grown in pots and a known number of hatched juveniles are applied to the plants. The final population is then derived from counting the number of cysts formed on the roots and the average number of eggs and juveniles in each cyst. The resistance of each variety is then calculated as a reproductive factor (*Rf*) as follows:

$$Reproductive\ factor\ (Rf) = \frac{Final\ population\ (Pf)}{Initial\ population\ (Pi)}$$

The initial population (*Pi*) is usually a known number of juveniles used to inoculate plants in the bioassay and the final population (*Pf*) is derived from counts of the number of cysts that develop and the average number of juveniles per cyst. Trap crops with an *Rf* of ≤0.1 thus denote a reduction in populations by 90% and varieties reaching this level are awarded Class 1 resistance. Any varieties reaching an *Rf* of 0.1-0.3 show a population reduction of 70-90% and are given Class 2 resistance designation (Bundessortenamt, 2014).

Resistant brassicaceous trap crops can be planted prior to sugar beet, typically after the harvest of a cereal, and grown from late summer into the winter. They do not require as careful management as a susceptible trap crop as their resistance limits BCN reproduction for as long as the soil temperature remains above 10°C, which is when egg hatch ceases (Dewar and Cooke, 2006).

Investigations using these trap crops have produced mixed results in past studies around the world. Research in the USA has produced mixed results, with some results showing decreases in BCN populations following the cultivation of trap crops and also subsequent yield improvement of sugar beet crops (Hafez, 1994), and similar findings have also been made in Germany (Müller, 1999). However, other work in the USA (Koch and Gray, 1997) produced mixed results with beneficial population reductions being found at only one site out of four. Recent findings from Germany however have been beneficial with Hauer et al. (2016) finding resistant mustard to lower BCN populations at five out of eight sites investigated and Kenter et al. (2014) finding resistant radish to lower populations of the nematode at twelve out of thirteen sites investigated; best results were obtained when the cover crop was sown in July rather than August. Finally, recently in Iran, mixed responses from trap crops were obtained with population reductions only being found at one site out of two trialed (Hemayati et al., 2017).

Whilst some studies have shown that brassica trap crops have promise, the results lack consistency and are discussed in further detail in Chapter 4. Despite these varied results, trap crops are widely used on over 40% of the sugar beet growing area in Germany (Hauer et al., 2016) and this could be for other benefits, such as prevention of soil erosion and nutrient leaching, which could be obtained from growing a cover crop, rather than solely for BCN control.

4.3.2 Biofumigation

Biofumigation typically utilises brassica intercrops, such as Indian mustard (*Brassica juncea*) and oil radish (*Raphanus sativus*), which produce glucosinolates (GSL) in their roots and

leaves (Back et al., 2018). Glucosinolates are a naturally occurring secondary metabolite found almost exclusively from *Brassicaceaeous* plant species (Matthäus and Luftmann, 2000) and over 130 different GSLs have now been found (Agerbirk and Olsen, 2012). Brassica intercrops are macerated when their GSL levels are greatest, usually at flowering (Taylor et al., 2014), and buried into the soil. The GSLs are released from the damaged cells and converted to toxic isothiocyanates (ITCs) and other volatile compounds by the enzyme myrosinase, which is found in separate cells (myrosin cells) within the leaves (Back et al., 2018).

To be effective, the water content, temperature and pH of the soil need to be optimum and the growth stage of the plant sufficient to provide sufficient GSL levels, and enable hydrolysis of the GSLs into ITCs to occur (Back et al., 2018). Ngala et al., (2014) demonstrated that a GSL concentration of 2.0 mol m^{-2} can provide 70% control of *Globodera pallida* in field plots. The process of macerating and incorporating the material is also costly and time consuming, as ideally the maceration, incorporation and sealing of the soil should be completed in one pass of a tractor (Back et al., 2018) to reduce the volatilisation and loss of ITCs to the atmosphere. The high costs associated with, seed, labour and fuel may be justifiable in a potato crop, however, it may not be economically sound to use biofumigation for BCN control, especially as there is limited evidence of biofumigation being effective against BCN.

Other biofumigation methods exist, such as partial biofumigation where the shoot material of the crop is not incorporated (Ngala et al., 2014) or instead applying preserved brassica seed meals (Morra and Borek, 2010). Partial biofumigation, caused by leaching of biofumigant compounds from roots of biofumigant species (Back et al., 2018) has been found to work on

potato cyst nematode, *Globodera pallida* and reduce the final population levels (Barker et al., 2014) by reducing the viability of encysted eggs (Lord et al., 2011; Ngala et al., 2014). Seed meals are produced as a by-product following oil extraction from seeds of crops such as oilseed rape (*B. napus*) and mustard (*B. juncea* and *Sinapis alba*) (Snyder et al., 2009) and are highly concentrated sources of GSLs. Seed meals can be applied over a specific area of a field infested with a pest or spread over and incorporated into an entire infested field. When water is applied, ITCs are released and fumigate the soil. Seed meals have been found to work against cockchafer larvae (*Melolontha melolontha* & *M. hippocastani*) due to the ITC release (Sukovata et al., 2014) and the findings of Lazzeri et al., (1993) indicate that *H. schachtii* J2s can be controlled using ITCs. Other nematode species have also been shown to be controlled through the application of seed meals such as *Meloidogyne incognita* (Curto et al., 2016), *Pratylencus penetrans* (Mazzola and Brown, 2010) and *Globodera pallida* (Dandurand et al., 2017).

4.4 Biological control

Fungal parasites, such as *Acremonium strictum* and *Fusarium oxysporum* have been recorded as fungal parasites of BCN in California (Nigh et al., 1980), however these were only able to infect eggs prior to their development into J2s and thus offer limited time for colonization of the fungus and therefore any control. Other fungi, such as *Datylella oviparasitica*, have been found to naturally occur in suppressive soils (Gao and Becker, 2002). When microplots were then inoculated to mimic these conditions *D. oviparasitica* was able to suppress BCN populations throughout numerous generations (Olatinwo et al., 2006) by over 90% versus a non-suppressive control soil. This investigation used fumigated soil and did not evaluate the possibilities of amending non-fumigated soils with fungi.

Crump and Kerry (1987) found *Cylindrocarpon destructans* was a frequently occurring fungal pathogen of *H. schachtii* in the UK. This fungal pathogen infects immature females and prevents them developing into mature cysts. The work of Dackman et al. (1989) complements this finding with *C. destructans* not found to infect J2s. Both *C. destructans and A. strictum* colonise the roots and then infect the females as they emerge through the root cortex (Crump and Kerry, 1987; Nigh et al., 1980).

The complex interactions between plants, soil and fungi make studying this area and implementing commercial fungal inoculations difficult, whilst the implementation of a wide rotation prevents natural suppressive soils to develop. Therefore, whilst fungal pathogens may be useful in BCN control, they cannot be relied upon to provide consistent and effective control.

Pasteuria nishizawae is a bacterium which is parasitic against cyst nematodes and is marketed as a form of biological nematicide, under the trade name of CLARIVA™ for use against the soybean cyst nematode (SCN), *H. glycines* in North America (Back et al., 2018). Spores of *P. nishizawae* are applied to the seeds, which spread to the soil surrounding the plants. Spores then colonise J2s when they move through the soil and the bacterium reduces the fecundity of a female nematode, but does not prevent her from reproducing. CLARIVA™ may show promise for use against BCN in sugar beet crops (Schlatter, 2015) although BCN has not been confirmed as a host of *P. nishizawae* (Noel et al., 2005). Currently, CLARIVA™ has not yet been licensed for use in the European Union (Maria et al., 2018), but is used in the USA, Canada and Brazil for BCN & SCN control (Syngenta, 2018).

4.5 Weed control

Where long rotations exist, BCN populations can still be harboured on weed species. As listed in Table 1, the number of common species which host BCN include many weeds such as fat hen (*Chenopodium album*), field penny cress (*Thlaspi arvense*) and green pigweed (*Amaranthus hybridus*) amongst others.

Meinecke and Westphal (2014) conducted studies on a wide range of commercial crop species and common weeds, which are reported hosts of BCN in Germany both under controlled conditions and in the field. They found that very few of the species are suitable enough hosts to cause major population increases, namely *T. arvense* (field penny cress), and would require field populations to establish at high levels to justify weed management for BCN control. It is likely that similar conditions would be needed in the UK to justify weed management. However, growers should still be aware of which weeds may act as hosts.

5. BCN economics

Quantifying the economic losses caused by *H. schachtii* around the world is difficult. This is due to the different species it feeds from and how the amount of yield loss can vary year to year due to the climate and weather experienced by a crop, as well as the soil type and any resistance or tolerance exhibited by the host. Müller (1999) put the value of annual losses within Europe in the region of €90 million. This would be over €125 million per annum when adjusted for inflation in 2018. However, there is currently no worldwide figure available and in fact losses may now be far lower than this due to the successful adoption of the BCN tolerant varieties of sugar beet (Blok et al., 2018).

Within UK sugar beet production, with an estimated 6% of land infested with *H. schachtii* and losses from infestation in the region of 30%, an annual yield loss of over 150,000 tonnes of beet can be expected, with a value of over £3.8 million[1]. However, when considering the losses that will occur to brassica crops and the costs of controlling the nematode the actual economic cost may be even greater.

The amount of production lost emphasises the importance of conducting future research to maximise profitable production of sugar beet and maintain production of sugar beet on land infested with BCN.

6. Conclusion

The beet cyst nematode has been a problem for almost as long as sugar beet has been a commercialised crop and until recently, control options were limited. The tightening of rotations has the potential to make the situation worse. Now, as more modern breeding techniques become available, new forms of control are needed and are being developed to tackle this global problem. A resurgence in BCN research in recent years reinforces the need for such information to tailor any management approaches to UK conditions where our climate and varieties differ from those in continental Europe. Therefore, control options used in Europe need to be tested under UK conditions to confirm their operation in the field in the UK and against British populations of BCN.

[1] Total UK sugar beet area 116,000 Ha of which 6% is 6960 Ha. Mean Yield of 81.3 Tonnes per hectare. Yield loss is assumed to be 30%. Total losses therefore equal 169,754 tonnes and the value of this is £3,819,474 (at £22.50 per tonne).

7. Hypotheses

Following this review of the literature, the following hypotheses were devised to understand better the interactions between beet cyst nematode, sugar beet and brassica trap crops.

I. Tolerant varieties of sugar beet will exhibit distinctly different physiological characteristics to susceptible varieties, which explain how they can maintain yields in the presence of BCN

II. Lower yield potentials of BCN resistant sugar beet varieties can be explained by their physiological traits

III. Tolerant varieties of sugar beet allow similar levels of BCN multiplication to susceptible varieties

IV. Brassica trap crops can be used to reduce in field populations of BCN and hence provide a yield benefit to the following crop

V. Different varieties of sugar beet, radish and mustard stimulate different hatch responses of *H. schachtii*

8. Thesis overview

This thesis focuses on research in four areas. Each part was written up as an academic paper submitted to a relevant journal. Papers which have been accepted for publication are included in their published format, the remaining papers are included in their form submitted for review.

Chapter 2 – 'Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, *Heterodera schachtii* (Schmidt) under uninfested conditions'. Published in the European Journal of Agronomy [Accepted 17 May 2018].

This paper describes experiments conducted to understand more about the differences which exist between the susceptible, tolerant and resistant varieties and specifically looks at changing plant population densities to overcome less vigorous canopy development identified in BCN resistant varieties.

Chapter 3 – 'Testing varieties of sugar beet for resistance and tolerance to the beet cyst nematode, *Heterodera schachtii* (Schmidt)'. Submitted to Annals of Applied Biology

This paper explains the development of a new technique developed to test sugar beet varieties for resistance and tolerance to BCN under uniform infestation using BCN J2s which were used to inoculate plants grown in 600 litre boxes filled with top soil. Physiological responses, such as chlorophyll density and transpiration, to infestation were then measured as the plants grew and help explain how the tolerant varieties may overcome infestation with BCN.

Chapter 4 – 'Evaluating resistant brassica trap crops to manage *Heterodera schachtii* (Schmidt) infestations in Eastern England'. Published in Pest Management Science. [Accepted 5 July 2018].

This paper describes experiments conducted using brassica trap crops in infested fields in Norfolk during 2016 and 2017 and the subsequent population reductions seen by some of the varieties tested.

Chapter 5 – 'Hatching dynamics of the beet cyst nematode, *Heterodera schachtii* (Schmidt), following exposure to root leachates from cultivars of sugar beet, white mustard and radish'. Submitted to Nematology

This paper presents results from a series of experiments using root exudates from a range of sugar beet, radish and mustard varieties to measure different responses in terms of juvenile hatch and emergence from cysts.

To conclude, the results of all of the work conducted are summarised in a general discussion in Chapter 6.

Since the experimental chapters were each submitted for publication, references can be found at the end of each chapter.

9. References:

Agerbirk, N., Olsen, C.E., 2012. Glucosinolate structures in evolution. Phytochemistry 77, 16–45. https://doi.org/10.1016/j.phytochem.2012.02.005

Amiri, S., Subbotin, S.A., Moens, M., 2002. Identification of the beet cyst nematode Heterodera schachtii by PCR. Eur. J. Plant Pathol. 108, 497–506. https://doi.org/10.1023/A:1019974101225

Armstrong, M.J., Milford, G.F.J., Pocock, T.O., Last, P.J., Day, W., 1986. The dynamics of nitrogen uptake and its remobilization during the growth of sugar beet. J. Agric. Sci. 107, 145–154. https://doi.org/doi:10.1017/S0021859600066892

Asmus, G.L., Ferraz, L.C.C.B., 2002. Effect of population densities of Heterodera glycines race 3 on leaf area, photosynthesis and yield of soybean. Fitopatol. Bras. 27, 273–278.

Back, M.A., Cortada, L., Grove, I.G., Taylor, V., 2018. Field Management and Control Strategies, in: Perry, R.N., Moens, M., Jones, J.T. (Eds.), Cyst Nematodes. CABI, Wallingford, Oxfordshire, UK, pp. 305–336.

Barker, A., Roberts, A., Barker, H., 2014. The on-going commercialisation of biofumigants in UK agriculture, in: Back, M.A., Clarkson, J., Lazzeri, L., Michel, V., Reade, J. (Eds.), 5th International Symposium of Biofumigation. Association of Applied Biologists, Harper Adams University.

BBRO, 2018. Recommended list of sugar beet varieties 2019.

BBRO, 2009. Beet Cyst Nematode Technical Guide.

Blake, A., 2011. Drought highlights hidden nematode threat. Crop Prod. Mag. 56–57.

Blok, V.C., Tylka, G.L., Smiley, R.W., de Jong, W.S., Daub, M., 2018. Resistance Breeding, in: Moens, M., Perry, R.N., Jones, J.T. (Eds.), Cyst Nematodes. CAB International, Wallingford, Oxfordshire, UK, pp. 174–214.

British Sugar, 2018. What we do [WWW Document]. URL https://www.britishsugar.co.uk/about-us/what-we-do/ (accessed 5.9.18).

Bundessortenamt, 2014. Beschreibende Sortenliste: Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben & Zwischenfrüchte.

Cogman, R., Morris, N., 2009. Beet Cyst Nematode - Living with the enemy. Br. Sugar Beet Rev. 77, 10–12.

Cooke, D.A., 1993. Nematode Parasites of Sugar Beet, in: Evans Trudgill, D L, Webster, J M, K. (Ed.), Plant Parasitic Nematoses in Temperate Agriculture. CAB International, Cambridge, pp. 133–170.

Cooke, D.A., 1991. The effect of beet cyst nematode, Heterodera schachtii, on the yield of sugar beet in organic soils. Ann. Appl. Biol. 118, 153–160. https://doi.org/10.1111/j.1744-7348.1991.tb06093.x

Cooke, D.A., 1987. Beet Cyst Nematode (Heterodera Schachtii Schmidt) and its Control on Sugar Beet. Agric. Zool. Rev. 2, 135–183.

Cooke, D.A., 1984. The relationship between numbers of Heterodera schachtii and sugar beet yields on a mineral soil, 1978-81. Ann. Appl. Biol. 104, 121–129. https://doi.org/10.1111/j.1744-7348.1984.tb05594.x

Cotten, J., Cooke, D.A., Darlington, P., Hancock, M., 1992. Surveys of Beet Cyst Nematode (Heterodera-Schachtii-Schmidt) in England. Ann. Appl. Biol. 120, 95–103.

Crump, D.H., Kerry, B.R., 1987. Studies on the population dynamics and fungal parasitism of Heterodera schachtii in soil from a sugar-beet monoculture. Crop Prot. 6, 49–55. https://doi.org/http://dx.doi.org/10.1016/0261-2194(87)90028-7

Curto, G., Dallavalle, E., Matteo, R., Lazzeri, L., 2016. Biofumigant effect of new defatted seed meals against the southern root-knot nematode, Meloidogyne incognita. Ann.

Appl. Biol. https://doi.org/10.1111/aab.12275

Dackman, C., Chet, I., Nordbringhertz, B., 1989. Fungal parasitism of the cyst nematode Heterodera schachtii - Infection and enzymatic activity. Fems Microbiol. Ecol. 62, 201–208. https://doi.org/10.1111/j.1574-6968.1989.tb03694.x

Dandurand, L.-M., Morra, M.J., Zasada, I.A., Phillips, W.S., Popova, I., Harder, C., 2017. Control of Globodera spp. Using Brassica juncea Seed Meal and Seed Meal Extract. J. Nematol. 49, 437–445.

Dewar, A.M., Cooke, D.A., 2006. Pests, in: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing Limited, Oxford, pp. 316–350.

Draycott, A.P., 2006. Introduction, in: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing, Oxford, UK, pp. 1–8.

Evans, K., Rowe, J.A., 1998. Distribution and economic importance, in: Sharma, S.B. (Ed.), The Cyst Nematodes. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1–30.

FAO, 2009. Agribusiness Handbook - Sugar beet - White Sugar. Rome, Italy.

FAOSTAT, 2014. Production - Crops (UK sugar beet).

Fenwick, D.W., 1940. Methods for the Recovery and Counting of Cysts of Heterodera schachtii from Soil. J. Helminthol. 18, 155. https://doi.org/10.1017/S0022149X00031485

Gamel, S., Letort, A., Fouville, D., Folcher, L., Grenier, E., 2017. Development and validation of real-time PCR assays based on novel molecular markers for the simultaneous detection and identification of Globodera pallida, G. rostochiensis and Heterodera schachtii. Nematology 19, 789–804. https://doi.org/10.1163/15685411-00003086

Gao, X., Becker, J.O., 2002. Population development of both sexes of Heterodera schachtii is

diminished in a beet cyst nematode-suppressive soil. Biol. Control 25, 187–194. https://doi.org/10.1016/s1049-9644(02)00061-0

Gracianne, C., Petit, E.J., Arnaud, J.F., Porte, CatherineSpatial distribution and basic ecology of Heterodera schachtii and H. betae wild populations developing on sea beet, B. vulgaris ssp. maritima, Renault, L., Fouville, D., Rouaux, C., Fournet, S., 2014. Spatial distribution and basic ecology of Heterodera schachtii and H. betae wild populations developing on sea beet, Beta vulgaris ssp. maritima. Nematology 16, 797–805. https://doi.org/10.1163/15685411-00002809

Hafez, S.L., 1994. The use of green manure crops in a sugarbeet rotation for sugarbeet cyst nematode management. J. Nematol. 26, 548 (Abstr.).

Handoo, Z.H., Subbotin, S.A., 2018. Taxonomy, Identification and Principal Species, in: Perry, R.N., Moens, M., Jones, J.T. (Eds.), Cyst Nematodes. CABI, Wallingford, Oxfordshire, UK, pp. 365–398.

Hauer, M., Koch, H.J., Krüssel, S., Mittler, S., Märländer, B., 2016. Integrated control of Heterodera schachtii Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. Appl. Soil Ecol. 99, 62–69. https://doi.org/10.1016/j.apsoil.2015.11.017

Hemayati, S.S., Jahad-e Akbar, M.-R., Ghaemi, A.-R., Fasahat, P., 2017. Efficiency of white mustard and oilseed radish trap plants against sugar beet cyst nematode. Appl. Soil Ecol. 119, 192–196. https://doi.org/10.1016/J.APSOIL.2017.06.017

Hooper, D.J., 1986. Handling, fixing, staining and mounting nematodes, in: Laboratory Methods for Work with Plant and Soil Nematodes. HM Government, London, United Kingdom, pp. 59–80.

Jaggard, K.W., Qi, A., 2006. Agronomy, in: Sugar Beet. Blackwell Publishing Ltd, pp. 134–168.

https://doi.org/10.1002/9780470751114.ch7

Jones, F.G.W., 1956. Soil populations of Beet eelworm (Heterodera schachtii) in relation to cropping. Ann. Appl. Biol. 44, 25–56. https://doi.org/10.1111/j.1744-7348.1956.tb06845.x

Kenter, C., Lukashyk, P., Lukashyk, P., Daub, M., Ladewig, E., 2014. Population dynamics of heterodera schachtii schm. and yield response of susceptible and resistant sugar beet (beta vulgaris L.) after cultivation of susceptible and resistant oilseed radish (raphanus sativus L.) | Populationsdynamik von heterodera schacht. J. Cultiv. Plants 66, 289–299. https://doi.org/10.5073/JFK.2014.09.01

Kerr, S., 2012. Sugar beet varieties for 2013. Br. Sugar Beet Rev. 80, 14–18.

Kerr, S., 2009. Sugar Beet varieties for 2010. Br. Sugar Beet Rev. 77, 10–14.

Koch, D.W., Gray, F.A., 1997. Nematode-resistant oil radish for Heterodera schachtii control in sugar beet-barley rotations. J. Sugar Beet Res. 34, 31–43.

Koenning, S.R., Sipes, B.S., 1998. Biology, in: Sharma, S.B. (Ed.), The Cyst Nematodes. Chapman & Hall, London, pp. 156–190.

Lange, W., De Bock, T.S.M., 1994. Pre-breeding for Nematode Resistance in Beet. J. Sugar Beet Res. 31, 13–26.

Lazzeri, L., Tacconi, R., Palmieri, S., 1993. In Vitro Activity of Some Glucosinolates and Their Reaction Products toward a Population of the Nematode Heterodera schachtii. J. Agric. Food Chem. 41, 825–829. https://doi.org/10.1021/jf00029a028

LIZ, 2016. LIZ - Ausfallraps-Manager [WWW Document]. URL http://raps.rheinmedia.de/raps/index.php?region=17&datum=2017-07-08&enddatum=2017-10-12 (accessed 5.9.18).

Lord, J.S., Lazzeri, L., Atkinson, H.J., Urwin, P.E., 2011. Biofumigation for Control of Pale

Potato Cyst Nematodes: Activity of Brassica Leaf Extracts and Green Manures on Globodera pallida in Vitro and in Soil. J. Agric. Food Chem. 59, 7882–7890. https://doi.org/10.1021/jf200925k

Maria, A., Domenica, A., Stefania, B., Giulia, B., Alba, B., Daniela, B., Laszlo, B., Harry, B., Arianna, C., Daniele, C.M., Federica, C., Chloe, D.L., Mark, E., Zoltan, E., Gabriella, F., Lucien, F., Marina, G., Luna, G., Alessio, I., Frederique, I., Samira, J., Dimitra, K., Renata, L., Christopher, L., Oriol, M.J., Paula, M., Ileana, M., Tunde, M., Alexandre, N., Laura, P., Manuel, P.M.J., Ragnor, P., Hermine, R., Angela, S., Miguel, S., Rositsa, S., Rachel, S., Alois, S., Franz, S., Juergen, S., Csaba, S., Jose, T., Andrea, T., Anne, T., Benedicte, V., Alessia, V., Laura, V., 2018. Peer review of the pesticide risk assessment of the active substance Pasteuria nishizawae Pn1. EFSA J. 16, e05159. https://doi.org/10.2903/j.efsa.2018.5159

Märländer, B., Hoffmann, C., Koch, H.J., Ladewig, E., Merkes, R., Petersen, J., Stockfisch, N., 2003. Environmental situation and yield performance of the sugar beet crop in Germany: Heading for sustainable development. J. Agron. Crop Sci. https://doi.org/10.1046/j.1439-037X.2003.00035.x

Matthäus, B., Luftmann, H., 2000. Glucosinolates in Members of the Family Brassicaceae: Separation and Identification by LC/ESI-MS-MS. J. Agric. Food Chem. 48, 2234–2239. https://doi.org/10.1021/jf991306w

Mazzola, M., Brown, J., 2010. Efficacy of Brassicaceous Seed Meal Formulations for the Control of Apple Replant Disease in Conventional and Organic Production Systems. Plant Dis. 94, 835–842. https://doi.org/10.1094/PDIS-94-7-0835

Meinecke, A., Westphal, A., 2014. Quantitative reproductive potential of Heterodera schachtii on weeds typical for late summer fallow in sugar beet rotations. Weed Res.

n/a-n/a. https://doi.org/10.1111/wre.12116

Moens, M., Perry, R.N., Jones, J.T., 2018. Cyst Nematodes - Life Cycle and Economic Importance, in: Moens, M., Perry, R.N., Jones, J.T. (Eds.), Cyst Nematodes. CAB International, Wallingford, Oxfordshire, UK, pp. 1–26.

Moriarty, F., 1966. Decline of a field population of beet eelworm (Heterodera schachtii). Nematologica 12, 349-.

Morra, M.J., Borek, V., 2010. Glucosinolate preservation in stored Brassicaceae seed meals. J. Stored Prod. Res. 46, 98–102. https://doi.org/http://dx.doi.org/10.1016/j.jspr.2009.12.001

Müller, J., 1999. The economic importance of Heterodera schachtii in Europe. Helminthologia 36, 205–213.

Müller, J., Rehbock, K., Wyss, U., Rehbock, K., 1981. Growth of Heterodera schachtii with remarks on amounts of food consumed. Rev. Nematol. 4, 227–234.

Müller, J., Rumpenhorst, H.J., 2000. Testing of crop cultivars for resistance to noxious organisms at the Federal Biological Research Center. Belin, Germany.

Neher, D.A., 2010. Ecology of Plant and Free-Living Nematodes in Natural and Agricultural Soil. Annu. Rev. Phytopathol. https://doi.org/10.1146/annurev-phyto-073009-114439

Ngala, B.M., Haydock, P.P.J., Woods, S., Back, M.A., 2014. Biofumigation with Brassica juncea, Raphanus sativus and Eruca sativa for the management of field populations of the potato cyst nematode Globodera pallida. Pest Manag. Sci. n/a-n/a. https://doi.org/10.1002/ps.3849

Nicol, J.M., Turner, S.J., Coyne, D.L., Nijs, L. den, Hockland, S., Maafi, Z.T., 2011. Current Nematode Threats to World Agriculture, in: Genomics and Molecular Genetics of Plant-Nematode Interactions. https://doi.org/10.1007/978-94-007-0434-3_2

Nigh, E.A., Thomason, I.J., Vangundy, S.D., 1980. IDENTIFICATION AND DISTRIBUTION OF FUNGAL PARASITES OF HETERODERA-SCHACHTII EGGS IN CALIFORNIA. Phytopathology 70, 884–889. https://doi.org/10.1094/Phyto-70-884

Noel, G.R., Atibalentja, N., Domier, L.L., 2005. Emended description of Pasteuria nishizawae. Int. J. Syst. Evol. Microbiol. 55, 1681–1685. https://doi.org/10.1099/ijs.0.63174-0

Olatinwo, R., Borneman, J., Becker, J.O., 2006. Induction of Beet-Cyst Nematode Suppressiveness by the Fungi Dactylella oviparasitica and Fusarium oxysporum in Field Microplots. https://doi.org/10.1094/PHYTO-96-0855

Perry, R.N., 2002. Hatching, in: Lee, D.L. (Ed.), The Biology of Nematodes. Taylor & Francis, London.

Perry, R.N., Moens, M., 2013. Glossary, in: Plant Nematology. CAB International, Wallingford, Oxfordshire, UK, pp. 523–531.

Radice, A.D., Powers, T.O., Sandall, L.J., Riggs, R.D., 1988. Comparisons of Mitochondiral DNA from the sibling species Heterodera glycines and Heterodera schachtii. J. Nematol. 20, 443–450.

Reuther, M., Lang, C., Grundler, F., 2017. Nematode-tolerant sugar beet varieties - Resistant or Susceptible to the Beet Cyst Nematode Heterodera schachtii?, Sugar industry.

Roberts, P.A., Thomason, I.J., 1981. Sugarbeet Pest Management: Nematodes. University of California.

Schlatter, C., 2015. Clariva™ seed treatment nematicide, A breakthrough for sugar beet production, in: 75th IIRB Congress. IIRB, p. 101.

Schmitz, A., Tartachnyk, I., Kiewnick, S., Sikora, R., Kühbauch, W., 2006. Detection of *Heterodera schachtii* infestation in sugar beet by means of laser-induced and pulse amplitude modulated chlorophyll fluorescence 8, 273–286.

https://doi.org/doi:10.1163/156854106777998755

Sharma, S.B., Sharma, R., 1998. Hatch and emergence, in: Sharma, S.B. (Ed.), The Cyst Nematodes. Chapman & Hall, London, pp. 191–216.

Shepard, A.M., 1970. Extraction and estimation of Heterodera, in: Southey, J.F. (Ed.), Laboratory Methods for Work with Plant and Soil Nematodes. HMSO, London.

Smith, H.J., Gray, F.A., Koch, D.W., 2004. Reproduction of Heterodera schachtii Schmidt on resistant mustard, radish, and sugar beet cultivars. J. Nematol. 36, 123–130.

Snyder, A., Morra, M.J., Johnson-Maynard, J., Thill, D.C., 2009. Seed meals from brassicaceae oilseed crops as soil amendments: Influence on carrot growth, microbial biomass nitrogen, and nitrogen mineralization. HortScience.

Southey, J.F., 1986. Principles of sampling for nematodes, in: Laboratory Methods for Work with Plant and Soil Nematodes. HMSO, London, United Kingdom, pp. 1–4.

Stevens, M., Liu, H.-Y., Lemaire, O., 2006. Virus Diseases, in: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing, pp. 256–285.

Sukovata, L., Jaworski, T., Lazzeri, L., Kolk, A., 2014. Mustard seed meal against Melolontha grubs - the first trials, in: Back, M.A., Clarkson, J., Lazzeri, L., Michel, V., Reade, J. (Eds.), 5th International Symposium of Biofumigation. Association of Applied Biologists, Harper Adams University.

Syngenta, 2018. Clariva pn Seed Treatment [WWW Document]. URL http://www.syngenta-us.com/seed-treatment/clariva-pn (accessed 5.18.18).

Taylor, F., Kenyon, D., Giela, A., Keenan, G., Rosser, S., 2014. Analysis of isothiocyanates formed by Brassica spp. using Gas Chromotography Mass Spectrometry. Asp. Appl. Biol. 126, 85–91.

Trudgill, D.L., Evans, K., Faulkner, G., 1972. A fluidising Column for Extracting Nematodes

from Soil. Nematologica 18, 469–475. https://doi.org/10.1163/187529272X00070

Turner, S.J., Subbotin, S.A., 2013. Cyst nematodes, in: Perry, R.N., Moens, M. (Eds.), Plant Nematology. CABI, Malta, pp. 109–143.

Vandenbossche, B.A., 2016. Effect of temperature on the interactions between beet cyst nematodes (Heterodera schachtii and Heterodera Betae) and sugar beet. Georg-August-University Göttingen.

Winfield, A.L., Enfield, M.A., Foreman, J.H., 1987. A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. Ann. Appl. Biol. 111, 223–231. https://doi.org/10.1111/j.1744-7348.1987.tb01449.x

World Conservation Monitoring Centre, 1992. Global Biodiversity: Status of Earth's living resources, 1st ed. Chapman & Hall, London.

Wright, A., Stevens, M., Back, M., Sparkes, D., 2017. Interactions between Heterodera schachtii and Sugar Beet. Asp. Appl. Biol. 134, 81–88.

Wyss, U., Stender, C., Lehmann, H., 1984. Ultrastructure of feeding sites of the cyst nematode Heterodera-schachtii Schmidt in roots of susceptible and resistant raphanus-sativus L. var oleiformis pers cultivars. Physiol. Plant Pathol. 25, 21–37.

Zheng, L., Ferris, H., 1991. Four types of dormancy exhibited by eggs of Heterodera schachtii. Rev. Nematol. 14, 419–426.

Chapter 2

Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, *Heterodera schachtii* (Schmidt) under uninfested conditions

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Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, *Heterodera schachtii* (Schmidt) under uninfested conditions

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ABSTRACT

The beet cyst nematode (BCN) is a problem to sugar beet growers around the world and can cause severe yield losses. Recently, varieties of sugar beet have been developed which are either tolerant to damage caused by BCN, or alternatively are resistant to BCN. Little is understood about these varieties and how they may have different physiological characteristics when compared with varieties of sugar beet that are susceptible to BCN. This study assessed a range of nine varieties, which were tolerant, susceptible or resistant to BCN, in pot and hydroponic tank investigations to measure differences in their canopy, early rooting and yield traits in the absence of BCN. Two field experiments, using four varieties which were susceptible, resistant or tolerant to BCN, then followed to test the hypothesis that increasing the plant population density (PPD) allows a BCN resistant variety to achieve a greater yield.

In the pot and hydroponic experiments, it became clear that the varieties had different growth habits. The resistant variety yielded the least sugar and had the smallest canopy per plant. In the field experiments, which were not infested with BCN, in both years the resistant variety also showed a delayed canopy expansion compared to the other varieties. The rate of expansion could be increased by increasing the PPD. In 2016 this increased PPD resulted in higher yields of the resistant variety. However, due to better canopy development in the following year, a yield penalty was found in 2017 at higher PPDs. Understanding how different varieties need different PPDs may make resistant varieties a more economical option to cultivate in the future. However, the levels of impurities, particularly sodium impurities, in the resistant plants may still make them a less favourable choice to grow.

The light tolerant varieties showed a distinct increased rooting and canopy expansion rate compared to the other variety types, while the tolerant varieties showed similar rooting and canopy traits to the susceptible varieties but had different yield responses to increased seed rate.

1. Introduction

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is widely grown across the world as a source of sucrose. Like all crops, sugar beet suffers threats to achieving maximum yield due to a range of pests and diseases and careful management of these threats is required to limit yield loss.

One pest which poses a threat to sugar beet crops all over the world is the beet cyst nematode (BCN), *Heterodera schachtii* (Schmidt). Commonly found in sugar beet crops grown on sandy, loamy or organic soils, the nematode can cause severe yield losses, especially in water limited conditions (Cooke, 1987). In Europe alone, BCN was estimated to cause annual losses of over €90 million (Müller, 1999). However, with modern varieties this figure may be much lower. BCN can go unnoticed at low population densities below the plant's tolerance threshold to damage. Yield will still be lost even if no symptoms are displayed such as stunted plant development canopy wilting and yellowing of leaves (Dewar and Cooke, 2006) and therefore infestations are probably more widespread than expected by sugar beet growers and levels of yield loss are difficult to quantify. Control of BCN has traditionally been limited to the enforcement of long rotations, of over five years between host species (Koch and Gray, 1997), either through government intervention or contract clauses with sugar processors

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(Cooke, 1987; Ministry of Agriculture, Fisheries and Food, 1977). Nematicides have also been an option for control, but have now been withdrawn from sale due to concerns about their harmful effects (Dewar and Cooke, 2006; Hauer et al., 2016). Other options, such as biofumigation and resistant brassica cover cropping may also provide control for BCN. However, these techniques can produce variable results (Hauer et al., 2016; Held et al., 2000 Hemayati et al., 2017; Lazzeri et al., 1993).

Advances in sugar beet breeding have led to the development of varieties of sugar beet which are tolerant, light tolerant or resistant to infestation by BCN. Tolerant varieties, which can compensate for losses to infestations of BCN and allow economically viable yields on infested land, were developed by introgressing genes such as *HsBvm-1* from *Beta vulgaris* ssp. *maritima*, a close relative of sugar beet (Stevanato et al., 2015). Introduced to the UK in 2009, the market share of these tolerant varieties has grown annually from 0.59% in 2009 up to 6.69% in 2017 (M Culloden Pers. Comm. – Head of Agriculture, British Sugar). Whilst these varieties have both gained in popularity and yield potential over this period, there is much that is not understood about their physiology and appropriate uses in the field. It is hypothesised that they may have higher levels of photosynthetic assimilation to counteract losses to the BCN or greater levels of early root growth to grow away from infested patches of soil. Varieties marketed as 'light tolerant' were previously available in the UK. Whilst they have since been superseded by higher yielding fully tolerant varieties, they were marketed as having a greater yield potential than tolerant varieties, but would only be beneficial to use in fields with low BCN populations (Kerr and Stevens, 2014). Whilst popular at the moment, tolerant varieties may be of limited use in the long term as they still cause the build-up of BCN populations in the soil (Hauer et al., 2016; Krüssel and Warnecke, 2014). Resistant varieties (which can actively reduce BCN populations) have been available to growers in continental Europe since the mid-1990 s (Müller, 1999; Zhang et al., 2008) and were developed by introgressing the $HS1^{pro1}$ gene from *Patellifolia procumbens* into sugar beet (Panella and Lewellen, 2007). The resistance mechanism enables the sugar beet to recognise the invading nematode during the development of its feeding cell (syncytium). The hypersensitive response results in the death of cells surrounding the syncytium and the nematode is deprived of nutrients which prevents successful BCN reproduction. As the nematode is prevented from viably reaching mature stages, when greater and more damaging feeding occurs (Müller et al., 1981), the yield of the crop is also protected. The final populations in the soil are lowered when a resistant variety is grown and therefore these varieties may be a good option for growers with BCN infestations who need to plant other host species, such as oilseed rape or vegetable brassicas, in their crop rotations and want to reduce their BCN population levels.

BCN infestation is usually very patchy in fields and rarely is it found in all parts of a field (Cooke, 1987). Therefore, growing a tolerant or resistant variety may have a negative impact on overall field yield due to their potentially lower yield performance in the absence of BCN and associated higher seed costs of such varieties (British Sugar, 2017). This study aimed to better understand the performance of a range of sugar beet varieties in terms of early rooting habits, canopy expansion and size, photosynthetic activity and their subsequent yield and quality. The experiments were conducted in the absence of any BCN infestation to understand if any physiological differences between the varieties could be identified in uninfested conditions and compare yield without the associated losses from BCN.

2. Materials and methods

2.1. Pot experiment

An experiment was established in an unheated glasshouse on 11 May 2015. Nine varieties of sugar beet, varieties 1–9 detailed in Table 1, were grown in five blocks, organised as a randomised block design, with two replicates of each variety in each block (n = 90).

Seeds of each variety were sown into five litre pots filled with a 20:80 mixture of sterilised Kettering loam (24% clay content) (Boughton, Kettering, UK) and coarse sand mixed to create a loamy sand soil texture. Three seeds of each variety were planted in each pot and thinned to one plant at 8 days after sowing (DAS). Plants were given 1.2 g of nitrogen fertilizer each using ammonium nitrate (NH_4NO_3) and a 0N-36P-36 K fertilizer with additional trace elements (Hortifeeds, Lincoln, UK) was used to meet all of the plants nutritional requirements. All fertilizer was applied to the pot prior to sowing. Plants were supplied with regular irrigation to prevent water stress throughout the experiment.

Leaf and canopy expansion were measured during the canopy expansion phase of the plants. Canopy expansion was measured using a digital camera (Canon Eos 1100D fitted with 18–55 mm Lens, Canon Inc. Japan) mounted on a copy stand from which canopy cover could be derived by the thresholding of green pixels using ImageJ (Rasband, 2016).

A Li-Cor LI6400XT (Li-Cor Inc. Lincoln, NE, USA) was used to measure photosynthetic assimilation (A_{max}), under the following conditions: a saturating photosynthetically active radiation (PAR) level of 1200 µmol m^2/s, CO_2 set to 400 µmol/mol, flow rate of 500 µmol/s and block temperature of 18 °C. Measurements were made on a fully expanded leaf and on each day they were completed between 10.00 and 14.00 h. The chamber was clipped onto the leaf to be measured in the upper half and conditions allowed to stabilise before the gas exchange data were logged. These measurements were repeated regularly throughout the season (57, 64 and 71 DAS on Leaf 5 and 108 and 122 DAS on Leaf 10).

At 148 DAS, after 2497 °C days above a base temperature of 3 °C (Gummerson, 1986) had accumulated, the plants were harvested. The leaves were then removed from the root and the leaf area of each plant measured using a Li-Cor LI-3100 leaf area meter (Li-Cor Inc. Lincoln, NE, USA). Roots were washed to remove any soil and fibrous roots. The storage root was then weighed and divided in half. One half was dried to determine root biomass and the other half processed into a brei sample for sugar & quality analysis (Asadi, 2005) using a Thermomix TM31 food processor (Vorwerk, Wuppertal, Germany) until the beet sample became a paste. This paste was transferred into a brei tray and frozen at −20 °C until sugar and content of potassium, sodium and amino nitrogen impurities could be determined at the BBRO tare house facility at British Sugar's Wissington Beet Sugar Factory. Sugar content was determined using polarimetry, sodium and potassium impurities by flame photometry and amino nitrogen impurities by colourimetry.

2.2. Hydroponic tank experiment

The same nine varieties grown in the pot experiment were tested in hydroponic pouches to investigate differences in early rooting. A randomised block design of 36 blocks, each with two replicates of each variety was established (n = 648).

Seeds were directly sown into pouches set up according to Atkinson et al., (2015) on 23 October 2015 (Fig S1). Conditions in the controlled environment room (CER) were maintained at 18 °C day and 8 °C night and a photoperiod of 16 h. The tanks into which the pouches were suspended were initially filled with 2 litres of ¼ strength Hoaglands No. 2 Basal Salt mixture (Sigma Aldrich, Gillingham, Dorset, UK) and then were topped up using deionised water only. After 21 days in the CER the pouches were removed and the roots of the seedlings photographed using a digital camera (Canon Eos 1100D fitted with 18–55 mm Lens, Canon Inc. Japan) and copy-stand. The photographs were then analysed using RootReader2D version 2.3 (Clark et al., 2013) to measure primary and lateral root lengths.

Table 1
Details of the varieties of sugar beet grown in the experiments described in this paper. They differ in their susceptibility and yield tolerance to infestations with *H. schachtii*. Varieties one to nine were grown in the pot and hydroponic experiments. In the field trials varieties two, three, seven and ten were grown. The table also details which breeder developed each variety and the year in which they were initially listed for cultivation in the UK (if applicable).

Variety Number	*H. schachtii* host Status[a]	Trait Description	Year first registered for cultivation in UK	Breeder
1	Susceptible	These plants support high levels of BCN reproduction (Perry and Moens, 2013) but yield well in non-infested conditions	2011	SVH
2	Susceptible		2011	STR
3	Light Tolerant	Able to compensate for damage caused by low levels of BCN infestation	2014	SYN
4	Light Tolerant		2010	SYN
5	Tolerant	Trait is not related to resistance. Tolerant plants are able to sustain growth & yield when parasitized by BCN (Perry and Moens, 2013)	2013	SVH
6	Tolerant		2013	BTS
7	Tolerant	Has increased yield performance relative to an infested susceptible plant (BBRO, 2014)	2015	SVH
8	Tolerant		2012	STR
9	Resistant[b]	The ability of a plant to inhibit reproduction of BCN relative to a susceptible plant that supports high levels of reproduction (Perry and Moens, 2013)	n/a	SYN
10	Resistant[b]		n/a	SYN

[a] As claimed by sugar beet breeders upon submission into recommended list (RL) trials.
[b] These varieties have never been commercially registered in the UK. Breeder key: SYN – Syngenta SVH – SES Vander Have BTS – Betaseed STR – Strube.

2.3. Field experiments

Field experiments were sown at The University of Nottingham's Sutton Bonington Campus on 7 April 2016 and on 10 April 2017. Both fields were of a freely draining slightly acid loamy soil type (Hallett et al., 2017). In both years the same four varieties of sugar beet were sown: one susceptible to BCN, one light tolerant, one tolerant and a new BCN resistant variety (varieties 2, 3, 7 and 10 respectively from Table 1), as the resistant variety previously used was not available. These varieties were selected as they were commercially available in the year of sowing except the resistant variety used was a coded variety under development and not commercially available in any market. Each variety was sown at rates of either 119,000, 153,000 or 211,000 seeds per hectare in a Latin square design (n = 48) with four replicates of each treatment.

Plots containing six rows of sugar beet (7.5 × 3 m in total, 50 cm row spacing) were sown with a Wintersteiger Monoseed K seed drill (Wintersteiger AG, Reid im Innkreis, Austria). Plots were fertilized with 120 kg ha^{-1} of nitrogen using ammonium nitrate (NH_4NO_3) in both years. Doses of N were split 1/3, applied prior to emergence, and 2/3 applied before the two true leaves stage. In 2016, at 49 DAS, the plots sown at 153,000 seeds ha^{-1} were thinned manually to reduce the population due to a malfunction with the drill. This ensured three distinct population densities were established.

Canopy cover was monitored using a digital camera fitted with a wide-angle lens (Canon EOS1100D and 10–18 mm lens). The lens' zoom was fixed at 10 mm and the camera mounted on a rig to hold the camera 1.2 m above the soil and 2.25 m from the edge of the plot. At this height, rows two, three and four (which were to be harvested) filled the image. Each photograph captured 2.7 m of row length (8.1 m in total). Images were taken from either end of the plots with the combined area covering 72% of the harvested plot area. The use of a laptop allowed for remote imaging of each plot and storage of the photographs. At the four-leaf stage the photographs were used to count established plants in the plots. Photographs were taken on a weekly basis during the canopy expansion phase of growth. The green area of each image was estimated using the threshold calculated by ImageJ (Rasband, 2016) to measure canopy cover.

Canopy development was modelled using a three-parameter log-logistic model fitted in R (R Core Team, 2016; Ritz et al., 2015) using the calculated values for each plot from the image analysis. The model then estimated maximum cover, slope and the inflection point of each plot's canopy. Inflection point (IP) denotes the time when the canopy reached 50% maximum canopy cover and is therefore a measure of the speed at which the canopy expands. A larger and more vigorous canopy has a lower IP than a smaller and slower canopy (Fig. 1).

The plots were harvested on 4 October in both years. Three rows were harvested using a Garford Victor harvester (Garford Farm Machinery Ltd, Peterborough, UK) to determine yield and impurity levels at the BBRO tare house. An additional ten beet from row 5 were harvested by hand to measure total biomass of each variety. The ten beet were weighed, subsampled to five, then these leaves and storage roots were washed to remove soil and lateral roots, and then chopped before being dried at 70 °C until constant weight.

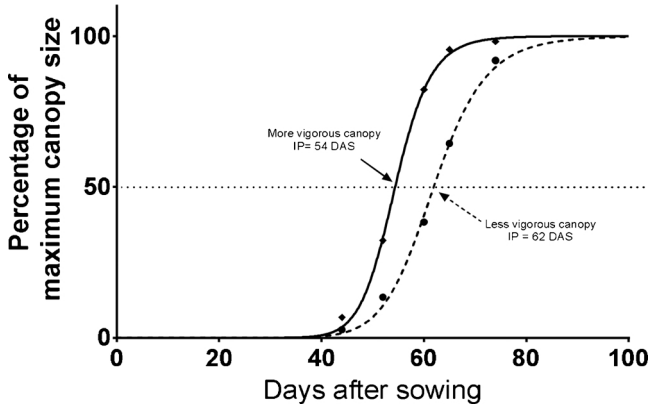


Fig. 1. Two contrasting canopy development curves as measured in the field experiment at Sutton Bonington in 2017. Data from images taken in the field were used to calculate canopy cover. These data were then inputted into a model to calculate the inflection point (IP) of the different canopies and this figure illustrates how a more vigorous canopy (—) of the susceptible variety at the highest seed rate reached the IP eight days earlier than the less vigorous canopy of the resistant variety at a low seed rate (—). Markers show actual data measured from image analysis (♦ = susceptible, • = resistant).

2.4. Data analysis

Genstat 17th Edition was used for appropriate ANOVAs for each experimental design, regression and comparison of regression analysis (VSN international, Hemel Hempstead, UK). Graphs were prepared using GraphPad Prism v.7 (GraphPad Software Inc. La Jolla, CA, USA). Tukey's multiple comparison test was used to compare the results of the ANOVAs reported in the tables and graphs.

3. Results

3.1. Pot experiment

Significant differences in canopy expansion were observed between the varieties when grown in the glasshouse (Table 2). At 28 DAS the

Table 2
Canopy cover of nine varieties of sugar beet which have differing susceptibility to *H. schachtii*. The plants were grown in pots in a glasshouse. Canopy cover was measured in cm^2 per plant and measured over three time points (28, 35 and 42 days after sowing) during canopy expansion.

	Variety									
DAS	1	2	3	4	5	6	7	8	9	*P*
28	144 bc	171 c	159 bc	170 bc	166 bc	129 ab	130 abc	166 bc	96 a	< 0.001
35	351 ab	394 b	361 ab	366 ab	422 b	341 ab	364 ab	423 b	299 a	0.007
42	695	677	714	689	677	680	696	672	635	n.s.

Same letters indicate no significant difference at P = 0.05 using Tukey's multiple comparison.

resistant variety (9) had the smallest canopy cover and was significantly smaller than all varieties except 6 and 7 ($P < 0.001$). At 35 DAS the resistant variety still had the smallest cover and was significantly lower than varieties 2,5 and 8 ($P = 0.007$). At 42 DAS there were no significant differences between the varieties, but the resistant variety remained the smallest.

When total leaf area was measured at harvest (Fig. 2a) varieties 2, 3, 4 and 6 had greater leaf areas than the remaining varieties and the resistant variety (9) still had the smallest leaf cover of all ($P < 0.001$). In terms of photosynthetic assimilation of CO_2, the resistant variety had similar A_{max} values to the susceptible and most tolerant varieties, however the light tolerant varieties (3 and 4) and tolerant variety number 8 had the lowest mean A_{max} values (Fig. 2b).

When the plants were harvested there were also significant differences in sugar yield (Fig. 2c). Variety 9 yielded the least, but was only significantly lower than variety 5 ($P = 0.022$). A significant difference in root:shoot ratio was also found from the biomass data. Variety 6 had the lowest ratio ($P < 0.001$) (Fig. 2d).

The resistant variety had the second lowest percentage of sugar in the root but was only significantly lower than variety 5 ($P = 0.006$, Fig. 3). All varieties had between 16.79 and 17.79% sucrose content. The resistant variety produced significantly greater levels of sodium impurities than all of the other varieties ($P < 0.001$). Apart from variety 6, all varieties produced similar amino nitrogen impurities to the resistant variety ($P = 0.001$). Significant differences were found in relation to the levels of potassium impurities measured too ($P < 0.001$) Varieties 8 and 3 produced the lowest (26.47 and 26.33 mg per 100 g of beet) and variety 2 the greatest (30.14 mg per 100 g of beet).

3.2. Hydroponic tank experiment

Image analysis of the three-week old seedlings showed significant differences in root growth between varieties (Fig. 4). The two light tolerant varieties (varieties 3 and 4) had longer roots than the other varieties that were tested ($P < 0.001$). Consistent rankings of the total root length and primary root length show the differences in the varieties are driven mainly by the differences in the length of the primary root. This is further supported by no significant differences being found in the ratio of primary to lateral roots of any of the varieties.

3.3. Field experiments

Both years experienced similar levels of total rainfall and thermal time over the course of the experiments (Fig. 5). In 2016, June rainfall was exceptionally high (three times the long term mean) which resulted in delayed and reduced total expansion of the canopies. April, May and June were also warmer in 2017 (Fig. 5) and therefore significantly different responses were observed in terms of both canopy development and subsequent yield.

3.3.1. Canopy development & yield responses

In 2016, the maximum canopy size, estimated by the log-logistic model, was found to differ significantly between the seed rates and varieties (Table 3a) but there was no interaction between seed rate and variety. The lowest seed rate produced an estimated final mean canopy cover of 78.6%, the intermediate seed rate produced a mean canopy cover of 89.5% and the highest seed rate produced the largest mean canopy cover of 93.8% ($P < 0.001$). The varieties produced distinctly different maximum canopy covers, with the resistant variety producing the lowest level of 83.8% followed by the susceptible variety at 87.6%, then the tolerant variety at 88.5% and finally the greatest mean cover was achieved by the light tolerant variety at 89.3% ($P = 0.031$).

In 2017, with much warmer conditions during canopy expansion, the canopy model predicted that all treatments would reach or exceed 99% canopy closure (Table 3b). There was a significant response to seed rate only, with the lowest seed rate predicted to produce the largest canopy. However, the averages for the different seed rates shows that all would meet or exceed 100% canopy cover.

In 2016, (Fig. 6a) a positive yield response to increasing PPD was found. Three distinct responses are shown; the tolerant and resistant varieties showed the same response to increasing the population, although their intercepts differed, reflecting the different yield potential of these varieties. The susceptible variety had the shallowest slope, indicating that it would benefit least from increasing PPD and the light tolerant variety had the steepest slope as it responded greatest to increasing PPD ($P = 0.046$).

In 2017, (Fig. 6b) the opposite response to increased PPD was found. The yield responses of all varieties showed a negative relationship to increased PPDs. The varieties all responded similarly (equal slopes) although there were different yield potentials indicated by their significantly different intercepts. The tolerant and resistant varieties had greater yield potential across all populations in 2017 ($P < 0.001$) than the light tolerant and susceptible varieties.

Using the inflection point (IP) as a measure of the vigour of the canopy, a significant negative relationship was found in 2016 between IP and sugar yield ($P = 0.013$) in response to the increased PPD (Fig. 6c). Similarly to the response to PPD, three distinctly different responses in the relationship between IP & subsequent yield were found. The susceptible variety had the shallowest response to the change in IP (by increasing PPD) and yield, whilst the resistant and tolerant variety show a more negative response and the light tolerant variety has the most negative response. The tolerant, light tolerant and susceptible varieties had more vigorous canopy expansion at all seed rates than the resistant variety.

In 2017, the opposite response to PPD and variety was found (Fig. 6d). Variety response can be seen to be the same as 2016, with delayed canopy development exhibited by the resistant variety. However, this resulted in a yield benefit rather than penalty. Parallel responses again can be seen and all varieties, excluding the tolerant variety, had similar intercepts ($P < 0.001$).

3.3.2. Biomass partitioning

In 2016, the higher seed rates produced less storage root biomass in relation to the canopy biomass across all varieties ($P = 0.028$, data not shown). In 2017, root biomass production was not found to differ across the treatments. However, there are trends, although not significant, relating to the amount of canopy biomass produced (data not shown).

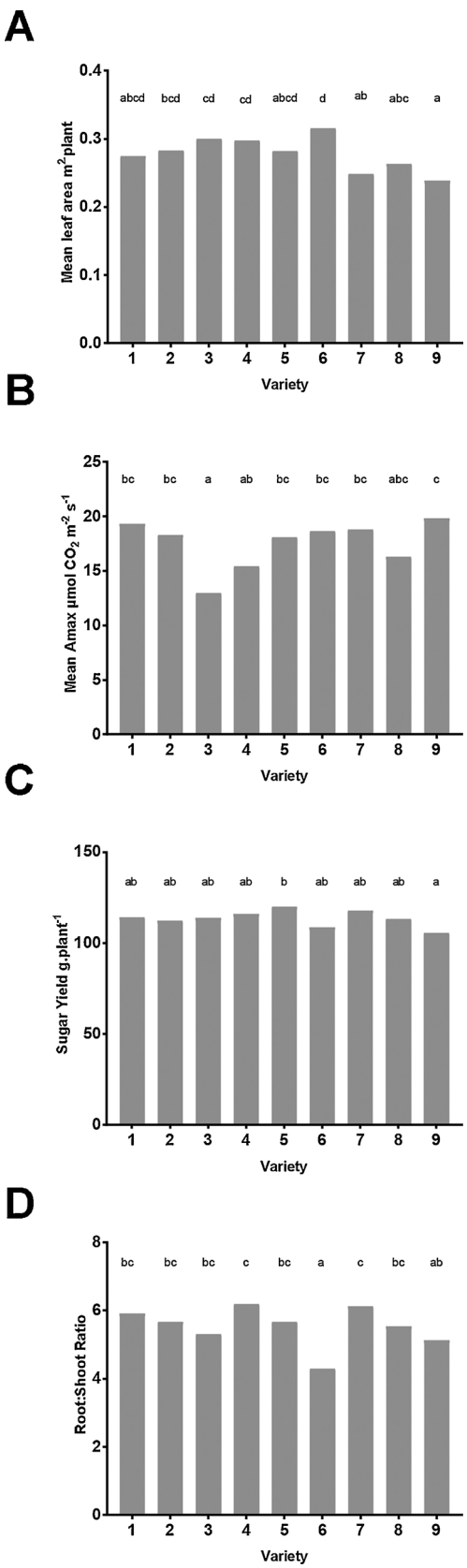


Fig. 2. Results from the pot experiment conducted in 2015 to compare differences between a range of sugar beet varieties which vary in their susceptibility to *H. schachtii*. A shows the mean leaf area per plant (m^2) at harvest ($P < 0.001$), B displays the mean CO_2 assimilation (µmol $CO_2 m^{-2} s^{-1}$) measured between 57 and 122 days after sowing ($P < 0.001$) from the different varieties. C shows the mean mass of sugar (g of sucrose per plant) produced by each plant at harvest ($P = 0.022$) D shows the mean ratio of root to shoot biomass produced by each variety ($P < 0.001$). All plants were grown in the absence of *H. schachtii*. Different lower case letters represent significantly different results at P = 0.05.

The higher seed rates produced higher levels of canopy biomass (P = 0.052) and the resistant variety consistently produced less canopy biomass at all PPDs than the other varieties (P = 0.076).

3.3.3. Root sucrose concentration and impurities

Sucrose levels in the roots show similar patterns in both years. In 2016, the light tolerant variety showed a significantly lower level of sucrose than the other varieties ($P < 0.001$). In 2017, the susceptible and tolerant varieties had significantly higher sucrose concentrations than the light tolerant and resistant varieties ($P < 0.001$) (Fig. 7a). In both years the resistant variety had higher sodium impurity levels than the susceptible variety. The light tolerant variety had the highest levels in 2016 ($P = 0.001$) and the resistant variety the highest in 2017 ($P \leq 0.01$) (Fig. 7b). Amino nitrogen and potassium impurity levels were not significantly different in either year between the varieties (data not shown).

4. Discussion

The results from these experiments have gained an insight into how BCN tolerant, resistant and susceptible varieties grow and develop their roots and canopies. As all experiments were conducted under BCN free conditions further work is required to understand if these varieties respond differently under infestation.

4.1. Canopy

Interception of light is directly related to yield of sugar beet (Jaggard and Qi, 2006). In the pot experiment we found that the resistant variety had similar photosynthetic rates to other varieties and therefore it seems likely that its reduced yield was due to the smaller canopy, and thereby reduced light interception, and the overall smaller size of the plants produced (since the root:shoot ratio was not different to most of the varieties). Less vigorous canopy development by the BCN resistant variety was also identified in both years of field experiments, indicated by their delay in reaching the IP.

Our results indicate that introducing tolerance traits to sugar beet has not led to significant reduction in canopy vigour, whereas the same cannot be said for the resistant varieties tested. The lower vigour and overall canopy size per plant seen in the pot experiment was also observed in the field. This reduced vigour might be a result of the breeding process to introgress the $HS1^{pro-1}$ gene from *P. procumbens*, to introduce resistance to BCN, and associated linkage drag of undesired genes (Flint-Garcia, 2013). Alternatively, the breeding lines used in the 1990s when these varieties were developed (Müller, 1999) may have had less vigorous canopies than today's elite cultivars.

Our investigations have revealed differences in the way that modern BCN resistant varieties develop their canopies compared to susceptible and tolerant cultivars. Understanding the lower level of canopy vigour and growth may assist these varieties in being approved for cultivation in the UK. The results of these field experiments will prove useful in this

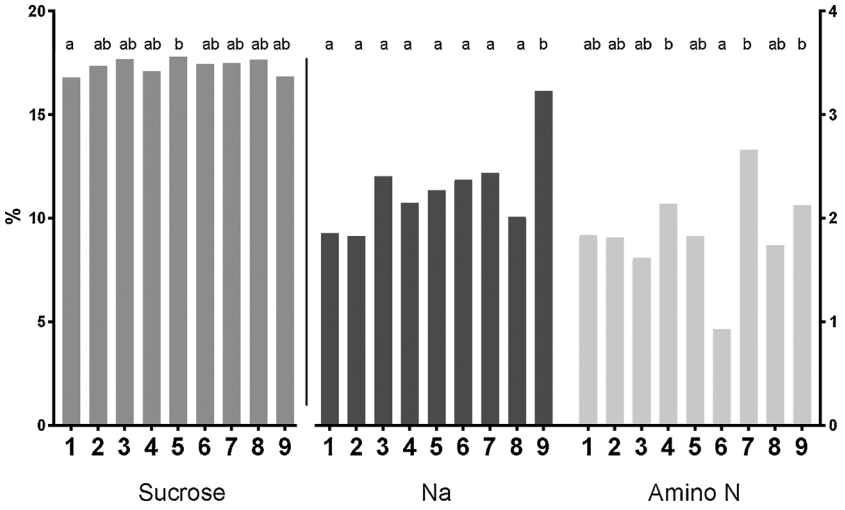


Fig. 3. The nine varieties of sugar beet, which differ in their susceptibility to *H. schachtii*, were grown in a pot experiment at Sutton Bonington. After the roots were harvested a brei paste sample was made. The brei sample was measured for sucrose content and impurities of sodium and amino nitrogen (measured in mg of impurity per 100 g of fresh beet). Significant differences were found between the varieties for sucrose content ($P = 0.006$) and impurities (Sodium $P < 0.001$ and amino N $P = 0.003$). Same letters above bar represent no significant difference between variety at $P = 0.05$.

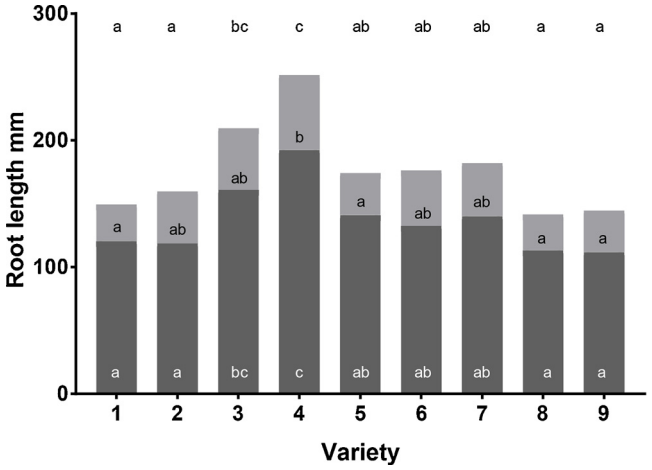


Fig. 4. A hydroponic tank experiment was conducted to investigate differences in the early rooting development of nine varieties of sugar beet which vary in their susceptibility to *H. schachtii*. Primary root lengths ■ and lateral root lengths ■ were measured of each plant using computer image analysis ($P < 0.001$ for all datasets) Lower case letters within the bars show differences between the respective root measurements. letters above the bars show differences between the total root length (total height of the bars) measurements. All differences at $P = 0.05$.

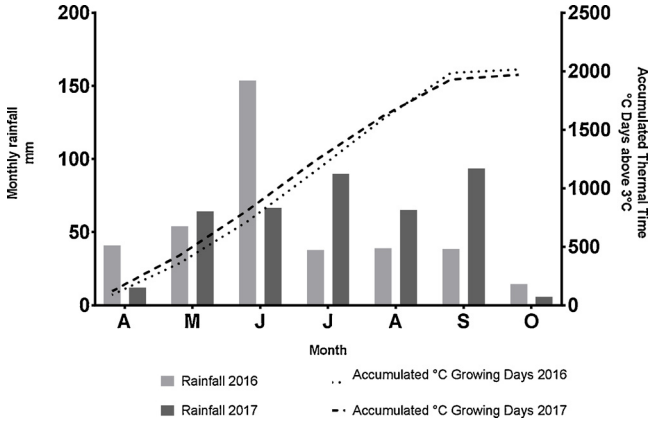


Fig. 5. Temperature and rainfall were measured throughout the experiment using the meteorological station at Sutton Bonington during the field experiments investigating the response of four sugar beet varieties, which varied in their susceptibility to *H. schachtii*, to increased plant populations. The data clearly show that fewer degree days were received in 2017 and a greater amount of rainfall in June 2016 than 2017. Data for April and October include only the days when plants were in the ground.

case, as they consistently show that BCN resistant varieties have a delayed time to reach their IP and that increasing the seed rate can accelerate the rate of canopy development. Sowing at a higher seed rate would incur extra seed costs so would require consideration as to whether the additional return would be worth the extra investment in seed.

In 2016, when canopy expansion was limited due to the excessive wet weather, the trend was for greater yield under higher PPDs, likely due to the larger canopy development which occurred before the wet weather caused canopy expansion to stop. The opposite was the case when weather conditions led to rapid canopy closure in 2017. More data is required to confirm that the yield penalty when growing a BCN resistant variety compared to a tolerant or susceptible variety can be overcome with a greater PPD. The opposing yield responses in the two years of field experiments require further experiments with more BCN resistant varieties, seed rates and replicated over more sites and seasons to test the generality of this response.

The canopy biomass response seen in 2017 is likely a result of shade avoidance (Ballaré and Pierik, 2017). At the higher PPDs plants were shaded by each other and had to compete with each other for light. Excessive canopy growth resulted and extra resources were invested into the canopy and not used for root growth and yield. In this case, the resistant variety, with its less vigorous canopy, seems to have benefitted and yielded better than expected. As the canopy was smaller there was less competition for light and each plant could dedicate more resources to developing roots, and therefore more yield, rather than having to grow excessively large canopies in an attempt to outcompete neighbouring plants. In years less favourable to canopy expansion than 2017, sowing the resistant variety at a higher rate may be justified in order to ensure better yields and overcome the less vigorous canopy trait.

4.2. Rooting

In oilseed rape (*Brassica napus*), positive relationships have been reported between primary root length in hydroponic pouches and field emergence and yield, as well as between lateral root density in hydroponics and the in-leaf concentration of calcium and zinc in field (Thomas et al., 2016). Furthermore, Bussell et al., (2016) found a relationship between the number of lateral roots produced in hydroponic pouches and nitrogen uptake of sugar beet grown in pots. The lack of significant variation in rooting between tolerant, susceptible and resistant varieties in our hydroponic experiment highlights the possibility that if rooting traits differ between susceptible and tolerant varieties

Table 3
Mean maximum canopy values estimated using a three-parameter log-logistic model. In 2016 significant differences were found between the varieties and seed rates, but no interaction between the two. In 2017 there were only significant differences found between the seed rate. Different lower case letters adjacent to the means signify a significant difference at P = 0.05.

A-2016

		Variety					
		Susceptible	Light Tolerant	Tolerant	Resistant	Mean	P
Seed Rate (Seeds ha^{-1})	119000	82.0	81.7	78.9	72.0	78.6 a	< 0.001
	153000	89.9	90.2	90.3	87.7	89.6 b	
	211000	90.9	96.1	96.2	91.8	93.8 c	
Mean		87.6 ab	89.3 b	88.5 ab	83.8 a		
P		0.031					

B-2017

		Variety					
		Susceptible	Light Tolerant	Tolerant	Resistant	Mean	P
Seed Rate (Seeds ha^{-1})	119000	104.4	102.6	101.5	104.3	103.2 b	
	153000	100.7	100.3	100.5	101.4	100.7 a	0.001
	211000	101.1	100.1	99.7	99.4	100.1 a	

they may require infestation with *H. schachtii* to become apparent. It was hypothesised that tolerant varieties would root deeper or more rapidly than a susceptible variety as a method to grow into areas of soil which are not so heavily infested with *H. schachtii*. This was not evident in the hydroponic system and an alternative method of screening for early rooting differences may be needed, as introducing the nematode

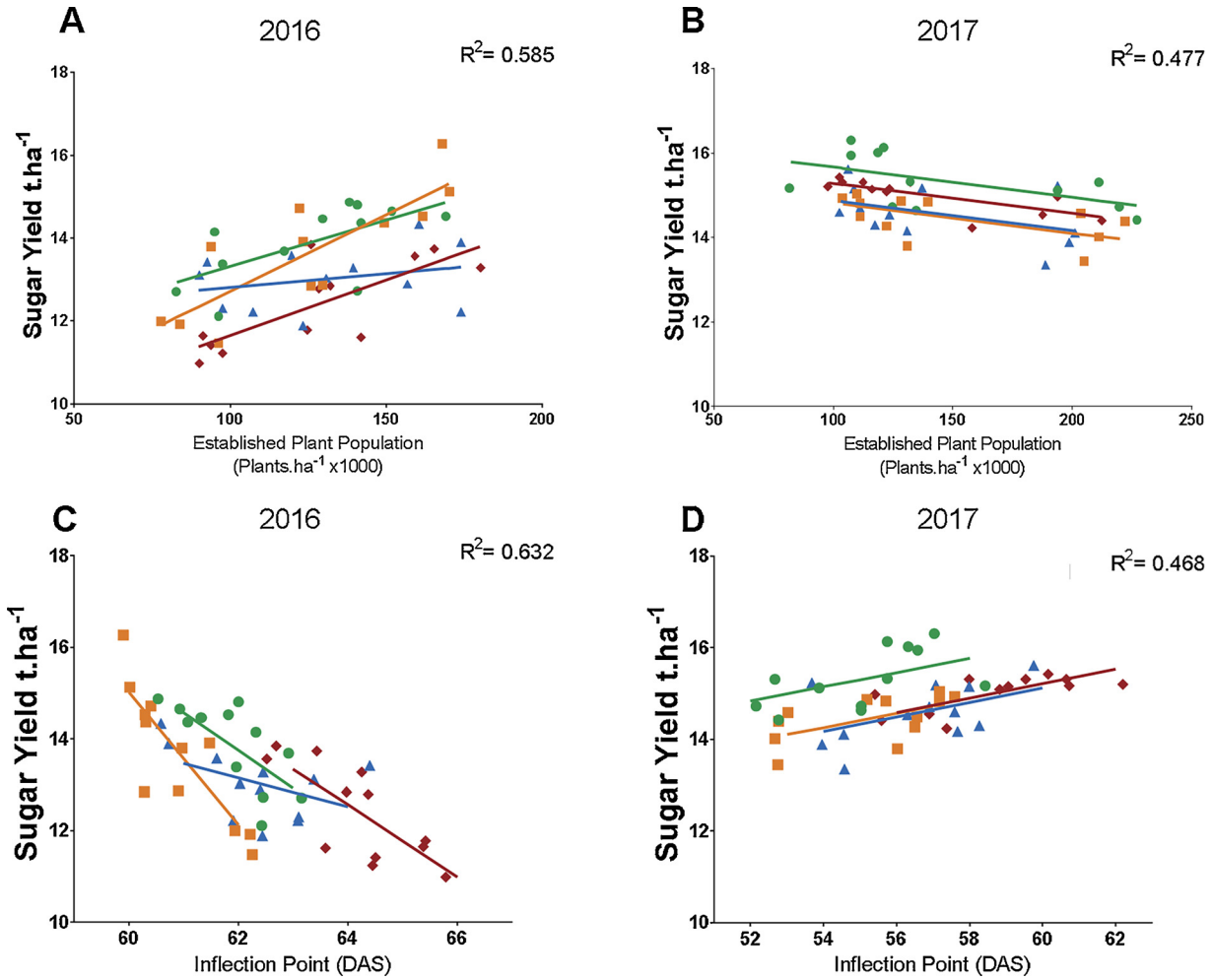


Fig. 6. Regression relationships between the established plant populations (A - 2016 & B - 2017) upon sugar yield and the response of the increasing inflection point (IP) of each variety (C -2016 & D - 2017) from the field experiments at Sutton Bonington. Resistant — Susceptible — Light tolerant — Tolerant —.

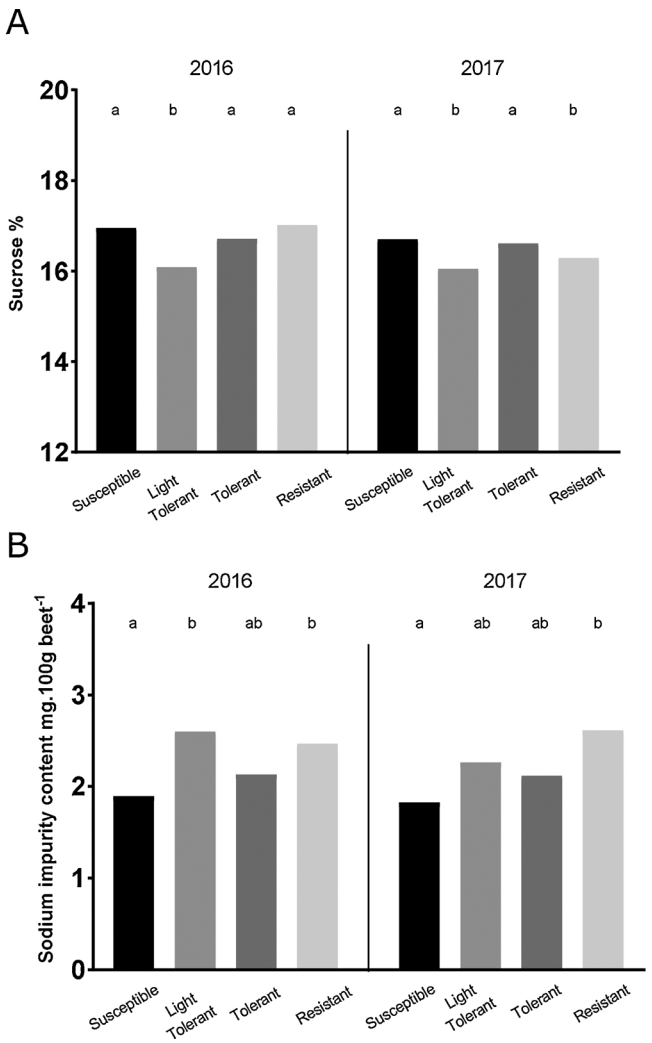


Fig. 7. Mean levels of sucrose in the storage root (A) ($P < 0.001$ for both years) and sodium impurities (B) ($P = 0.006$ in 2016, $P = 0.014$ in 2017) in the sugar beet measured in the samples at harvest from the field experiments at Sutton Bonington. Significant differences were only detected between the varieties and seed rate had no effect on sucrose % or sodium impurities measured. Lower case letters which are different indicate a significant difference at $P = 0.05$.

would require the plants to be grown in soil rather than hydroponics. However, the light tolerant varieties did show enhanced root growth in the pouches compared to the other varieties tested. It is possible that the enhanced root growth observed in the light tolerant varieties may be the mechanism which allows these varieties to outyield susceptible sugar beet varieties at low BCN population. Growing the variety in infested conditions would be needed to test this hypothesis.

4.3. Impurities

The impurity levels found in the pot experiment and two years of field experiments show that the resistant varieties always had significantly greater levels of sodium than the susceptible control varieties. Sugar beet is known to be able to use sodium as a replacement osmoticum in cases of insufficient potassium availability (Subbarao et al., 2003) and the elevated levels may be due to the varieties being more closely related to wild *P. procumbens* and therefore have different osmotic requirements than the elite susceptible varieties usually grown in the UK. However, the enhanced sodium levels seen in the resistant varieties might also be due to the resistance mechanism to BCN. The higher concentration of sodium may make conditions less favourable to the nematode when they establish their feeding cell (syncytium) (Kyndt et al., 2013) in the roots of the sugar beet and assist with the destruction of the syncytium via the hypersensitive response (Heijbroek et al., 1983; Huang, 1998).

The associated sugar losses due to raised impurity levels must still be considered (Dutton and Huijbregts, 2006) as BCN resistant varieties would be less favourable to sugar processors due to this. Although, if it could be demonstrated that resistance and impurity levels are not associated, breeding could be expected to develop BCN resistant varieties with reduced impurity levels in the future.

5. Conclusions

Tolerant varieties appear to have equally vigorous canopy expansion patterns compared to susceptible varieties. This finding is supported by yield results from sugar beet variety trials (BBRO, 2017). However, no obvious physiological differences have been measured during this investigation to highlight BCN tolerance mechanisms, and they may only reveal themselves in BCN infested conditions. The importance of quantifying canopy development has been shown and the techniques used during this research may be used in other aspects of sugar beet variety breeding.

Most importantly, this study has revealed more about the agronomy of BCN resistant varieties. It has demonstrated that they have a less vigorous canopy which can be overcome and manipulated by adjusting the plant population density. More experiments on more sites and over a range of seasons would reveal if an increased seed rate could be justified and calculate exactly how much higher the seed rate of a BCN resistant variety needs to be to compensate for the delayed canopy expansion. This is vital to assist such varieties becoming listed for use in new markets, such as the UK, and allow growers to benefit from their active control mechanisms for BCN and maintain and enhance yields on BCN infested fields regularly cultivated with sugar beet.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eja.2018.05.005.

References

Asadi, M., 2005. Beet-Sugar Handbook. http://dx.doi.org/10.1002/9780471790990.

Atkinson, J.A., Wingen, L.U., Griffiths, M., Pound, M.P., Gaju, O., Foulkes, M.J., Le Gouis, J., Griffiths, S., Bennett, M.J., King, J., Wells, D.M., 2015. Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. J. Exp. Bot. 66, 2283–2292. http://dx.doi.org/10.1093/jxb/erv006.

Ballaré, C.L., Pierik, R., 2017. The shade-avoidance syndrome: multiple signals and ecological consequences. Plant. Cell. Environ. http://dx.doi.org/10.1111/pce.12914.

BBRO, 2017. Sugar Beet Recommended Variety List for 2018. [WWW Document]. URL. Date Last (Accessed: 13 March 2018). https://bbro.co.uk/media/1251/2018-sbt-rl-varieties-tables-final-27mar2017.pdf.

BBRO, 2014. BBRO Recommended Sugar Beet Variety List 2015. [WWW Document]. URL. Date Last (Accessed: 13 March 2018). https://bbro.co.uk/media/1148/2015-rl-table-final.pdf.
British Sugar, 2017. Sugar Beet Seed Prices 2018. Peterborough, United Kingdom. .
Bussell, J., Sparkes, D., Mooney, S., Broadley, M., 2016. Identifying rooting traits for optimal nutrient uptake. In: 75th IIRB Congress. International Institute for Beet Research. pp. 70.
Clark, R.T., Famoso, A.N., Zhao, K., Shaff, J.E., Craft, E.J., Bustamante, C.D., Mccouch, S.R., Aneshansley, D.J., Kochian, L.V., 2013. High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant., Cell. Environ. 36, 454–466. http://dx.doi.org/10.1111/j.1365-3040.2012.02587.x.
Cooke, D.A., 1987. Beet cyst nematode (*Heterodera Schachtii* Schmidt) and its Control on Sugar Beet. Agric. Zool. Rev. 2, 135–183.
Dewar, A.M., Cooke, D.A., 2006. Pests. In: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing, pp. 316–358.
Dutton, J., Huijbregts, T., 2006. Root quality and processing. In: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing, pp. 409–442.
Flint-Garcia, S.A., 2013. Genetics and consequences of crop domestication. J. Agric. Food Chem. 61, 8267–8276. http://dx.doi.org/10.1021/jf305511d.
Gummerson, R.J., 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. J. Exp. Bot. 37, 729–741. http://dx.doi.org/10.1093/jxb/37.6.729.
Hallett, S.H., Sakrabani, R., Keay, C.A., Hannam, J.A., 2017. Developments in land information systems: examples demonstrating land resource management capabilities and options. Soil Use Manage. 514–529. http://dx.doi.org/10.1111/sum.12380.
Hauer, M., Koch, H.J., Krüssel, S., Mittler, S., Märländer, B., 2016. Integrated control of *Heterodera schachtii* Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. Appl. Soil. Ecol. 99, 62–69. http://dx.doi.org/10.1016/j.apsoil.2015.11.017.
Heijbroek, W., Roelands, A.J., De Jong, J.H., 1983. Transfer of resistance to beet cyst nematode from Beta patellaris to sugar beet. Euphytica 32, 287–298. http://dx.doi.org/10.1007/BF00021437.
Held, L.J., Jennings, J.W., Koch, D.W., Gray, F.A., 2000. Economics of trap cropping for sugarbeet nematode control. J. Sugar Beet Res. 37, 45–55.
Hemayati, S.S., Jahad-e Akbar, M.R., Ghaemi, A.R., Fasahat, P., 2017. Efficiency of white mustard and oilseed radish trap plants against sugar beet cyst nematode. Appl. Soil. Ecol. 119, 192–196. http://dx.doi.org/10.1016/j.apsoil.2017.06.017.
Huang, J., 1998. Mechanisms of resistance. In: Sharma, S.B. (Ed.), The Cyst Nematodes. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 353–368.
Jaggard, K.W., Qi, A., 2006. Agronomy. Sugar Beet. Blackwell Publishing Ltd, pp. 134–168. http://dx.doi.org/10.1002/9780470751114.ch7.
Kerr, S., Stevens, M., 2014. Sugar beet varieties for 2015. Br. Sugar Beet Rev. 82, 16–19.
Koch, D.W., Gray, F.A., 1997. Nematode-resistant oil radish for *Heterodera schachtii* control in sugar beet-barley rotations. J. Sugar Beet Res. 34, 31–43.
Krüssel, S., Warnecke, H., 2014. Effects of sugar beet varieties on population dynamics of *Heterodera schachtii* (Schmidt), vol. 138. Sugar Ind, pp. 60–67.
Kyndt, T., Vieira, P., Gheysen, G., de Almeida-Engler, J., 2013. Nematode feeding sites: unique organs in plant roots. Planta 238, 807–818. http://dx.doi.org/10.1007/s00425-013-1923-z.
Lazzeri, L., Tacconi, R., Palmieri, S., 1993. In vitro activity of some glucosinolates and their reaction products toward a population of the nematode *Heterodera schachtii*. J. Agric. Food Chem. 41, 825–829. http://dx.doi.org/10.1021/jf00029a028.
Ministry of Agriculture, Fisheries and Food (Ed.), 1977. The Beet Cyst Nematode Order. Ministry of Agriculture, Fisheries and Food, pp. 988.
Müller, J., 1999. The economic importance of *Heterodera schachtii* in Europe. Helminthologia 36, 205–213.
Müller, J., Rehbock, K., Wyss, U., 1981. Growth of *Heterodera schachtii* with remarks on amounts of food consumed. Rev. Nematol. 4, 227–234.
Panella, L., Lewellen, R.T., 2007. Broadening the genetic base of sugar beet: introgression from wild relatives. Euphytica 383–400. http://dx.doi.org/10.1007/s10681-006-9209-1.
Perry, R.N., Moens, M., 2013. Glossary. Plant Nematology. CAB International, Wallingford, Oxfordshire, UK, pp. 523–531.
R Core Team, 2016. R Development Core Team. R A Lang. Environ. Stat. Comput. https://doi.org/http://www.R-project.org.
Rasband, W., 2016. ImageJ. U. S. Natl. Institutes Heal, Bethesda, Maryland, USA.
Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PLoS One 10. http://dx.doi.org/10.1371/journal.pone.0146021.
Stevanato, P., Trebbi, D., Panella, L., Richardson, K., Broccanello, C., Pakish, L., Fenwick, A.L., Saccomani, M., 2015. Identification and validation of a SNP marker linked to the Gene HsBvm-1 for nematode resistance in sugar beet. Plant. Mol. Biol. Rep. 33, 474–479. http://dx.doi.org/10.1007/s11105-014-0763-8.
Subbarao, G.V., Ito, O., Berry, W.L., Wheeler, R.M., 2003. Sodium - a functional plant nutrient. CRC. Crit. Rev. Plant. Sci. 22 (5), 391–416. http://dx.doi.org/10.1080/07352680390243495.
Thomas, C.L., Graham, N.S., Hayden, R., Meacham, M.C., Neugebauer, K., Nightingale, M., Dupuy, L.X., Hammond, J.P., White, P.J., Broadley, M.R., 2016. High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (*Brassica napus* L.). Ann. Bot. 118, 655–665. http://dx.doi.org/10.1093/aob/mcw046.
Zhang, C.L., Xu, D.C., Jiang, X.C., Zhou, Y., Cui, J., Zhang, C.X., Chen, D.F., Fowler, M.R., Elliott, M.C., Scott, N.W., Dewar, A.M., Slater, A., 2008. Genetic approaches to sustainable pest management in sugar beet (Beta vulgaris). Ann. Appl. Biol. 152 (2), 143–156. http://dx.doi.org/10.1111/j.1744-7348.2008.00228.x.

Addendum to Chapter 2

The ground cover estimated values stated in Table 3 are in percent

To support the description of the lines in Figure 6 see the table below:

Table of equations for Figure 6

Plant Population

Variety	2016				2017			
	Intercept	SE	Slope	SE	Intercept	SE	Slope	SE
Tolerant	11.08	1.1	2.23E-05	8.65E-06	16.375	0.271		
Light Tolerant	9.014	0.926	3.7E-05	7.18E-06	15.524	0.273	-7.1E-06	1.59E-06
Susceptible	12.16	1.02	6.51E-06	7.6E-06	15.591	0.264		
Resistant	8.97	1	2.68E-05	7.64E-06	15.995	0.257		

Inflection point

Variety	2016				2017			
	Intercept	SE	Slope	SE	Intercept	SE	Slope	SE
Tolerant	64.9	16.7	-0.825	0.27	6.68	1.97		
Light Tolerant	101.9	15.6	-1.448	0.256	5.79	1.98	0.1567	0.0357
Susceptible	32.8	12.6	-0.317	0.202	5.71	2.02		
Resistant	62.8	13.7	-0.785	0.213	5.81	2.1		

SE = Standard Error.

Chapter 3

A method to assess varieties of sugar beet for resistance and tolerance to *Heterodera schachtii* (Schmidt)

This article has been prepared as a journal article, but has not yet been submitted for review.

A METHOD TO ASSESS VARIETIES OF SUGAR BEET FOR RESISTANCE AND TOLERANCE TO *HETERODERA SCHACHTII (*SCHMIDT)

Assessing sugar beet varieties for *H. schachtii* tolerance/resistance

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Abstract:

The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, causes significant losses to sugar beet (*Beta vulgaris* ssp. *vulgaris*) crops across the globe. This study has developed a method, which with future refinement, could be used to test tolerance of sugar beet varieties to damage from BCN, and their resistance to BCN population multiplication.

Micro-plots were established using large plastic boxes, filled with top soil, in which 16 sugar beet were grown. Six varieties were used, which were selected for their reported tolerance or resistance to BCN. Half of the boxes were inoculated with hatched second stage BCN juveniles when the sugar beet were at the six leaf stage, the other boxes remained uninfested controls. Plants were grown for a full 'field' season and harvested before yield analysis was conducted and final BCN populations assessed. Physiological measurements of the beet were taken, including stomatal conductance, photosynthetic activity and leaf chlorophyll density to investigate tolerance mechanisms of the varieties.

Two of the tolerant varieties, when infested, showed an initial reduction and then recovery in their leaf chlorophyll levels over time compared to the susceptible variety which maintained lower chlorophyll levels when infested for the duration of the experiment. Consistent differences also were detected in the levels of stomatal conductance in both years with one tolerant variety showing an increase when infested and the resistant variety showing a decrease when infested. Despite these differences, no significant interaction between variety and infestation in terms of yield was found, although the varieties did have different yield potentials.

The nematode population results indicate that varieties of beet marketed as tolerant were in fact partially resistant to BCN. Quantification of their levels of resistance would assist growers' variety choices to manage BCN infestations. The soil box method, with further refinement, will enable the tolerance/resistance mechanisms to be studied further and used to uniformly assess future varieties of sugar beet.

Keywords:
Variety choice, *Beta vulgaris*, Beet cyst nematode, integrated pest management, physiology

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1. Introduction

It is estimated that annually nematodes cause over $80bn of losses to crops around the world (Nicol et al., 2011). One such nematode pest is *Heterodera schachtii* Schmidt, the beet cyst nematode (BCN), which has been a recognised pest of sugar beet crops since the mid-19th century. Beet cyst nematode is found worldwide, in over 40 countries (CABI, 2018), and in all major beet sugar growing regions (Dewar & Cooke, 2006). In Europe, it is estimated to cause annual losses to sugar beet crops in excess of €90 million (Müller, 1999), although with new tolerant and resistant varieties the levels of loss may be much lower (Blok et al., 2018). The nematode has a preference for organic, loamy and sandy soils and can cause yield losses of up to 60% in sugar beet crops, especially in situations of high infestation combined with drought (Cooke, 1987).

Since the withdrawal of licensed nematicides for use against BCN (Dewar & Cooke, 2006), there are limited control options available. Long rotations without suitable host species, which allow for the natural population decline over time (Cooke, 1987), were previously

enforced. For example, in Britain, infested fields were limited by law to cultivation of a BCN host species no more than one in five years (MAFF, 1977). However, such orders have been revoked and rotations can now be as frequent as one in three years of BCN host species. Therefore, other methods to manage BCN infestations are required to prevent future population build-up. Some methods, such as resistant brassica trap cropping (Hauer et al., 2016b; Wright et al., 2018a; Koch & Gray, 1997) or biological control (Schlatter, 2015) may be used, but increasingly, sugar beet variety choice is an important way to manage BCN and its impact on yield.

Since 2009, BCN tolerant varieties have been available in the UK. These have been developed by a range of sugar beet breeders by introgressing genes, such as *HsBvm-1* from the wild sugar beet relative *Beta vulgaris* ssp. *maritima* (Stevanato et al., 2015; Lange & De Bock, 1994). Such varieties protect the yield of the beet crop, on fields with BCN infestations, through traits which overcome or compensate for the damage caused by the BCN, although their exact mechanisms are unknown. Uptake of tolerant varieties has been successful and they now make up around 6% of the UK sugar beet area (Wright et al., 2018b) which is the same area estimated to be infested with BCN (Wright, 2017) indicating that growers with known BCN infestations are selecting tolerant varieties. However, it is anticipated that the actual area infested with BCN is greater than 6% but populations are currently at sub-clinical levels and therefore susceptible plants do not exhibit infestation symptoms. Tolerant varieties are believed to be a good solution to protect against the potential £3.5 million of estimated annual losses of sugar beet crops to BCN in the UK (Wright et al., 2017). However, there is still much more which needs to be understood about such varieties, such as their yield, quality

and ability to host BCN, and also any differences which exist between the tolerant varieties available to growers.

In the UK, tolerant varieties are submitted into recommended list trials operated by British Beet Research Organisation (BBRO) and British Society of Plant Breeders (BSPB) which are conducted on fields without BCN infestations. Therefore, yields of tolerant varieties are currently only formally assessed under non-infested conditions and the yields of the tolerant varieties under infestation are not independently evaluated. Instead, breeders own data are used to support tolerance claims, generated from a range of tests such as bioassays and field trials, but there is no standard method to compare the varieties. This is due to the complexity and size needed for sugar beet variety trials and the time needed to sample fields for BCN before and after growing the sugar beet, as well as the difficulty in finding fields with near homogenous distribution of BCN population densities. Therefore, a method to assess the yield performance of the tolerant varieties under a uniform infestation of BCN, and their effect on BCN fecundity, would provide a novel solution for variety selection.

Sugar beet varieties which are resistant, and unable to host BCN successfully, have been in use in Europe since the late 1990s (Müller, 1999) when the *HS1*^{pro1} gene was successfully introgressed into sugar beet from *Patellifolia procumbens* (Panella & Lewellen, 2007). Whilst their popularity with growers is lower than tolerant varieties, they do offer the advantage that they are able to actively kill invading juvenile nematodes by destroying their syncytium (feeding cell), preventing the nematode from reproducing (Cai et al., 1997) and reducing soil populations. However, a yield penalty associated with these resistant varieties exists (Dewar & Cooke, 2006; Blake, 2011), and they are not widely cultivated (Märländer et al., 2003).

Recent publications state that more modern resistant varieties may now have increased yield potentials (Hauer et al., 2016b; Wright et al., 2018b) so they may be more widely used in the future.

Plants infested with nematodes have previously been found to display altered physiological traits when compared with uninfested plants. For example, soybean *glycine max* plants, have been found to have reduced levels of leaf chlorophyll and photosynthetic activity when infested with the soybean cyst nematode (SCN), *Heterodera glycines* (Asmus & Ferraz, 2002). In contrast, other traits, such as stomatal conductance did not differ between the plants, regardless of the level of infestation of SCN. Recent studies in sugar beet infested with BCN have also shown similar results, with reduced chlorophyll levels in infested plants (Hauer et al., 2016a).

This study aimed to develop a method which can assess both the tolerance (yield and quality), and also the resistance to BCN (nematode population development), of sugar beet varieties under uniform infestation using micro-plots. Alongside the measurements of the final yield and final BCN population (*Pf*), comparisons were made of the responses of the different varieties in terms of chlorophyll content, photosynthetic activity and stomatal conductance during the growing season to test the hypothesis that the physiology of the plants is manipulated by the infestation of BCN and that tolerant varieties possess distinct characteristics allowing them to yield well even in the presence of BCN.

2. Materials and Methods

2.1 Experimental design

In 2016 and 2017, micro-plot experiments were established at the University of Nottingham's Sutton Bonington Campus, developed from a similar technique previously under development at Brooms Barn research station (Burks et al., 2011). Dolav 1000-ACE Boxes (Internal dimensions 585 mm high, 1128 mm wide, 928 mm deep) (Dolav, Watton, Norfolk, UK) were arranged in a randomised block design (2016 n=40, 2017 n=48) with four blocks. Each block consisted of ten boxes (increased to 12 in 2017) which were filled with 605 litres of sandy loam top soil (Marketed as 'Sports10', British Sugar, Peterborough, UK). The boxes were set up outside on a level concrete apron and the plants were rain fed with additional irrigation to avoid severe drought (see 2.1.2). In 2016, the boxes were filled on 24 March and subsequently allowed to settle for two weeks before being raked level prior to sowing. Sugar beet seeds of five varieties (Table 1) were sown into the box in a 4 x 4 grid (20 x 18 cm grid spacing) on 8 April 2016, resulting in sixteen plants in each box after thinning. In 2017, the boxes were filled on 24 February. Six varieties of beet (Table 1) were sown on 10 April and the grid spacing was increased to 22 x 26 cm to give each plant more space and sixteen plants were still sown. Each block had two boxes of each variety, one of which was infested with BCN as detailed in 2.2 and the other remained uninfested. The top soil had trace amounts of BCN, mostly unviable due to the soil recovery process from the beet sugar factory.

2.1.1 Agronomy

All boxes received the equivalent of 120 kg of nitrogen.ha^{-1} in 2016 using ammonium sulphate [$(NH_4)_2SO_3$], with the application split into 1/3 prior to sowing and the remainder prior to the plants reaching two true leaves. In 2017, ammonium nitrate was used [NH_4NO_3] and applied

at a rate of 80 kg of nitrogen.ha^{-1}: 50% applied pre and then the remaining 50% post emergence of the seedlings. The rate was reduced due to a large supply of available nitrogen in the top soil in 2017. Weeds, pests and diseases were managed through appropriate use of herbicides, insecticides and fungicides.

2.1.2 Irrigation

In 2016, the boxes were hand watered to prevent the crop from drought. A hose and lance was used to administer the water evenly across each box for the same period of time for each box. In 2017, an irrigation system was installed using dripline pipes and each box had 3 lengths of 1 metre pipe running between the rows of the sugar beet plants to deliver an even amount of water to each box when needed. Irrigation was administered when the

.

Table 1 - Descriptions of the varieties used in the box experiments conducted in 2016 and 2017 detailing their trait response to BCN, as described by their respective breeders.

Variety Number	BCN Status[+]	Trait Description	Breeder	Year first registered for cultivation in UK
1	Resistant	Plant able to inhibit BCN reproduction relative to a susceptible variety (Perry & Moens, 2013)	Syngenta	N/A
2	Light Tolerant	Able to yield well under a low infestation of BCN, but susceptible to poor yield under a high infestation (BBRO, 2014)	Syngenta	2014
3	Susceptible	Supports a very high level of reproduction of BCN, the counterpart of resistance and as a result yields poorly when grown in infested conditions (Perry & Moens, 2013)	Strube	2011
4	Tolerant (A)	Plant able to sustain growth and yield successfully in the presence of BCN. This trait is not related to resistance (Perry & Moens, 2013)	SES VanDerHave	2015
5	Tolerant (B)		Betaseed	2016
6[+]	Tolerant (C)		Strube	2012

[+] Only grown in 2017

+ As claimed by breeders when submitted to recommended list (RL) variety trials

uninfested susceptible variety boxes showed symptoms of wilting

2.2 BCN inoculation

2.2.1 Inoculation wells

To inoculate the plants with juveniles, four wells were made around each plant in a cross pattern, one well at the end of each cross and between 30-50 mm from the beet plant. A 1000µl pipette tip, which had had the most tapered 17 mm part removed, was used as the well and placed into the soil by about 20 mm into which hatched juveniles in solution were added.

2.2.2 Juvenile extraction

Juveniles were hatched from cultured cysts grown in a controlled environment room. Cysts were cultured on oilseed rape (*Brassica napus*) and were extracted from the soil using a Wye Washer (Winfield et al. 1986). Juveniles were then hatched from the cysts using an adapted method of the IRS (Institute for Sugar Beet Research, The Netherlands) as follows: the extracted BCN cysts were placed onto four 220 mm diameter milk filters: two Hygia FavoritII filters (NIFA, Leeuwarden, The Netherlands) and two Type S475-30 filters (Lekko B.V., Veenendaal, The Netherlands) layered alternately. These were held between two rings of plastic and supported by a mesh base. The rings and filters were then placed in a plastic saucer of 25 cm diameter. The cysts were added and the saucer filled with water until the top of the milk filters became damp. The cysts were then placed on a bench in the laboratory and covered. After three days the water, which now contained many thousands of juveniles, was decanted off. In order to obtain only viable juveniles the water was then poured onto a single milk filter (Hygia favoritll filter). Over a four hour period the juveniles were allowed to migrate through the filter and then the water, which contained the viable juveniles was removed. The

density of the juveniles was checked under the microscope and diluted to provide 4 ml of inoculum per plant, i.e. 64 ml per box per inoculation.

2.2.3 Juvenile inoculation

The juveniles were held in suspension in a beaker of water using a mobile magnetic stirrer (Stuart SM27, Cole-Palmer, Staffordshire, UK). A pipette was then used to extract 50 ml of juvenile solution (Eppendorf Multipette M4 and 50ml combitip, Eppendorf, Hamburg, Germany) and dispensed in 1 ml doses into each well.

The hatching of the juveniles was repeated over three periods in each year. In 2017, the same method was used but the infestation level was increased and occurred earlier (Table 2). The plants were inoculated when at the six leaf stage.

2.3 Physiological measurements

In 2016, an AP4 porometer (Delta T devices, Cambridge, UK) was used to measure stomatal conductance at regular intervals between June and August. In 2017 the range of measurements were increased through the use of a SPAD 502plus (Konica Minolta Inc. Tokyo, Japan) to measure leaf chlorophyll content and a Li-Cor LI6800 (Li-Cor Inc. Lincoln, NE, USA) to measure photosynthetic activity of the plants. The LI6800 was set-up as follows: a flow of 500 µmol/s, chamber temperature of 20°C, a saturating photosynthetically active radiation (PAR) light intensity of 1200µmol m^2/s, CO_2 400 µmol/mol and relative humidity at 50%. Photosynthetic measurements occurred between 9 and 14 October 2017, with a different block being measured each day between 09.00 and 12.00 on days without rain or strong winds. Parameters used from the data generated by the LI6800 were instantaneous rates of

CO_2 assimilation and transpired water, which were then used to calculate instantaneous water use efficiency (iWUE) of the sugar beet plants (Condon et al., 2002).

The central four plants in the middle of the boxes were used for all physiological assessments. Measurements were only conducted on healthy, fully expanded leaves and leaves at the same stage of development were used on each date.

Table 2 – Inoculation doses and timings (days after sowing) of inoculation of second stage BCN juveniles (J2s) in 2016 and 2017 .

	Inoculation 1 J2 per plant (DAS)	**Inoculation 2 J2 per plant (DAS)**	**Inoculation 3 J2 per plant (DAS)**	**Total inoculation per plant (J2)**
2016	736 (59)	1232 (62)	640 (67)	2608
2017	1472 (48)	3200 (51)	1096 (53)	5768

2.4 Harvest

Beet were harvested on 15 November 2016 and 8 November 2017 respectively. Fifteen beet were lifted and bagged prior to being sent for sugar and impurity analysis at the BBRO tarehouse at the Wissington Beet Sugar factory in Norfolk, UK. The remaining beet was retained to determine biomass. The sample was weighed dirty and then washed and reweighed which allowed the soil tare to be calculated. Sugar content of the beet was calculated using polarimetry, impurity levels of the beet were determined using flame photometry (sodium and potassium impurities) and colourimetry (amino nitrogen) according to standard methods (ICUMSA, 2007). Using the impurity data the alkalinity ratio produced by each sample was calculated as the ratio of combined sodium and potassium impurities to the amino nitrogen impurities (Dutton & Huijbregts, 2006). Higher alkalinity ratios lead to

poorer quality beet which makes extracting sugar more difficult, and results in sugar being lost to molasses during extraction (C Parker, Pers. Comm – Development Manager, British Sugar).

2.5 BCN final population determination

The soil in the centre of each infested box (approximately central 50 x 50 cm) was sampled for BCN two weeks after harvest in both years. Twenty soil cores (25 mm diameter, 150 mm depth) were collected from each box using an AMS EZ-Eject soil probe (AMS Inc. American Falls, ID, USA) to assess BCN populations in the top section of the box as this was the area into which the juveniles were inoculated into. The soil was thoroughly mixed and passed through a 4 mm mesh sieve. A 200 ml subsample of soil was removed and the cysts extracted using a Wye Washer (Winfield et al., 1987). Cysts were then counted, crushed (Reid, 1955), diluted in 50 ml of water and the mean egg and juvenile contents of 1 ml of the solution determined using a stereo microscope (Leica M80, Leica, Wetzlar, Germany) and counting slide (Fenwick, 1951).

2.6 Data analysis

Data were analysed using GenSTAT 17th edition (VSN international, Hemel Hempstead, UK) using appropriate two-way ANOVA and calculation of the least significant difference. SPAD data (leaf chlorophyll content) were analysed using a repeated measures ANOVA. Graphs were produced using Microsoft Excel (Microsoft Corp., Redmond, Washington, USA).

3. Results

3.1 Final BCN populations

The primary aim of this study was to quantify any differences in the final populations of BCN achieved on the varieties studied, using a uniform source of inoculum. In both years, significant and consistent differences in the final populations of BCN were found (Fig. 1). The resistant variety produced the lowest final population (*Pf*) of BCN compared to all other varieties. The tolerant varieties produced the next lowest *Pf*, and were not significantly different to each other, or to the resistant variety, in either year. In 2016, the susceptible variety produced the second greatest *Pf*, which was significantly greater than the tolerant and

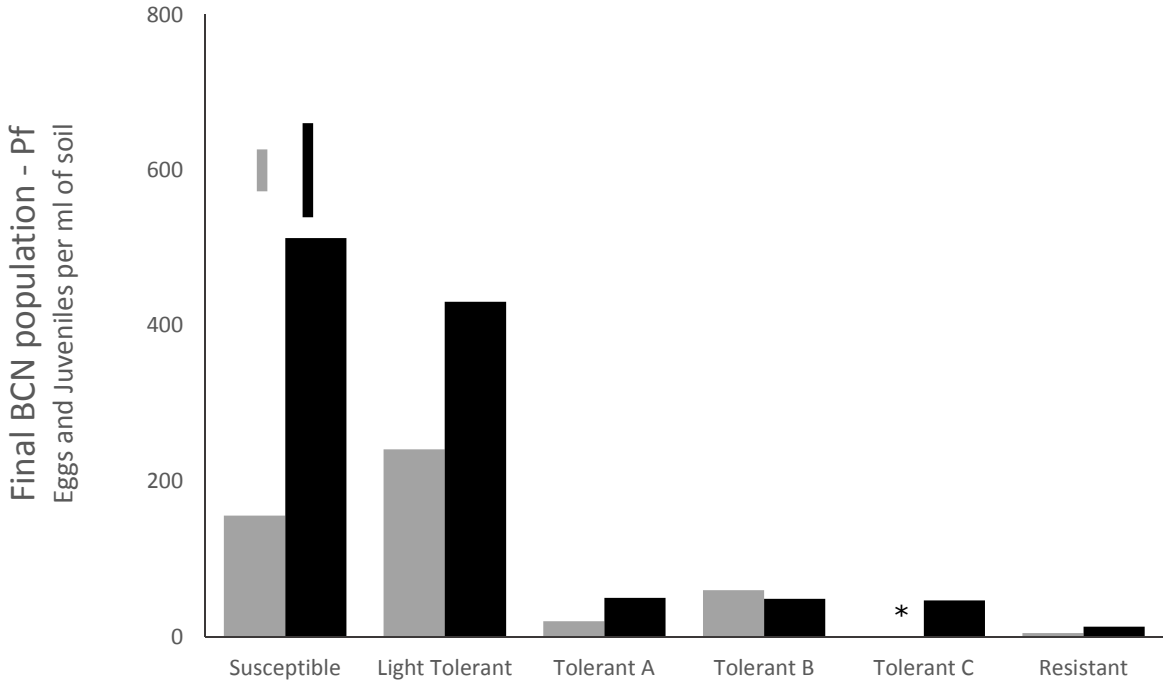


Figure 1 – Final populations (Pf) of BCN from soil in the infested boxes in 2016 and 2017. Soil was sampled from the central area of the box two weeks after harvest of the sugar beet plants. Error bars show LSD at 5% (P<0.001 for both years). * indicates that there is no data for this variety in 2016 as it was not grown that year.

resistant varieties but significantly lower than the light tolerant variety. In 2017, the light tolerant variety produced significantly more BCN than the tolerant and resistant varieties ($P<0.001$), but fewer, although not significantly less, than the susceptible variety.

3.2 Physiological results

3.2.1 Leaf chlorophyll content

Significantly different responses to infestation were measured in terms of chlorophyll content over time in the 2017 experiment (Fig 2). When infested, the susceptible variety had consistently lower levels of chlorophyll over the course of all measurements. Conversely, the resistant variety only showed a significant reduction in chlorophyll content, in response to infestation on one date (21 July). The light tolerant variety showed no response to infestation on the first two measurement dates but the leaves of the infested plants had significantly lower levels of chlorophyll for the last three measurements (25 and 30 July and 4 August) and showed no sign of recovery.

Tolerant varieties A and C showed a recovery in chlorophyll levels, when infested, after an initial decline. In tolerant variety A, the differences were no longer evident after the 21 July and in tolerant variety C, the differences had disappeared by the final measurement on 4 August. Tolerant variety B showed a different trend, with the chlorophyll content of the infested plants always being lower than non-infested plants and significantly different at the three measurement dates, including the final measurement, and showed no sign of chlorophyll level recovery.

3.2.2 Stomatal conductance

Whilst there was a high level of variation within the stomatal conductance measurements in both years, at several time points significant responses were detected.

In 2016, a significant interaction was found between variety and infestation in the first two (Fig 3a and 3b) of five measurements taken when tolerant variety A was shown to increase its stomatal conductance in response to BCN infestation on both occasions (P=0.014 at 7 July, P=0.014 at 13 July). During the later measurements there were no significant interactions which may be due to the beet suffering from water stress.

In 2017, tolerant variety A again showed a similar trend to 2016 at the first assessment (14 July) which was to increase stomatal conductance when infested with BCN (Fig 3c), and once again the resistant variety showed a decrease in stomatal conductance (P=0.048). At the final two measurements there were no significant interactions.

3.2.3 Photosynthesis measurements

Measurements were taken towards the end of the growing season in 2017, between the 9 and 14 October, to investigate any differences in photosynthesis, transpiration and water use efficiency of the plants. No significant interaction was measured in terms of photosynthesis or transpiration levels between the infested and non-infested plants or between the varieties themselves. However, when iWUE was calculated, significant varietal differences were found (Fig 4) (P= 0.008). Tolerant variety A had significantly reduced levels of iWUE than tolerant variety B and the susceptible variety, and tolerant variety C showed a reduced iWUE compared to other varieties except tolerant variety A.

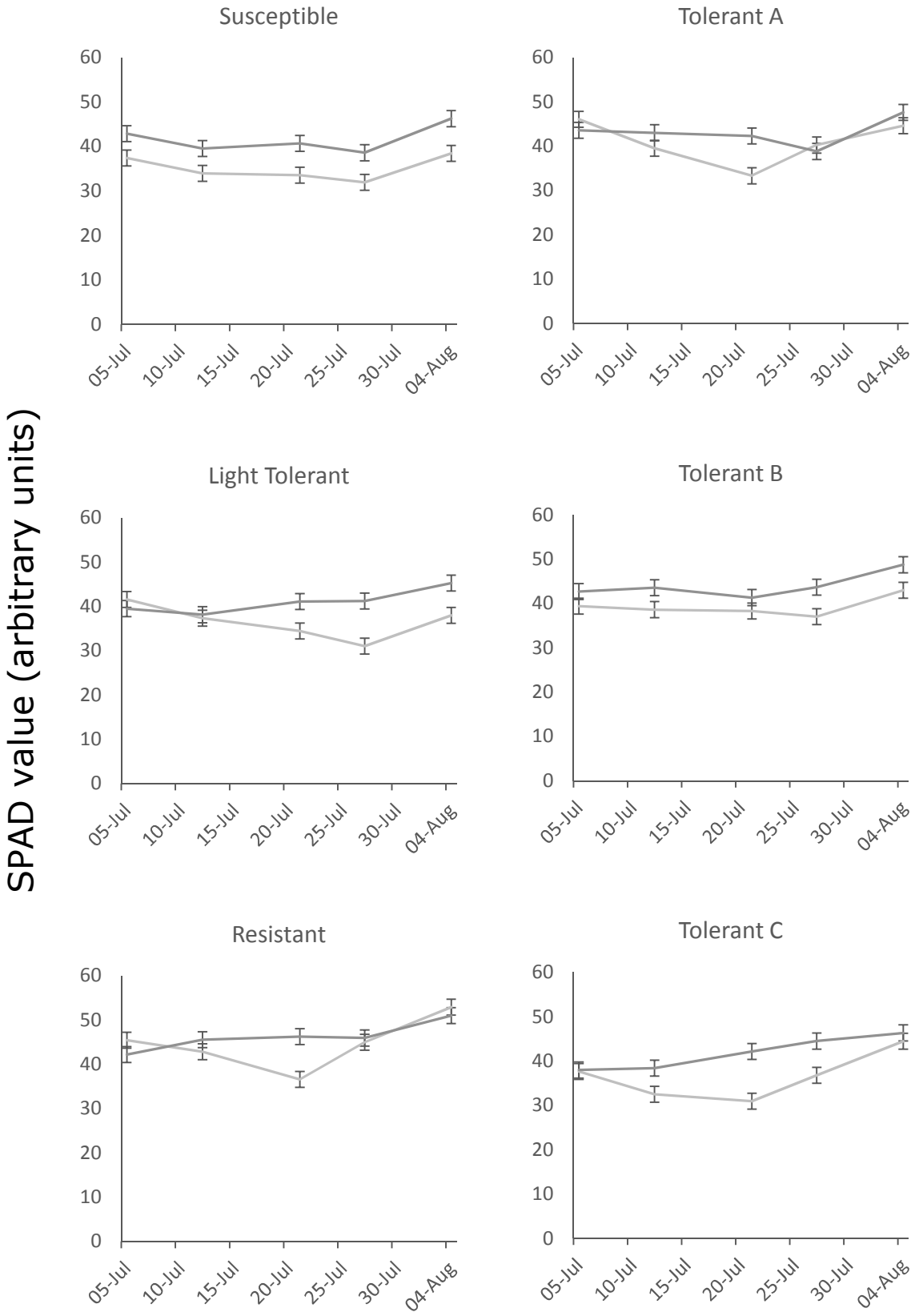


Figure 2 – Chlorophyll measurements (SPAD) taken throughout July and August 2017 on the six varieties of sugar beet being grown (Infested Non infested). Variety x Infestation x Time interaction P=0.014, Error bars show LSD at 5%.

3.3 Sugar beet yield and quality

There was no significant interaction between variety and infestation in either year of the experiment. However, significant yield differences between the varieties were found, and this reflected their differing yield potentials (Table 3). Whilst not significant, the susceptible variety had a 5% yield penalty when infested in 2016 but this was not evident in 2017, possibly because of regular irrigation. Interestingly, the resistant variety yielded just as well as some of the tolerant varieties in both years.

Table 3 –Yields of sucrose of infested and non-infested sugar beet plants and mean yields (kg of sugar per box) from the box experiments at Sutton Bonington in 2016 and 2017.

	2016			2017		
Variety	Non Infested	Infested	Mean	Non infested	Infested	Mean
Susceptible	2.59	2.46	2.52	2.68	2.67	2.67
Light Tolerant	2.62	2.61	2.62	2.72	2.65	2.69
Tolerant A	2.37	2.25	2.31	2.56	2.62	2.59
Tolerant B	2.59	2.62	2.61	2.89	3.00	2.94
Tolerant C*				2.53	2.57	2.55
Resistant	2.37	2.48	2.43	2.72	2.58	2.65
P	-	-	<0.001	-	-	<0.001
LSD			0.123			0.106

* Tolerant C only grown in 2017.

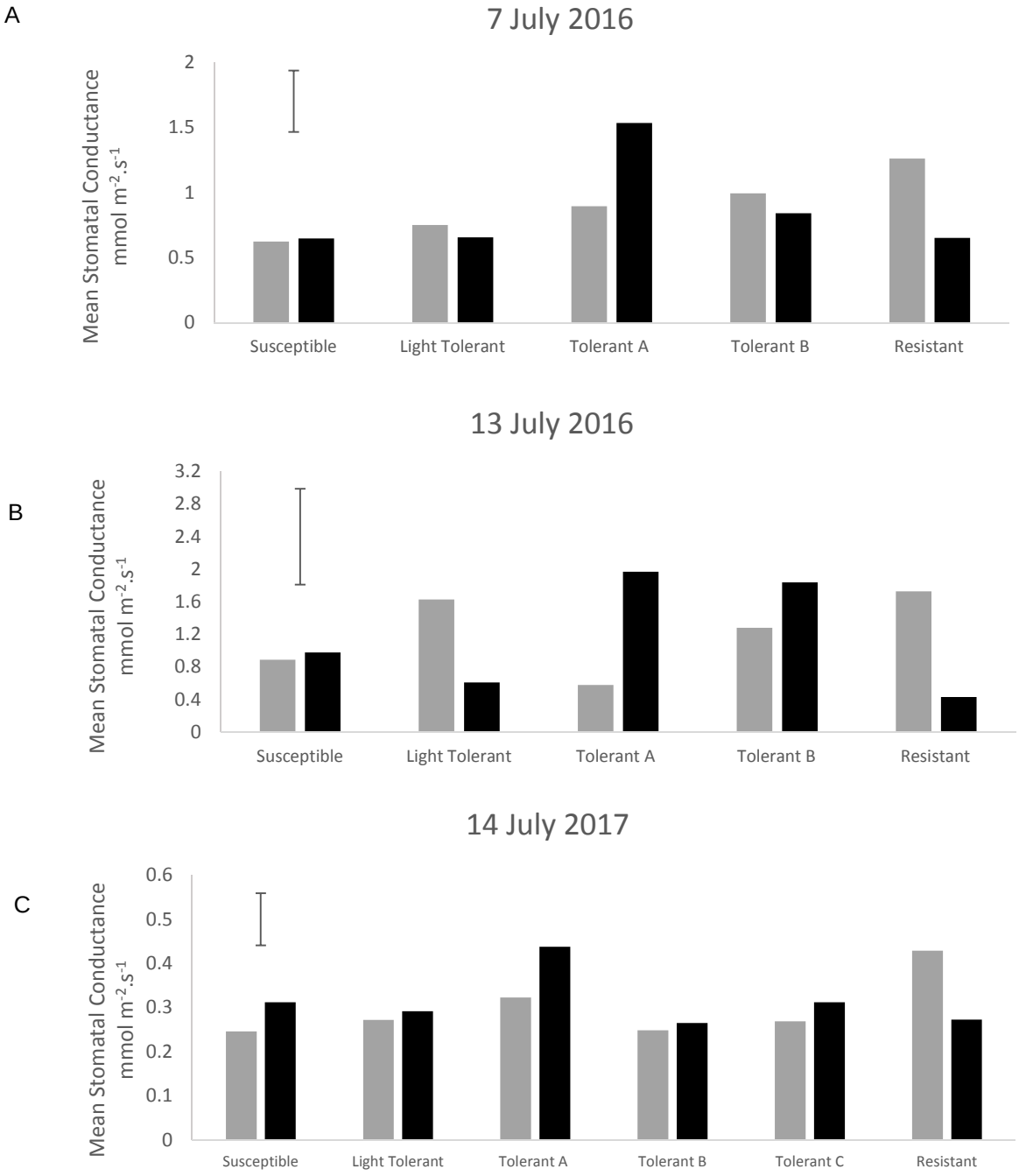


Figure 3 – Mean stomatal conductance from the infested ■ and non infested ■ plants grown in the boxes in 2016 (A and B) and 2017 (C). Only data from dates where a significant variety x infestation interaction was detected are shown. Error bars show LSD at 5%, P= 0.014 for A and B, P =0.049 for C.

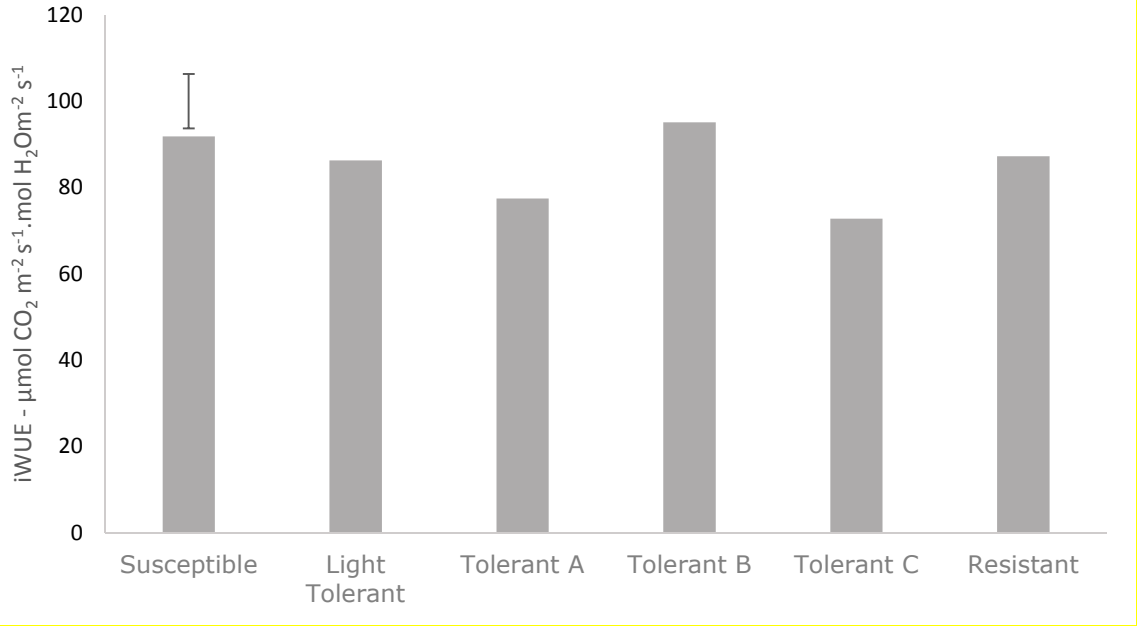


Fig 4. Instantaneous water use efficiency (iWUE) measured from the six sugar beet varieties grown in 2017 using a Li-Cor Li6800 during October (P= 0.008). Error Bar shows LSD at 5% significance.

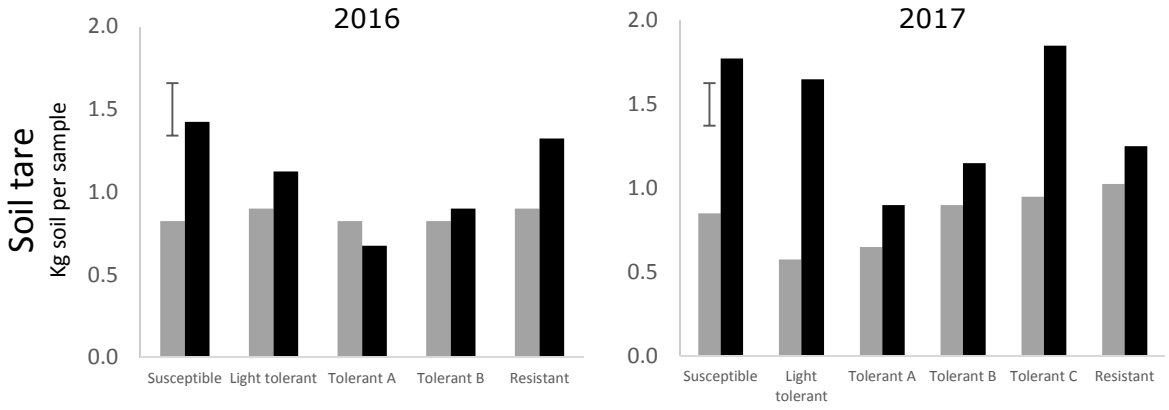


Figure 5 – Mean soil tare (mass of soil retained on the storage roots) from the 15 beet storage roots from each box. Non infested plants- ■ Infested plants - ■. Error bars show LSD at 5% (2016 P =0.018, 2017 P<0.001)

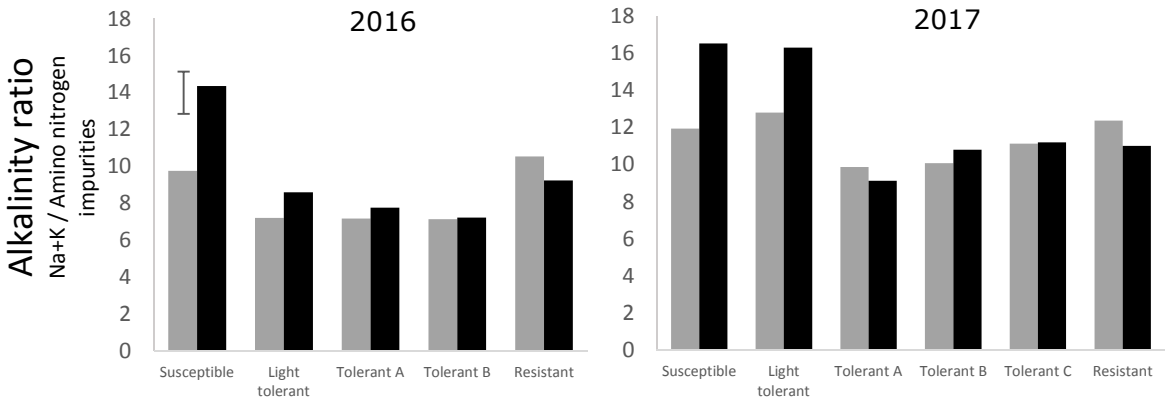


Figure 6 – Alkalinity ratio of the impurities found in the roots of the sugar beet. Error bar shows LSD at 5% in 2016 when significant differences were found (P=0.013). Non infested – ■ Infested - ■

In both years the susceptible variety showed a significant increase in soil tare when infested. Tolerant variety C in 2017 showed a significant increase in soil tare, along with the light tolerant variety whereas tolerant varieties A and B did not increase soil tare when infested with BCN in either year (Fig. 5). The light tolerant variety only showed a significantly higher soil tare in 2017. The resistant variety also shows a significant increase in soil tare in 2016 when infested. In 2016, the alkalinity ratio of the susceptible variety increased when infested with BCN while the other varieties showed no significant response to infestation. In 2017, a similar trend was observed, however it was not significant (Fig. 6). Analysis of the individual impurities showed that potassium impurity levels drove differences observed in the alkalinity ratio, since sodium and amino nitrogen levels had no significant variety and infestation interaction.

4 Discussion

Our investigation has developed a method which allows sugar beet to grow for` an entire season, and therefore let BCN populations develop over multiple generations. The use of hatched juveniles allows for even inoculation and direct comparison between varieties without the need for soil sampling prior to sowing sugar beet. Our results relating to the final populations of BCN which developed on the sugar beet plants agree with findings of Hauer et al. (2016) who also showed that tolerant sugar beet resulted in reproduction of BCN but to a lesser extent than a susceptible variety, apart from on fields with very high infestations where the susceptible, tolerant and resistant varieties all caused a population reduction. Westphal (2013) also found that susceptible varieties produce a significantly higher *Pf* than tolerant varieties, and resistant varieties result in a *Pf* significantly lower than both the tolerant and susceptible varieties. Likewise, it has been previously shown that tolerant varieties vary in nematode reproduction (Reuther et al., 2017), as seen in our results with the light tolerant variety producing higher populations than the tolerant varieties.

Quantification of the reproduction rates of BCN will allow sugar beet growers to decide which varieties may be better for long term BCN management. The light tolerant variety would build the *Pf* to a significantly higher level than a tolerant variety, and therefore pose a greater risk to future cropping. From our results, it appears that BCN tolerant varieties should be classified as partially resistant as the *Pf* is lower than the susceptible variety, but they do not show complete resistance. This may be due to the breeding selection process as mass selection for resistance for BCN was applied to lines bred from *B. vulgaris* ssp. *maritima* (Lange & De Bock, 1994) and then the lines with the highest levels of resistance were crossed with high yielding lines to develop the tolerant varieties. This conclusion is supported by our data and also data

from Reuther et al. (2017) who proposed that tolerant varieties fall into a spectrum of resistance, however, from our findings we must doubt whether the light tolerant variety has any form of tolerance and probably should be considered as another susceptible variety.

Consistent reproduction on the tolerant varieties may also be useful for population forecasting. Whilst only a selection of the tolerant varieties available were used in these experiments, using further varieties and a range of different inoculation timings, and doses, may provide information to tailor variety choice to specific field population levels and balance the potential yield of these varieties with their ability to increase BCN *Pf*.

Gopal et al. (2017) found that the penetration of roots of sugar beet by invading BCN juveniles was not affected by variety tolerance or resistance to BCN. However, root staining revealed that the ratio of female:male nematodes which develop is significantly reduced in resistant varieties compared to a susceptible variety, and tolerant varieties also result in an increased proportion of males to females (Gopal et al., 2017), and would lead to fewer cysts developing which was also seen in our results. It seems likely therefore that tolerant varieties interfere with the establishment of the syncytia of developing BCN juveniles to cause these changes.

The different responses of the varieties to infestation, in terms of leaf chlorophyll, appear to be linked to BCN reproduction (Fig 1). The varieties with the greatest levels of BCN reproduction (susceptible and light tolerant) show large and significant reductions in their chlorophyll levels when infested, especially at the later measurements and this is consistent with findings in Germany in infested field sites (Hauer et al., 2016a). It is likely that the nitrates which are usually re-assimilated from older to new leaves (Cariolle & Duval, 2006) are instead

being diverted to the roots and ultimately the syncytium of the feeding nematodes. As female nematodes require 29 times the nutrition of a male (Müller et al., 1981), those varieties with the lower *Pf* and fewer cysts would have resulted in a reduced nutrient demand from the host plants. The varieties which show recovery of chlorophyll levels tended to support fewer nematodes, and therefore less feeding would have occurred, leaving more nitrates available for the plant. The exception is tolerant variety B, which although produced a relatively low final *Pf*, the infestation with BCN still had a sustained and negative effect on leaf chlorophyll levels.

Chlorophyll level reductions may not have resulted in a negative impact on yield. It has been shown that a decrease in nitrogen in a crop does not change the light conversion coefficient (Jaggard & Qi, 2006) and may explain why a significant decrease in yield in the susceptible variety was not detected. A greater or earlier infestation of the plants may be required to elicit such a response and reduce nitrogen levels sufficiently to cause a negative impact on yield, as is often seen on infested plants in the field. In a study of *Heterodera glycines* on susceptible soybean (*Glycine max*) a reduction in leaf chlorophyll level was found which increased with inoculum density (Asmus & Ferraz, 2002) and it is possible that a similar response may be seen in sugar beet. Experiments with a range of inoculum levels would be needed to test this.

Although additional hand watering and irrigation was administered, the boxes dried out very rapidly on hot days and, when watered in the mornings, the plants would become stressed by the afternoon and therefore differ from plants grown in the field which have deeper root systems and access to greater amounts of water. The excessive drought stress experienced

by the plants grown in boxes may mean that they all became droughted and closed their stomata to conserve water, thus reducing stomatal conductance (Morillo-Velarde & Ober, 2006). However, our data suggest that BCN tolerant variety A may be able to increase stomatal conductance to counteract the negative consequences of infestation when there is no apparent drought stress, but this effect does not continue when the plants do become droughted, as the plant then conserves water. Tolerant varieties B and C did not show this response and neither did the susceptible or light tolerant varieties. The resistant variety shows the opposite response with reduced stomatal conductance when infested, which may be negative as it will limit photosynthetic activity. This response could be due to damage caused to the roots as part of the hypersensitive response to kill off the feeding juveniles and interruptions in rooting development and thus less water is taken up by the plant. It may be concluded that the different varieties have different stomatal conductance habits which then respond differently to infestation of BCN such as the increases seen in tolerant variety A and the decreases in the resistant variety.

We found no significant interaction between variety type and BCN infestation relating to water use efficiency (WUE), this finding is supported Rajabi et al., (2009) who showed that WUE of sugar beet varies due to genotype and other evidence by that Hauer et al. (2015) who also demonstrated that BCN infestation doesn't appear to affect the WUE of sugar beet, and that drought stress and nematode stress cannot be considered comparable. If the iWUE values we recorded represent total plant WUE, then tolerant varieties A and C were less efficient at using water in exchange for assimilated CO_2 than the other varieties, and would yield less sugar per unit of water transpired. These results correlate well with the yield of the varieties at harvest (Table 3). Earlier photosynthetic measurements, would have been

beneficial, especially in the warmer summer months when the nematodes would have been more active, however this was not possible due to the equipment not being available.

Our data agree with recent findings that modern resistant varieties may have an increased yield potential compared to their older counterparts and now yield similarly to susceptible varieties (Hauer et al., 2016b; Kenter et al., 2014; Wright et al., 2018b). Tolerant variety B shows the greatest yield potential of the tolerant varieties and this may be related to the higher iWUE measured; this variety may therefore also perform better under drought conditions: more experiments with this variety would be needed to confirm this.

The drought stress experienced by the plants may explain why we were unable to detect a significant response to BCN infestation, even in the susceptible variety in terms of sugar yield in either year. To use this method as a yield screen an alternative method of watering might be required.

Another important factor to measure at harvest is the soil tare, which and can be increased by excessive lateral rooting caused by disruption of auxin transport by the developing syncytia of the nematodes (Grunewald et al., 2009). Our findings relating to soil tare are important to consider in regards to the quality of sugar beet and grower returns. Understanding those varieties that retain the least amount of soil can benefit growers when selecting which varieties to grow as there are extra costs to clean and then deliver beet with high soil tares. The lack of response of two tolerant varieties may be explained by differences in the development of roots of tolerant sugar beet varieties. Mutants of *Arabidopsis* have been shown to prevent the development of large cysts of BCN when they lack proteins for auxin

transport due to the failure of the syncytial development (Grunewald et al., 2009). Tolerant varieties of sugar beet could use similar alternative auxin transport pathways which may explain both the lack of increased soil tare (caused by excessive lateral rooting) as well as their reduced reproduction of BCN. The fact we found significantly distinct responses between the tolerant varieties indicate various tolerance traits have been introduced to the varieties by different breeders.

The response seen in terms of alkalinity ratio demonstrates that the quality of the sugar beet can be negatively affected by an infestation with BCN, especially in the susceptible variety tested. This finding does not agree with Kenter et al. (2014) who found no effect of nematode infestation on the technical quality of beet produced when a susceptible and resistant variety were studied. However, in our study, the tolerant and resistant varieties appear to maintain their quality when infested. It seems likely that these interactions are very dependent on both the varieties and the climates in which they are grown. From our data we can conclude that, whilst the overall sugar yield shows no significant effect from the infestation in either year, the higher soil tares and impurities produced from infested plants result in lower overall yields, whereas a tolerant variety that maintains its quality and would be preferred by both sugar beet growers and processors alike.

Additional refinements need to be made to simulate more field-like nematode populations. Since our method achieved inoculations of less than one juvenile per ml of soil (considering only the top 150 mm of soil), higher and earlier doses may be needed to cause significant nematode damage to the plants. We delayed inoculations until the plants were well established at six weeks old, so that the root systems would be large enough to host a vast number of juveniles invading simultaneously, however, this may also then have limited the

impact of the nematodes especially as Griffin (1981) demonstrated the infestation of six week old plants showed the least impact on biomass compared to plants inoculated at zero, two or four weeks old. In addition, Olthof (1983) showed previously that infested soils result in more damage to sugar beet plants than when they are inoculated, so infested soil may prove more effective in the future to refine our method, although would be less uniform. Further research into techniques of testing will allow for optimisation of the infestation technique to produce significant yield differences.

5 Conclusions

Evidence from our experiments suggest that tolerant varieties possess mechanisms to adjust their physiological responses to the environment in response to infestation with BCN. These findings help to explain how tolerant sugar beet varieties are able to yield well, even when infested with BCN. The ability of the varieties to maintain lower soil tares and reduced impurity levels may be due to the tolerant varieties hosting fewer feeding female nematodes, and these results suggest that the tolerant varieties studied are also partially resistant to BCN. The ability of this method to uniformly test the resistance mechanism of the varieties to nematode infestation, may help to develop a larger scale variety test for BCN tolerant varieties. With further refinement to the inoculation method, such as earlier and more prolonged infestation, as well as multiple levels of inoculation, this would help growers and agronomists with information on the most suitable variety to grow under particular infestation levels. The boxes also allow for a much smaller area of land to be used compared to conventional sugar beet trials, improving the uniformity of the test and removing the need for in-field soil testing before planting the sugar beet. The method may also be useful for variety testing for other crop species, such as potatoes where the potato cyst nematodes

(*Globodera pallida* and *G. rostochiensis*) cause losses to potato crops worth up to £50 million per annum in the UK alone (Watts, 2013) .

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7 References

Asmus G.L., Ferraz L.C.C.B. (2002) Effect of population densities of Heterodera glycines race 3 on leaf area, photosynthesis and yield of soybean. *Fitopatologia Brasileira*, **27**, 273–278.

BBRO (2014) BBRO Recommended Sugar Beet Variety List 2015 Ed BBRO. , 1. Available at: https://bbro.co.uk/media/1148/2015-rl-table-final.pdf.

Blake A. (2011) Drought highlights hidden nematode threat. *Crop Production Magazine*, 56–57.

Blok V.C., Tylka G.L., Smiley R.W., de Jong W.S., Daub M. (2018) Resistance Breeding, In *Cyst Nematodes*, pp. 174–214. Eds M. Moens, R. N. Perry, and J. T. Jones. Wallingford, Oxfordshire, UK: CAB International.

Burks E., Morris N., Stevens M. (2011) Beating Cyst Nematode. *British Sugar Beet Review*, **79**, 26–29.

CABI (2018) Heterodera schachtii (beet cyst eelworm). *Invasive Species Compendium*. Available at: https://www.cabi.org/isc/datasheet/27036 [Accessed February 17, 2018].

Cai D.G., Kleine M., Kifle S., Harloff H.J., Sandal N.N., Marcker K.A., KleinLankhorst R.M., Salentijn E.M.J., Lange W., Stiekema W.J., Wyss U., Grundler F.M.W., Jung C. (1997) Positional cloning of a gene for nematode resistance in sugar beet. *Science*, **275**, 832–834.

Cariolle M., Duval R. (2006) Nutrition - Nitrogen, In *Sugar Beet*, pp. 169–184. Ed A. P. Draycott. Blackwell Publishing.

Condon A.G., Richards R.A., Rebetzke G.J., Farquhar G.D. (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Science*, **42**, 122–131.

Cooke D.A. (1987) Beet Cyst Nematode (Heterodera Schachtii Schmidt) and its Control on Sugar Beet. *Agricultural Zoology Reviews*, **2**, 135–183.

Dewar A.M., Cooke D.A. (2006) Pests, In *Sugar Beet*, pp. 316–350. Ed A. P. Draycott. Oxford: Blackwell Publishing Limited.

Dutton J., Huijbregts T. (2006) Root Quality and Processing, In *Sugar Beet*, pp. 409–442. Ed A. P. Draycott. Blackwell Publishing.

Fenwick D.W. (1951) A New Modification of the McMaster Slide for Use in Potato-root

Eelworm Investigations. *Journal of Helminthology*, **25**, 173–176. Available at: http://dx.doi.org/10.1017/S0022149X00018824.

Gopal H.K.K., Roeb J., Hallmann J., Vidal S. (2017) Resistance and tolerance in different sugar beet genotypes against the beet cyst nema-tode Heterodera schachtii, In *10th Young Scientists Meeting,* p. 57. Siebeldingen, Germany,.

Griffin G.D. (1981) The Relationship of Plant Age, Soil Temperature, and Population Density of Heterodera schachtii on the Growth of Sugarbeet. *Journal of Nematology*.

Grunewald W., Cannoot B., Friml J., Gheysen G. (2009) Parasitic nematodes modulate PIN-mediated auxin transport to facilitate infection. *PLoS Pathogens*, **5**.

Hauer M., Koch H.-J., Mittler S., Windt A., Krüssel S., Schlinker G., Wollenweber D., Rustemeyer C., Märländer B. (2016a) Catch crop cultivation, variety choice, N fertilization: effects on N supply and yield of sugar beet and on nematode infestation. *Sugar industry*, **141**, 44–52.

Hauer M., Koch H.J., Krüssel S., Mittler S., Märländer B. (2016b) Integrated control of Heterodera schachtii Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. *Applied Soil Ecology*, **99**, 62–69.

Hauer M., Koch H.J., Märländer B. (2015) Water use efficiency of sugar beet cultivars (Beta vulgaris L.) susceptible, tolerant or resistant to Heterodera schachtii (Schmidt) in environments with contrasting infestation levels. *Field Crops Research*, **183**, 356–364.

ICUMSA (2007) *ICUMSA Methods Book*. Berlin: Bertens.

Jaggard K.W., Qi A. (2006) Agronomy, In *Sugar Beet*, pp. 134–168. Blackwell Publishing Ltd. Available at: http://dx.doi.org/10.1002/9780470751114.ch7.

Kenter C., Lukashyk P., Lukashyk P., Daub M., Ladewig E. (2014) Population dynamics of heterodera schachtii schm. and yield response of susceptible and resistant sugar beet

(beta vulgaris L.) after cultivation of susceptible and resistant oilseed radish (raphanus sativus L.) | Populationsdynamik von heterodera schacht. *Journal of cultivated Plants*, **66**, 289–299.

Koch D.W., Gray F.A. (1997) Nematode-resistant oil radish for Heterodera schachtii control in sugar beet-barley rotations. *Journal of Sugar Beet Research*, **34**, 31–43.

Lange W., De Bock T.S.M. (1994) Pre-breeding for Nematode Resistance in Beet. *Journal of Sugar Beet Research*, **31**, 13–26.

MAFF (1977) The Beet Cyst Nematode Order 1977 Ed Ministry of Agriculture Fisheries and Food. *988*.

Märländer B., Hoffmann C., Koch H.J., Ladewig E., Merkes R., Petersen J., Stockfisch N. (2003) Environmental situation and yield performance of the sugar beet crop in Germany: Heading for sustainable development. *Journal of Agronomy and Crop Science*, **189**, 201–226.

Morillo-Velarde R., Ober E.S. (2006) Water Use and Irrigation, In *Sugar Beet*, pp. 221–255. Ed A. P. Draycott. Oxford, UK: Blackwell Publishing.

Müller J. (1999) The economic importance of Heterodera schachtii in Europe. *Helminthologia*, **36**, 205–213.

Müller J., Rehbock K., Wyss U., Rehbock K. (1981) Growth of Heterodera schachtii with remarks on amounts of food consumed. *Revue de Nematologie*, **4**, 227–234.

Nicol J.M., Turner S.J., Coyne D.L., Nijs L. den, Hockland S., Maafi Z.T. (2011) Current Nematode Threats to World Agriculture, In *Genomics and Molecular Genetics of Plant-Nematode Interactions*,

Olthof T.H.A. (1983) Effect of Plant Age and Transplanting Damage on Sugar Beets infected by Heterodera schachtii. *Journal of Nematology*.

Panella L., Lewellen R.T. (2007) Broadening the genetic base of sugar beet: Introgression from wild relatives, In *Euphytica*, pp. 383–400.

Perry R.N., Moens M. (2013) Glossary, In *Plant Nematology*, pp. 523–531. Wallingford, Oxfordshire, UK: CAB International.

Rajabi A., Ober E.S., Griffiths H. (2009) Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugar beet. *Field Crops Research*, **112**, 172–181. Available at: https://www.sciencedirect.com/science/article/pii/S0378429009000653#fig4 [Accessed August 8, 2018].

Reid E. (1955) A rolling method for opening cysts of potato root eelworm. *Plant Pathology*, **4**, 28–29. Available at: http://dx.doi.org/10.1111/j.1365-3059.1955.tb00730.x.

Reuther M., Lang C., Grundler F. (2017) *Nematode-tolerant sugar beet varieties - Resistant or Susceptible to the Beet Cyst Nematode Heterodera schachtii?*

Schlatter C. (2015) Clariva™ seed treatment nematicide, A breakthrough for sugar beet production, In *75th IIRB Congress*, p. 101. IIRB.

Stevanato P., Trebbi D., Panella L., Richardson K., Broccanello C., Pakish L., Fenwick A.L., Saccomani M. (2015) Identification and Validation of a SNP Marker Linked to the Gene HsBvm-1 for Nematode Resistance in Sugar Beet. *Plant Molecular Biology Reporter*, **33**, 474–479.

Watts W. (2013) *Integrated biomethods for the management of Globobodera pallida (Stone.)*. Harper Adams University.

Westphal A. (2013) *Vertical Distribution of Heterodera schachtii under Susceptible, Resistant, or Tolerant Sugar Beet Cultivars*.

Winfield A.L., Enfield M.A., Foreman J.H. (1987) A column elutriator for extracting cyst

nematodes and other small invertebrates from soil samples. *Annals of Applied Biology*, **111**, 223–231.

Wright A., Stevens M., Back M., Sparkes D. (2017) Interactions between Heterodera schachtii and Sugar Beet. *Aspects of applied biology*, **134**, 81–88.

Wright A.J.D. (2017) PhD Update- Beet Cyst Nematode. *British Sugar Beet Review*, **85**, 50–53.

Wright A.J.D., Back M.A., Stevens M., Sparkes D.L. (2018a) Evaluating resistant brassica trap crops to manage Heterodera Schachtii (SCHMIDT) infestations in eastern england. *Pest Management Science*. Available at: https://doi.org/10.1002/ps.5134.

Wright A.J.D., Bussell J.S., Stevens M., Back M.A., Sparkes D.L. (2018b) Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, Heterodera schachtii (Schmidt) under uninfested conditions. *European Journal of Agronomy*, **98**, 37–45. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1161030118301084 [Accessed May 30, 2018].

Chapter 4

Evaluating resistant brassica trap crops to manage *Heterodera schachtii* (Schmidt) infestations in eastern England

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Evaluating resistant brassica trap crops to manage *Heterodera schachtii* (Schmidt) infestations in eastern England

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Abstract

BACKGROUND: The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, is a plant parasitic nematode that causes severe yield losses in sugar beet. Resistant brassicas (radish and mustard) have been bred to be planted after harvest of the main crop, for example a cereal, and encourage BCN juvenile hatching. The resistant plants stimulate juvenile hatching but are not suitable hosts. Juveniles are unable to complete their lifecycle and thus populations are lowered. This research aimed to investigate the effectiveness of a range of brassicas in terms of BCN control when grown in infested fields in eastern England.

RESULTS: Experiments used four different radish cultivars, which differed in their resistance to BCN, and one resistant mustard variety. Field experiments were sown in early September in 2016 and 2017. Significant reductions in BCN populations were found only following resistant mustard and the radish with the greatest resistance level.

CONCLUSIONS: Further research is needed to understand how best to utilize brassicas and whether they are economically viable when alternative management options for BCN are available. Time of planting may be crucial to fully achieve their BCN-reducing potential.
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Keywords: beet cyst nematode; radish; mustard; nematode management; IPM

1 INTRODUCTION

The beet cyst nematode (BCN), *Heterodera schachtii* Schmidt, has been a well-known pest of sugar beet for over 150 years.[1] It can be found in all major sugar beet-producing areas of the world[2] and is a major problem for growers with infestations, especially in years of limited water supply.[3] Yield losses can be severe (30–40% on susceptible cultivars[4]) and have been calculated at as high as £3.8 million per year in the UK.[5] However, modern varieties of sugar beet with BCN tolerance may be able to overcome the majority of the yield losses.[6] However, these tolerant varieties still lead to a build-up of BCN populations,[6] which pose a problem for future sugar beet crops in the infested field and increase the chance of infested soil transfer to other fields. BCN is also harmful to a wide range of crop species, particularly brassicas such as oilseed rape (*Brassica napus*)[7] and, therefore, alternative methods of reducing populations are important, especially as chemical options for BCN control are no longer available.[8]

Use of BCN-resistant brassicas has become commonplace in Europe. They are a popular option in countries such as Germany, where BCN infestations are widespread and may exceed 50% of the area cultivated with sugar beet.[4] The use of oilseed rape as a trap crop was proposed in the late 19th century by Professor Julius Kühn.[1] Oilseed rape, which is susceptible to BCN, is planted to stimulate juvenile emergence and root invasion in the summer prior to planting sugar beet in the following spring. The oilseed rape seedlings need to be destroyed before the nematodes reproduce. Therefore, use of susceptible trap crops is a risk for the grower, because failure to destroy the crop before a completed lifecycle could exacerbate an infestation and this method was not implemented in the 1800s.[1] However, tools are now available to advise growers when to destroy oilseed rape volunteer seedlings in Germany to maximize BCN population control.[9] The use of resistant brassica varieties that offer an option for BCN control with much less risk to the grower may be more suitable. Resistant brassicas stimulate juvenile hatching from cysts in the soil, and although they allow for root invasion, are resistant to the nematode, and prevent completion of its lifecycle, hence lowering populations within the soil.[10] Resistant varieties of both white mustard (*Sinapis alba*) and oil radish (*Raphanus sativus*) are now available for cultivation as a trap crop and could be incorporated as part of an integrated pest management (IPM) strategy for BCN control.

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Correction added on 1 November 2018 after initial online publication: in section 2.2, "Field experiments", the initial (*Pi*) and final population (*Pf*) have been inverted in the formula to calculate the Reproductive factor (*Rf*).

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The use of trap crops for BCN control has been investigated in many locations worldwide, with mixed results. Research in Germany in the 1990s found reductions in BCN populations using brassica trap crops and improvements in the yield of subsequent beet crops[1] and similar results were found by Hafez[11] in the USA. However, Koch *et al.*,[12] also in the USA, found significant reductions in BCN populations using oil radish at only one of four sites. Kenter *et al.*[13] found BCN populations were lowered at 12 of 13 sites in Germany where resistant radish variety 'Adagio' was planted, and the best results were obtained where the cover crop was sown in July rather than August. Hauer *et al.*[6] found resistant mustard reduced populations of BCN at five of eight environments tested between 2013 and 2014, also in Germany. Mustard varieties Luna and Accent, and oil radish variety Colonel (the latter two are evaluated here) were found to reduce BCN populations at one site in Iran but did not produce significant reductions at a second site.[14] It appears, therefore, that the results of trap crop use are highly variable and further research into their use is needed, especially as different results are obtained by different researchers in different countries and in different years. Findings from one country cannot simply be applied to another and, likewise, the findings from one variety of trap crop cannot be expected to carry over to another variety.

It is clear that gaps remain in our understanding of the use of trap crops because their effects are variable between years and the countries in which the experiments are conducted, due to different climatic conditions and weather patterns, as well as time of sowing. We therefore decided to conduct two field trials, over 2 years, to see how a range of commercially available trap crop varieties might reduce BCN populations on infested fields in eastern England. To do this, on-farm field experiments were conducted in fields with known infestations of BCN.

2 MATERIALS AND METHODS

2.1 Brassica variety selection

Following recommendations from a seed merchant, five brassica treatments were selected for sowing in a field experiment. Four of the treatments reportedly showed some resistance to BCN in bioassays performed on the varieties in Germany.[6] These were: one Class 1 radish (with ≥ 90% resistance to BCN), two Class 2 radishes (70–90% resistance to BCN) and one Class 2 mustard. The final brassica treatment was a tillage radish with no reported resistance to BCN. A fallow treatment was also included in the experimental design. Details of the brassica treatments are listed in Table 1.

Table 1. Descriptions of the varieties of radish and mustard used in the field experiments in Norfolk, UK

Species	Cultivar name	BCN resistance class*	Seed rate kg ha^{-1}	Treatment name
Raphanus sativus	Colonel	1	22	Class 1 Radish
R. sativus	Defender	2	22	Class 2 Radish A
R. sativus	Bokito	2	20	Class 2 Radish B
R. sativus	Early Mino	-	12	Susceptible Radish
Sinapis alba	Accent	2	18	Class 2 Mustard

*Class 1 is stated as having a reproductive factor (*Rf*) of ≤ 0.1 and class 2 a reproductive factor (*Rf*) of between 0.1 and 0.3 when tested in official laboratory bioassays[6].

2.2 Field experiments

Fields with known infestations of BCN were selected in 2016 (Brettenham, Norfolk, 52°24′22.6296″ N, 0°51′1.4688″ E) and 2017 (Bridgham, Norfolk, 52°26′0.1932″ N, 0°52′9.5016″ E). Both fields are of a loamy sand soil type over chalk bedrock.[15] Each field was surveyed for BCN populations on a 50 × 50 m grid spacing comprising 40 soil cores (150 mm deep, 25 mm diameter) from which BCN populations were determined as described later. Once areas with the greatest populations had been identified, the field was lightly cultivated using a disc harrow (Simba Xpress 5.5, Simba International Ltd, Sleaford, UK) and the plot layout was marked on the field. Plots were 6 × 9 m and centred between wheelings in the field. In both years, 24 plots were sampled over the high population areas. Surveying and sampling took place immediately after harvest of the previous crop, which was winter wheat (*Triticum aestivum*) in both years. From each plot, 40 soil cores were taken in a zig-zag pattern, using an AMS EZ-Eject soil probe (AMS Inc., American Falls, ID, USA) (25 mm diameter), to a depth of 150 mm to create a bulk sample for each plot. The bulk sample was passed through a 4 mm sieve to remove large stones and the soil was mixed thoroughly before two 200-mL subsamples were taken. Both subsamples were weighed, and one was washed using a Wye washer to extract cysts.[16] The other subsample was dried at 105 °C for 24 h to determine the moisture content of the soil and the dry weight of the washed sample was calculated. Cysts from the sample were counted and then crushed using a Reid aluminium slide.[17] To determine the mean number of eggs per cyst, crushed cysts were diluted in 50 mL of water inside a measuring cylinder and mixed thoroughly using a glass pipette and pipette controller (Powerpette, VWR Int., Radnor, PA, USA). A 1-mL subsample was dispensed into a Fenwick slide[18] and the number of viable eggs and juveniles counted using a stereomicroscope (Leica M80, Leica, Wetzlar, Germany) and tally counter. The number of BCN eggs per gram of dry soil was determined for each plot using the cyst and egg data. Field experiments were established using randomized block designs with the field plots grouped into blocks of similar initial population (*Pi*) prior to sowing of the brassicas. Each block contained one replicate of each treatment (Table 1). Initial plot populations ranged from 2.1 to 14.8 eggs g^{-1} dry soil in 2016 (mean 7.8) and from 2.1 to 24.9 eggs g^{-1} dry soil in 2017 (mean 7.5).

Field experiments were sown on 2 September 2016 and 1 September 2017 using a Horsch Pronto 6 m seed drill (Horsch Maschinien GmbH, Schwandorf, Germany). The selected varieties of radish and mustard were drilled at the seed rates stated in Table 1 following guidance from a seed merchant. There was also a fallow treatment in which the drill and tractor passed over the plot but no seed was sown. Soil temperatures were monitored throughout using a temperature logger (Tinytag Plus 2, Gemini Data Loggers, Chichester, UK) buried 10 cm into the soil. Accumulated thermal time > 10 °C (a temperature at which egg hatch activity has been reported to cease[19]) was measured as 278 °C days in 2016 and 223 °C days in 2017 from sowing to destruction of the brassicas. All plots received 40 kg ha^{-1} of nitrogen as ammonium nitrate (NH_4NO_3) in liquid form prior to emergence of the seedlings using a Berthoud raptor FC crop sprayer (Berthoud Agricole SAS, Belleville, France).

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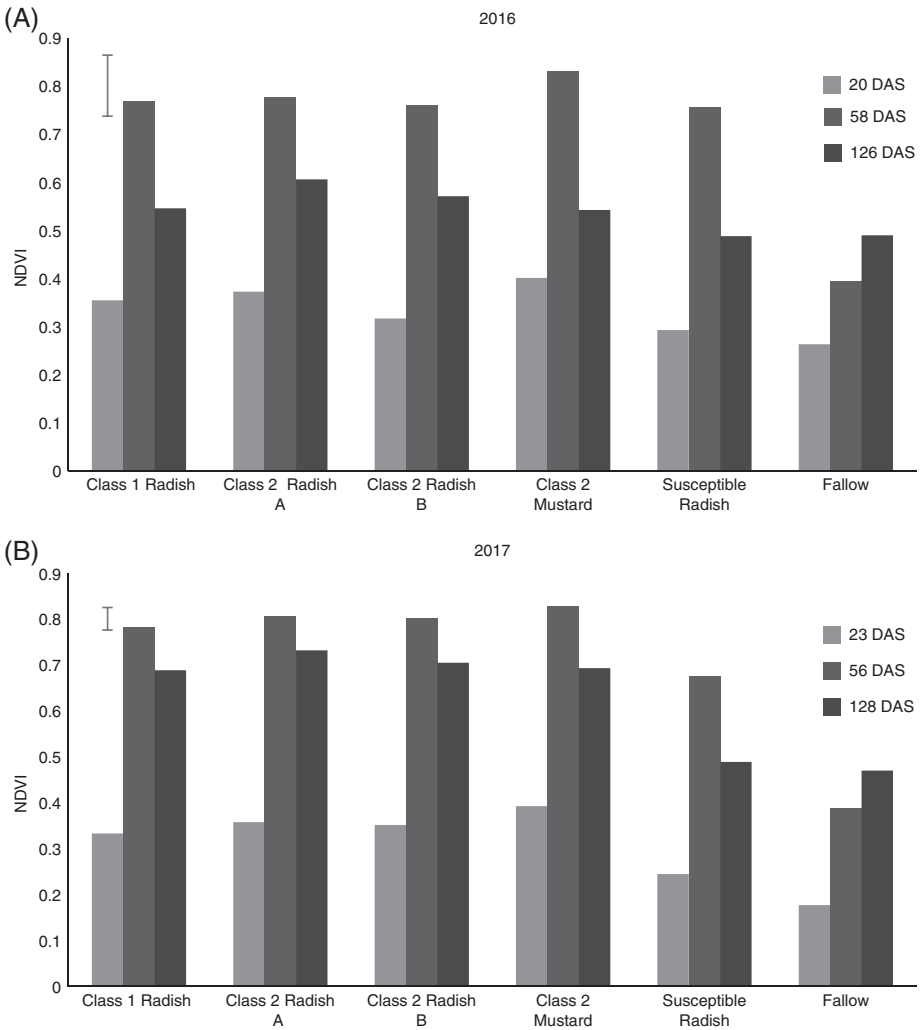


Figure 1. Normalized Difference Vegetative Index (NDVI) of brassica crops grown in fields infested with *Heterodera schachtii* in Norfolk, UK. NDVI was measured three times in each season (2016–2017: 20, 58 and 126 DAS; 2017–2018: 23, 56 and 128 DAS) Differences between treatments were found in both years ($P < 0.001$ for both years). Error bars shows LSD at 5% time × treatment interaction. Fallow treatments were not sterile so NDVI values for these plots show the development of weed plants.

Canopy spectral reflectance data were recorded during the brassica growth using a Crop Circle ACS-430 NDVI meter (Holland Scientific, Lincoln, NE, USA) to measure the Normalized Difference Vegetation Index (NDVI).[20] We used NDVI as a measure of trap crop growth because a higher NDVI equates to greater canopy ground cover and therefore represents greater crop growth, especially as this relationship has been demonstrated previously in brassica species.[21] Brassicas were destroyed early in the following January in both years using a 3 m tractor mounted flail (Maschio Gaspardo S.p.A., Campodarsego, Italy). Prior to flailing, a 50 × 50 cm quadrat was used to take four samples from each plot to determine shoot biomass. Samples were dried at 70 °C until constant weight and weighed. After flailing, each plot was resampled for BCN cysts as described previously to determine the *Pf* of each plot. The *Rf* was then calculated as follows:

$$\textit{Reproductive factor}\ (Rf) = \frac{\textit{Final population}\ (Pf)}{\textit{Initial population}\ (Pi)}$$

Experiment length (~ 16 weeks) followed standard farm practice in the UK regarding the cultivation of a cover crop and was to allow any frost-susceptible varieties of the cover crops to die off during the winter rather than result in large amounts of biomass that would have remained on the surface or required the use of herbicides to kill the trap crops. Likewise, the fallow treatments allowed weeds to grow to simulate normal fallow conditions on the fields.

2.3 Data analysis

Data were analysed using Genstat 17th edition (VSN International, Hemel Hempstead, UK). NDVI data were analysed using a repeated measures analysis of variance (ANOVA), and field data using a one-way ANOVA. Calculation of the least significant difference (LSD) at 5% significance was included in the ANOVA. Figures were prepared using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and GraphPad Prism version 7 (GraphPad Software Inc., La Jolla, CA, USA).

3 RESULTS

Similar trends in trap crop growth were observed in both years of the field experiments, as measured using NDVI (Fig. 1). NDVI

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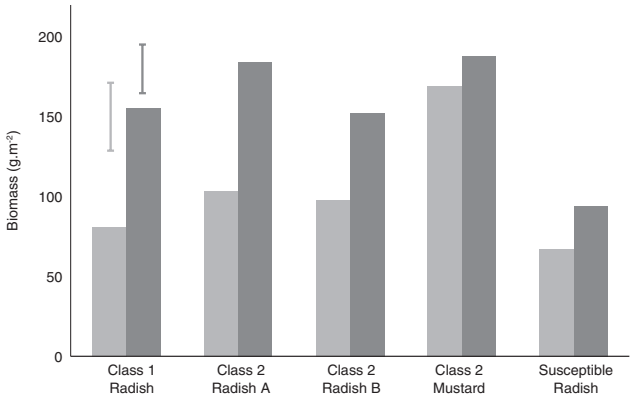


Figure 2. Aboveground biomass of the brassica trap crops in 2016 and 2017 in field experiments in Norfolk, UK. Significant differences were found in both years ($P < 0.001$ in both years) Error bars show LSD at 5% for each respective year. No samples were taken from the fallow treatments.

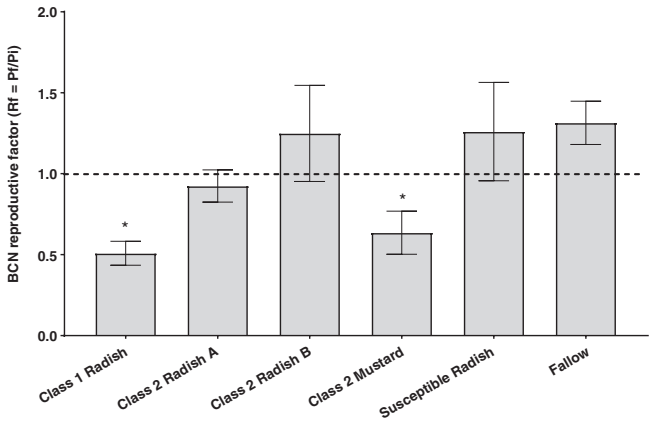


Figure 3. Combined *Heterodera schachtii* reproduction factor data from the brassica trap crops grown in field trials in 2016 and 2017. Significant differences were found between the treatments ($P = 0.01$) and significantly reduced populations from the fallow control treatment are denoted by *. The dashed line shows where $Pf/Pi = 1$, i.e. where no net BCN population change was measured. LSD at 5% significance = 0.53. Error bars show ± SEM.

increased between the first and second measurements, because the radishes or mustard plants were growing rapidly, and then declined by the third measurement, in early January. Only the fallow treatment showed a continual increase in NDVI due to the development of weeds on the fallow plots. In 2016, there were no significant differences in NDVI between trap crops but they had a greater NDVI at 58 days after sowing (DAS) than the fallow treatment. In 2017, the susceptible radish had a significantly lower NDVI than the other trap crops at every measurement and resulted in an NDVI at 128 DAS that was similar to that of the fallow treatment.

In 2016, the mustard had a significantly greater biomass than all of the other trap crops (Fig. 2, $P < 0.001$). In 2017, the mustard again showed the greatest biomass and was significantly greater than the biomass of Class 1 radish and Class 2 radish B. Class 2 radish A also shows significantly greater growth than Class 2 radish B. However, all resistant treatments resulted in greater growth than the susceptible radish in 2017 ($P < 0.001$).

When analysing the 2 years separately, populations of BCN were not significantly reduced by the Class 1 radish or Class 2 mustard compared with the fallow control (2016 $P = 0.085$, 2017 $P = 0.125$). Equally, the susceptible radish and the two Class 2 radish varieties did not lead to any clear reductions in BCN populations. However, when data from the 2 years were combined into a multiyear analysis, significant differences were found between the treatments ($P = 0.01$) (Fig. 3). Class 1 radish and Class 2 mustard showed significant mean population reductions compared with the fallow treatment but differences were not found from using the two Class 2 radish varieties or the susceptible radish.

4 DISCUSSION

Interest in the use of resistant brassicas for control of BCN has increased in recent years but limited data have been available on their effectiveness in UK field conditions. We investigated their use over two field seasons to understand how effective they are at reducing nematode populations and which types are most suited to climatic conditions in the UK.

The Class 1 radish and Class 2 mustard were the only two treatments to significantly reduce the BCN population when compared with the fallow control, and we hypothesize that these varieties stimulate significantly greater levels of BCN hatch than the other varieties. The finding of 30–40% reduction in the BCN population with these two varieties when grown for such a short amount of time, and when temperatures have begun to decline, is important to those dealing with BCN infestations, especially in the UK. Growing Class 1 radish and Class 2 mustard ahead of sugar beet should reduce the population of BCN that might infect seedlings and limit growth.

Differences in *Rf* caused by the brassicas are likely explained by a combination of their growth habit and differences in their ability to stimulate BCN juvenile hatch. All the brassica treatments achieved good levels of ground cover when measured by NDVI, and when the biomass was measured the resistant radishes all seemed to grow equally well in both years; the mustard also grew well. Therefore, none of the treatments were limited in their growth. Because root growth is strongly related to shoot growth,[22] we can expect that rooting was similar between the treatments and differences likely exist in the extent to which the different varieties stimulate BCN second stage juvenile (J2) hatch. Hatching stimulation must be considered when choosing which variety of trap crop to grow because this trait might be more important than the resistance rating. Current assessments for resistance to BCN using a bioassay and hatched juveniles might give misleading results because the ability to stimulate hatching is not assessed. Alternative methods may be needed, such as the use of laboratory hatch assays,[23,24] to screen future varieties for their hatching ability combined and confirm resistance to advise farmers with infested fields on which varieties to choose for maximum population control.

The BCN population data show an increase in numbers even under the fallow treatment. We expected populations to remain at levels very close to the *Pi* or to reduce slightly following the fallow treatment, as seen in other investigations,[14] although Hauer *et al.*[6] did find some plots with large increases in BCN populations under fallow conditions treated with straw mulch. The increases under fallow seen here could be due to a number of factors. First, BCN-susceptible weed species may have been growing and allowed the population to build up. Weed growth was observed and is reflected in the NDVI measurements on the fallow plots. Fat hen (*Chenopodium album*), a known host of BCN, is commonly found across the host farm.[25,26] However, fat hen is classed as a

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relatively poor host of BCN[27] and Meinecke *et al.*[25] concluded that common arable weeds do not require control for nematological reasons. Therefore, it seems unlikely that weeds are responsible for our observed population increases although we cannot be certain. Investigations into UK weed populations and their ability to host BCN would be useful to expand on our findings and possibly confirm whether the increased populations are due to weeds. A clean fallow treatment was not used here for two reasons, first, we wanted to investigate how a fallow field may cause changes to BCN populations, and second, it would not have been viable for the host farmer to regularly spray or cultivate the fallow plots. However, should similar experiments be conducted in the future it would be a good additional treatment and might provide further explanations to our observations.

We hypothesize that consolidation of the soil between the two sampling dates might have led to the population increases observed. The samples for *Pi* were taken immediately after cultivation of the field in summer. This created an unconsolidated soil surface throughout the area sampled. When the *Pf* samples were taken 4 months later, considerable soil weathering due to rainfall had occurred. This weathering led to the observed consolidation, i.e. the same mass of soil now filled a smaller volume, and so soil layers below the 150 mm summer sample depth (therefore not sampled for *Pi*) were included in the winter samples collected to determine *Pf*. This might have skewed our population counts, especially as BCN populations in deeper layers may be greater than at shallower soil layers.[28] If the findings of other investigations, showing no population build up under fallow[14] and our theory of soil consolidation are correct, it may be that both Class 2 radish treatments, and the susceptible radish, result in population declines that they were not significant enough to detect. However, the results could also be due to sampling error, which is inherently large in field experiments with nematodes[29,30] although this should have been equal between the plots because the same method was used throughout.

The need to survey the field for BCN populations and then sample each plot before planting the trap crops was very time-consuming and delayed establishment of the trap crops by several weeks during which sampling, cyst extraction and nematode counts took place. The effectiveness of the trap crops may be further improved by earlier establishment, which would benefit from warmer weather conditions and longer day lengths, thereby promoting more growth of the trap crops; because of these factors, earlier sowing has been shown to increase the effectiveness of trap crops and the growth of cover crops in Germany.[6,13,31] However, labour and equipment availability in July and August, when harvest of combinable crops is typically underway, may not allow for earlier sowing. Extension of cover crop growth through the winter is unlikely to have affected BCN populations because the temperature of the soil dropped below 10 °C, the temperature at which hatching of BCN juveniles ceases,[8] during early November in both years.

Population reductions, as a result of growing trap crops, may be useful only if they lead to increased yield in subsequent crops and can be accommodated within the farming rotation. Cooke[32] stated clearly that a trap crop should meet three criteria before being suitable for commercial use, and these stand true today. First, it should stimulate egg hatch, which we have shown to be the case with at least two of the varieties used. Second, reproduction of the nematode should be limited, which appeared to be the case because no varieties consistently showed major increases in populations. Finally, the trap crop should be agriculturally and economically acceptable. In our case, planting at the beginning of September fitted the system on the host farm because the cereal harvest was complete, and labour and machinery were available for drilling the brassicas. However, when seed, fertilizer application and the destruction of the trap crops in the winter are factored in, costs to the grower may be prohibitive and would require the trap crops to boost yields of the following sugar beet crop by at least 5 t ha^{-1} (Class 1 radish costs £2.40 per kg (Brown P, personal communication) at a seed rate of 22 kg ha^{-1} = £52.80 ha^{-1}. Costs of sowing, £24.37 ha^{-1}; fertilizing, £11.16 ha^{-1} (plus fertilizer £21.33); and flailing, £19.50 ha^{-1} giving total costs of £129.16 ha^{-1}. With the current beet price of £22.50 per tonne, yields need to increase by > 5.74 t.ha^{-1} following the trap crop to be economically viable. Costs are even higher with other treatments due to greater seed costs. [Costs calculated using standard figures].[34]).

The use of BCN-tolerant varieties of sugar beet is now commonplace for growers with infestations.[6,33] These varieties overcome the majority of yield losses, so returns to the grower are significantly greater than with a susceptible variety; however, losses are not entirely prevented and a negative relationship between *Pi* and sugar yield was found by Hauer *et al.*[6] Therefore, the addition of a trap crop to the rotation may be useful in these circumstances, but only if the additional yield benefit can meet the costs of cultivating the trap crop. Other factors relating to the planting of a trap crop must also be considered and these may be positive (e.g. improvements to soil structure, weed control, nutrient retention and prevention of soil erosion) or negative (e.g. providing a habitat over winter for other agriculturally important pests). Further research is required to consider the use of trap crops within arable farming systems.

5 CONCLUSIONS

Our experiments have shown that brassica trap crops may form part of an IPM strategy to manage BCN populations in eastern England. The Class 1 radish and Class 2 mustard treatments produced significant population reductions, whereas the two Class 2 radish treatments did not reduce the populations significantly. Understanding the costs of growing a trap crop and how these might be repaid in the following crop is needed to provide an agronomic benefit.

Further studies are needed to evaluate more of these resistant brassica trap crops over a wider range of seasons and soil types, but our results indicate that two of the varieties tested offer some form of BCN population management. However, these population reductions may soon be undone when a susceptible host species for BCN is planted. In addition, a yield benefit from the use of the trap crop needs to be identified prior to their widespread adoption in the UK for BCN control.

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REFERENCES

1 Müller J, The economic importance of *Heterodera schachtii* in Europe. *Helminthologia* **36**:205–213. Muller, J (reprint author), Biol Bundesanstalt Land & Forstwirtschaft, Inst Nematol & Wirbeltierkunde, Toppheideweg 88, D-48161 Munster, Germany. (1999).
2 Moens M, Perry RN and Jones JT, Cyst nematodes – life cycle and economic importance, in *Cyst Nematodes*, ed. by Moens M, Perry RN and Jones JT. CAB International, Wallingford, pp. 1–26 (2018).
3 Cooke DA, Beet cyst nematode (*Heterodera schachtii* Schmidt) and its control on sugar beet. *Agric Zool Rev* **2**:135–183 (1987).
4 Blok VC, Tylka GL, Smiley RW, de Jong WS and Daub M, Resistance breeding, in *Cyst Nematodes*, ed. by Moens M, Perry RN and Jones JT. CAB International, Wallingford, pp. 174–214 (2018).
5 Wright A, Stevens M, Back M and Sparkes D, Interactions between *Heterodera schachtii* and sugar beet. *Asp Appl Biol* **134**:81–88 (2017).
6 Hauer M, Koch HJ, Krüssel S, Mittler S and Märländer B, Integrated control of *Heterodera schachtii* Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. *Appl Soil Ecol* **99**:62–69 (2016).
7 Kakaire S, Grove IG and Haydock PPJ, Effect of temperature on the life cycle of *Heterodera schachtii* infecting oilseed rape (*Brassica napus* L.). *Nematology* **14**:855–867, [Kakaire, Stephen; Grove, Ivan G.; Haydock, Patrick P. J.] Harper Adams Univ Coll, Crop & Environm Res Ctr, Nematol & Entomol Grp, Newport TF10 8NB, Shrops, England. Kakaire, S (reprint author), Harper Adams Univ Coll, Crop & Environm Res Ctr, Nematol & E (2012).
8 Dewar AM and Cooke DA, Pests, in *Sugar Beet*, ed. by Draycott AP. Blackwell, Oxford, pp. 316–350 (2006).
9 LIZ, *LIZ - Ausfallraps-Manager* [Online]. (2016). Available: http://raps.rheinmedia.de/raps/index.php?region=17&datum=2017-07-08&enddatum=2017-10-12 [9 May 2018].
10 Back MA, Cortada L, Grove IG and Taylor V, Field management and control strategies, in *Cyst Nematodes*, ed. by Perry RN, Moens M and Jones JT. CAB International, Wallingford, pp. 305–336 (2018).
11 Hafez SL, The use of green manure crops in a sugarbeet rotation for sugarbeet cyst nematode management. *J Nematol* **548**:26 (1994).
12 Koch DW, Gray FA and Krall JM, Nematode-resistant oil radish for *Heterodera schachtii* control II sugarbeet–dry bean–corn rotations. *J Sugar Beet Res* **35**:63–75 (1998).
13 Kenter C, Lukashyk P, Lukashyk P, Daub M and Ladewig E, Population dynamics of *Heterodera schachtii* Schm. and yield response of susceptible and resistant sugar beet (*Beta vulgaris* L.) after cultivation of susceptible and resistant oilseed radish (*Raphanus sativus* L.). *J Cultiv Plants* **66**:289–299 (2014).
14 Hemayati SS, Jahad-e Akbar M-R, Ghaemi A-R and Fasahat P, Efficiency of white mustard and oilseed radish trap plants against sugar beet cyst nematode. *Appl Soil Ecol* **119**:192–196 (2017).
15 Hallett SH, Sakrabani R, Keay CA and Hannam JA, Developments in land information systems: examples demonstrating land resource management capabilities and options. *Soil Use Manag* **33**:514–529 (2017).
16 Winfield AL, Enfield MA and Foreman JH, A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. *Ann Appl Biol* **111**:223–231 (1987).
17 Reid E, A rolling method for opening cysts of potato root eelworm. *Plant Pathol* **4**:28–29 (1955).
18 Fenwick DW, A new modification of the McMaster slide for use in potato–root eelworm investigations. *J Helminthol* **25**:173–176 (1951).
19 Cooke DA, The effect of resistant cultivars of catch crops on the hatching of *Heterodera schachtii*. *Ann Appl Biol* **106**:111–120 (1985).
20 Sharma LK, Bu H, Denton A and Franzen DW, Active-optical sensors using red NDVI compared to red edge NDVI for prediction of corn grain yield in North Dakota, U.S.A. *Sensors (Switzerland)* **15**:27832–27853 (2015).
21 Cowley RB, Luckett DJ, Moroni JS and Diffey S, Use of remote sensing to determine the relationship of early vigour to grain yield in canola (*Brassica napus* L.) germplasm. *Crop Pasture Sci* **65**:1288–1299 (2014).
22 Vos J, Van Der Putten PEL, Hussein MH, Van Dam AM and Leffelaar PA, Field observations on nitrogen catch crops II. Root length and root length distribution in relation to species and nitrogen supply. *Plant and Soil* **201**:149–155 (1998).
23 Fatemy S and Abootorabi E, Hatching activity, invasion rate and reproduction of *Heterodera schachtii* on oilseed rape cultivars. *Nematol Mediterr* **30**:163–166 (2002).
24 Vandenbossche BA, *Effect of Temperature on the Interactions between Beet Cyst Nematodes (Heterodera schachtii and Heterodera betae) and Sugar Beet*. Georg-August-University, Göttingen (2016).
25 Meinecke A, Ziegler K, Bürcky K and Westphal A, Composition of the stubble weed flora and its role for *Heterodera schachtii* in the year preceding sugar beet production. *Weed Res* **54**:614–623 (2014).
26 BBRO, *Beet Cyst Nematode Technical Guide*. British Beet Research Organisation, Brooms Barn (2009).
27 Ahmad M, Sedaghatjoo S and Westphal A, Reproductive capacity of *Heterodera schachtii* on *Thlaspi arvense*, *Capsella bursa-pastoris* and varying populations of *Chenopodium album*. *J Plant Dis Prot* **123**:37–42 (2016).
28 Whitehead AG, Vertical distribution of potato, beet and pea cyst nematodes in some heavily infested soils. *Plant Pathol* **26**:85–90 (1977).
29 Southey JF, *Principles of Sampling for Nematodes, Laboratory Methods for Work with Plant and Soil Nematodes*. HMSO, London, pp. 1–4 (1986).
30 Been TH and Schomaker CH, Distribution patterns and sampling, in *Plant Nematology*, ed. by Perry RN and Moens M. CAB International, Wallingford, pp. 331–358 (2013).
31 Koch HJ, Windt A, Mittler S and Hauer M, Effect of weather variables on biomass production and N uptake of catch crops,and their influence on soil water and Nmin content in Northern Germany. *J Cultiv Plants* **69**:361–372 (2017).
32 Cooke DA, The relationship between numbers of *Heterodera schachtii* and sugar beet yields on a mineral soil, 1978-81. *Ann Appl Biol* **104**:121–129 (1984).
33 BBRO, *British Beet Research Organisation Reference Book*. British Beet Research Organisation, Norwich (2017).
34 Redman G, *The John Nix Pocketbook for Farm Management 2018*, 48th edn. Agro Business Consultants, Melton Mowbray (2017).

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Addendum to Chapter 4

The data used in the ANOVA to calculate the data shown in figure 3 were found to be normally distributed and therefore not transformed prior to analysis. Year was also included as a fixed factor in the analysis, with no significantly different responses found between the two years.

Chapter 5

Hatching dynamics of the beet cyst nematode, *Heterodera schachtii* (Schmidt) following exposure to root leachates from cultivars of sugar beet, white mustard and radish

The following chapter has been submitted to Nematology for review and consideration for publication

HATCHING DYNAMICS OF THE BEET CYST NEMATODE, *HETERODERA SCHACHTII* (SCHMIDT) FOLLOWING EXPOSURE TO ROOT LEACHATES FROM CULTIVARS OF SUGAR BEET, WHITE MUSTARD AND RADISH

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Abstract:

The hatching and emergence of juveniles from cysts of *H. schachtii* in response to various stimuli is well understood. These responses relate to means of optimising the chances of successful reproduction and survival of populations. However, the effect of different cultivars of host species is not so well understood. To gain a further understanding of any differences between cultivars of host species, we conducted a number of experiments using root leachates obtained from a range of varieties of sugar beet (*Beta vulgaris*), radish (*Raphanus sativus*) and white mustard (*Sinapis alba*). *H. schachtii* cysts were exposed to root leachate solutions in *in vitro* laboratory assays during which juvenile emergence from cysts was monitored weekly.

There were significant differences in the stimulation of hatch of juveniles in response to the different sugar beet cultivars. The resistant varieties of sugar beet consistently showed the lowest levels of hatching stimulation, whereas a light tolerant variety resulted in the greatest hatch.

The results obtained from the experiments using the brassica varieties showed very different responses. In the first experiment, the root leachates appeared to inhibit hatch of the juveniles. However, the latter two experiments showed that they do stimulate hatch of juveniles and that there were significant differences in hatch responses due to the age of the plant.

Keywords: beet cyst nematode, hatching, root leachates, *Beta vulgaris*, *Sinapis alba*, *Raphanus sativus*, IPM

1. Introduction

The beet cyst nematode, *Heterodera schachtii* (Schmidt) is a major pest of sugar beet (*Beta vulgaris* L.) crops around the world (Müller, 1999) and can cause yield losses of up to 60% in susceptible varieties (Blok et al., 2018; Cooke, 1987). It also has a wide host range (CABI, 2018) and can also cause damage to brassica crops such as oilseed rape (*Brassica napus*) (Kakaire et al., 2012). Careful management is required to monitor and limit yield losses caused by the nematode and prevent spread of infested soil to new fields.

The use of tolerant varieties of sugar beet in the management of *H. schachtii* infestations have become popular. Tolerant varieties are able to limit the yield losses caused by the feeding nematodes, although these varieties do not limit population multiplication (Hauer et al., 2016; Reuther et al., 2017). Varieties with tolerance are now widely available across European markets and are recommended for use in *H. schachtii* infested fields (BBRO, 2018). Tolerant varieties are favoured by growers since they yield better than truly *H. schachtii* resistant (which are able to actively lower *H. schachtii* populations) varieties of sugar beet, which experience a yield drag (Dewar & Cooke, 2006; Märländer et al., 2003). Another popular method of control is the use of a resistant trap crop (Müller, 1999; Cooke, 1985; Hauer et al., 2016). Trap crops for control of *H. schachtii* consist of varieties of radish (*Raphanus sativus*) or white mustard (*Sinapis alba*), which are commonly planted in infested fields during the summer prior to sugar beet cultivation (usually immediately after harvest of the previous crop) and are intended to reduce the populations of *H. schachtii* in the soil, ahead of the sugar beet. These trap crops are tested using bioassays to determine their resistance characteristics using hatched juveniles of *H. schachtii* (Hauer et al., 2016; Bundessortenamt, 2014) and therefore the ability of these varieties to stimulate the hatch of juveniles is not typically

assessed and this study aimed to assess the hatching dynamics of a range of these brassica trap crop varieties.

Previously, four distinct types of dormancy have been found in *H. schachtii* (Zheng & Ferris, 1991), and these increase the chances of some of the juveniles finding a suitable host and reproducing. Perry (2002) classified *H. schachtii* as possessing a hatch response which results in very large numbers of juveniles hatching in response to host root leachates but also having a large level of hatch to just water. This is in contrast with other cyst nematode species, such as the potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*), where hatching is modulated by a variety of hatching factors associated with the root diffusates of potatoes with very few hatching in response to water (Evans, 1983; Perry, 2002). In the case of *H. schachtii*, the various hatch responses (Zheng & Ferris, 1991) cause some juveniles to hatch soon after developing and others remain dormant until a suitable host is detected, and as a result, approximately 50% of a cyst's contents hatch every year (Jones, 1956). It is for this reason that a period of approximately five years between a host species being grown in an infested field can keep *H. schachtii* populations below damaging levels in sugar beet crops (BBRO, 2009; Koch & Gray, 1997). Where cultivation of sugar beet is required to be more frequent than recommended, or growers' rotations include other host species, the use of the resistant brassicas may be a useful tool in controlling *H. schachtii* damage to future crops.

It is also important to consider whether different varieties of sugar beet stimulate significantly different amounts of hatch of *H. schachtii* juveniles as a variety which results in reduced initial levels of hatch may be less prone to damage from invading juveniles. Previous studies usually have included just one or two examples of commercial varieties of host species being

investigated (Cooke, 1985; Steele et al., 1982; Kakaire et al., 2015). Therefore, differences which may exist between the different variety types have not been investigated in detail. Different *H. schachtii* hatching responses to various cultivars of the same species have previously been identified in oilseed rape (Fatemy & Abootorabi, 2002) and we hypothesise that different cultivars of other host crops may also vary in their ability to stimulate juvenile hatch.

We set out to understand how a range of modern cultivars of sugar beet, radish and one cultivar of white mustard may differ in their ability to stimulate hatch of juveniles of *H. schachtii* and whether these differences could be related to their reported tolerance, resistance or susceptibility to *H. schachtii*. To achieve these aims we performed a series of *in-vitro* experiments, which are reported in this manuscript.

2. Materials and methods

A total of four experiments were conducted to test the influence of leachates obtained from different varieties of sugar beet, radish and mustard (Table 1) upon the hatching dynamics of *H. schachtii*

- Experiment 1 assessed the level of hatch stimulated from twelve varieties of radish, nine of which are resistant to *H. schachtii.* It also included one resistant variety of white mustard.
- Experiment 2 tested *H. schachtii* hatching responses to nine varieties of sugar beet, which varied in their susceptibility to *H. schachtii.*
- Experiment 3 combined both brassica and sugar beet treatments to again investigate hatching responses. Three resistant varieties of radish, the resistant mustard and a

susceptible radish were grown alongside six varieties of sugar beet. Some of the sugar beet varieties had been used in Experiment 2 and some were newer varieties (BBRO, 2015). All brassica treatments had been previously grown in Experiment 1.

- Experiment 4 replicated only the brassica treatments from Experiment 3. However, the plants were grown for five, six and seven weeks to investigate the effect of age of the plants upon the stimulation of hatch

2.1 Root leachate collection

For all experiments, seeds of each variety were planted into 9cm Ø, 350ml plastic plant pots filled with sterilised coarse sand. The plants were grown in triplicate at 20°C day, 16°C night with a 16 hour photoperiod in a controlled environment room (CER). Plants were evenly watered using a dripper irrigation system and nutrition was provided using a complete fertiliser (Hortifeed standard 15-7-30, diluted 1g to 1l water, Hortifeeds, Lincoln, UK). Root leachates were collected from the plants after they had grown for six weeks (Experiments 1, 2 and 3). In Experiment 4, plants were grown for either five, six or seven weeks. Leachates were collected from the pots using a modified method of Shepard (1970). The irrigation was removed and the pots were saturated with deionised water 24 hours prior to leachate collection and conditions in the CER changed to 12°C in continuous dark. This was to allow the leachates to diffuse into the water and also reduce transpiration by the plants. The next day the pots were placed into a glass funnel over a beaker and then into the pot a further 200 ml of deionised water was applied, which was passed through the pot three times, being topped up with extra water if necessary. As a result, 600 ml of root leachate for each treatment was collected from the three replicates and they were combined to make one sample. A proportion of each sample was then filtered using a funnel and filter paper

(Whatmann 113). Filtered leachate solution was then diluted 1:1 with additional deionised water and 100 ml of each root leachate solution retained for use in the experiments. The diluted samples were stored at 3°C until needed for the hatching assay. For Experiment 1, the leachates were stored for eight weeks before starting the hatching assay. All of the other hatching assays began within one week of the root leachate solution (RLS) collection and preparation. Fresh RLS were prepared for each experiment.

2.2 Hatching assay set-up

The hatching assays were set up using a modified method of Danquah *et al.*, (2011). Plastic multi-well plates (with either 12 or 24 wells depending on the number of treatments for the experiment) were used. A blocked design was used with each plate used representing a block. Into one well in each plate 2ml of the RLS was pipetted to which ten cysts were added inside baskets created from the wide end of 1ml pipette tips onto which plastic netting (250 µm aperture) was fused using a heat plate and when filled the plates were then sealed with parafilm. The hatching assays ran for six weeks and during this time, the plates were kept in the dark inside a plastic box on the benchtop in the laboratory. A temperature logger (Tiny Tag Ultra 2, Gemini Data Loggers Ltd, Chichester, UK) was placed alongside the plates for the duration of the experiment to monitor temperature. For all experiments, temperatures were measured to be in the range of 20°C ± 4°C. Each week the RLS was removed so that the number of hatched juveniles could be counted under a stereomicroscope (Leica M80) and 2 ml of fresh RLS solution pipetted into the correct corresponding well.

Table 1 – List of treatments used in the hatch experiments. Radish and mustard treatments are listed with their corresponding resistance class, and sugar beet treatments with their reported variety type as recommended by their breeders.

Radishes (*R. sativus*)

Treatment	Variety	Resistance class*	Experiments
1	Colonel	1	1,3,4
2	Decapo	2	1
3	Defender	2	1,3,4
4	Melotop	2	1
5	Compass	2	1
6	Baracuda	2	1
7	Bokito	2	1,3,4
8	Anaconda	2	1
9	Romessa	2	1
10	Siletina	N/A	1
11	Silletta Nova	N/A	1
12	Early Mino	N/A	1,3,4

Mustard (*S. alba*)

Treatment	Variety	Resistance class*	Experiments
13	Accent	2	1,3,4

Sugar Beet (*B. vulgaris*)

Treatment	Variety	Variety Type+	Experiments
14	Sanetta	R	2
15	Coded Variety	R	3
16	Aurora	T	2,3
17	Thor	T	2,3
18	Mongoose	T	2
19	Pamina	T	2
20	BTS 755	T	3
21	Maddox	LT	2,3
22	Sentinel	LT	2
23	Cayman	S	2
24	Pasteur	S	All

Controls

Treatment	Type	Experiments
25	Sand	All
26	Deionised Water	All

* Resistance classes are measured under controlled tests in Germany before approval for cultivation (Bundessortenamt, 2014). Resistance class of 1 ≥ 90% resistance and class 2 = 70 to 90% resistance to *H. schachtii*.

+ Sugar beet varieties can be classed as Resistant (R), Tolerant (T), Light tolerant (LT) or Susceptible (S) to *H. schachtii* when tested in recommended list trial (BBRO, 2018)

After the six weeks had elapsed, the cysts were removed from the baskets and crushed to release the eggs and juveniles inside. The contents of the 10 cysts were diluted in 50 ml of water. The water was then thoroughly agitated using a 10 ml glass pipette and pipette controller (Powerpette, VWR International, Radnor, PA, USA) and then 1 ml of this solution was extracted and counted using the microscope to allow for the percentage hatch of the eggs to be determined.

All experiments contained five replicates of each treatment, except for the final experiment which contained four replicates. The cysts used in the experiments had been recently cultured on oilseed rape (*Brassica napus*) for Experiments 1-3 and susceptible sugar beet (*Beta vulgaris*) for Experiment 4. Cysts were obtained from the infested soil using a Wye Washer column elutriator (Winfield et al., 1987). Prior to extraction the soil containing the cysts was stored at 3°C and kept moist. After extraction all of the cysts were cleaned in deionised water prior to being placed into the plates. Only cysts which appeared viable were used in the experiments with damaged, small or empty cysts being discarded. The population used for the cultures originated from a *H. schachtii* infested field site near Thetford, Norfolk, UK (52° 24' 22.2156'' N 0° 51' 4.3992'' E).

2.3 Data analysis

All data were analysed using ANOVA in GenStat 17th Edition (VSN international, Hemel Hempstead, UK). Genstat was also used for calculation of least significant difference (LSD) values and also to conduct Duncan's multiple range test, both at 5% significance. Data were then presented in graphical format using GraphPad Prism v.7 (GraphPad Software Inc. La Jolla, CA, USA).

Curve fitting was performed using GraphPad Prism v.7 also. One phase association exponential curves were fitted to work out the hatching dynamic for each sample over time, *t*, using the following equation:

$$Y = Y0 + (Plateau - Y0) \times (1 - \exp(-K \times t))$$

Y is the proportion of hatched juvenile nematodes, and Y0 denotes the number of hatched juveniles at *t*0, i.e. zero. Plateau is point at which Y becomes static and therefore the hatching ceases. K is the reaction rate constant which is expressed in inverse days and is derived from the slope of the curve, and can be considered the hatching rate constant, hereby referred to as HR, of the response of the nematodes to the RLS (Fig 1). Faster hatching reactions to the RLS have a lower HR than slower nematode hatching reactions to the RLS. The HR value of each sample was then analysed by ANOVA. All curves were found to have an R^2 value of ≥0.9. Curves with ambiguous fits and which did not follow expected one phase association exponential curves were detected by the analysis software and excluded from further analysis.

3. Results

3.1 Experiment 1 – Brassica hatch responses

Exposure of *H. schachtii* to deionised water resulted in the greatest HR, which was significantly higher than treatments R10 and R5. Treatment R7 (Table 2), which had the lowest observed level of hatch of any treatment (Fig 2), was not significantly different to the deionised water control.

Hatching of all treatments increased each week, with the greatest levels of hatch occurring during the first half of the bioassay (Fig. 2). When the combined total levels of hatch were compared, the deionised water control clearly stimulated the greatest levels of hatch at 30% ($P<0.001$). Mean hatch for all brassica RLS treatments was 10.9% and all treatments were similar to each other except a significant difference detected between R12 (19.3%) and R7 (3.8%). The sugar beet treatment (SB S 24) resulted in a total hatch of 20.4%, which was similar to the deionised water control. The sand leachate control was found to have a significantly lower level of hatch than the deionised water.

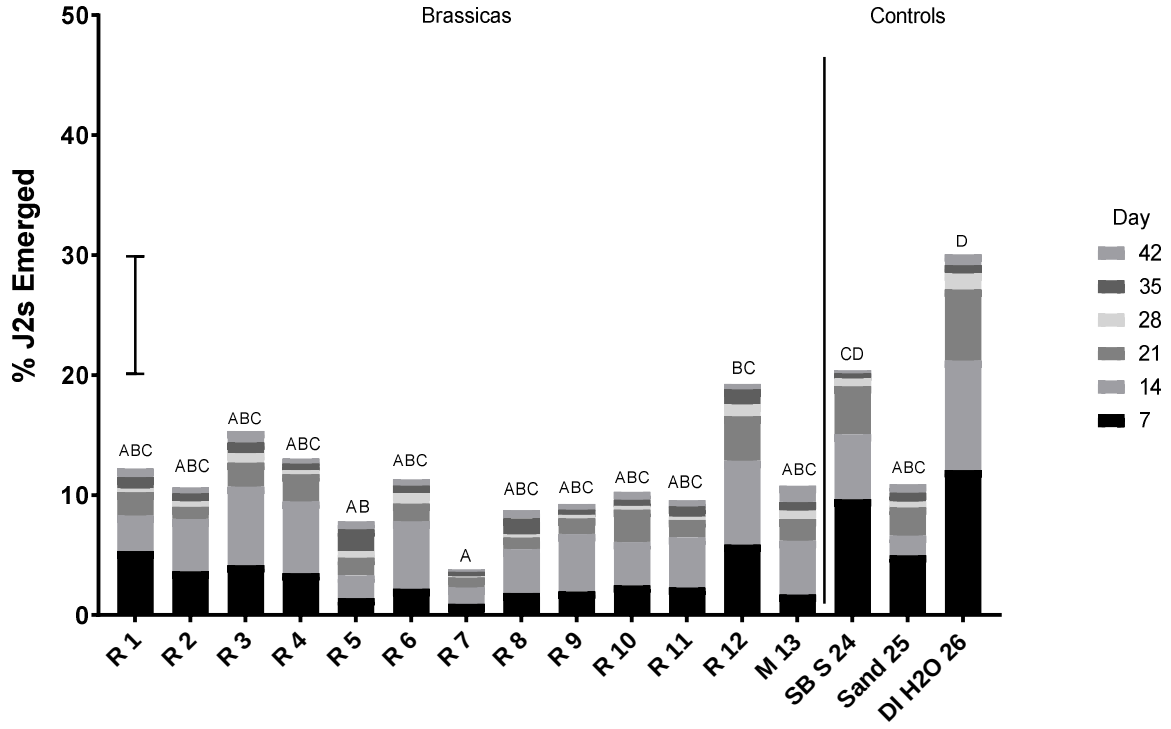


Figure 1 – Results of experiment using brassica treatments after six weeks of exposure to root exudate solutions. Treatments are either of radish (*R. sativus*) [R] or mustard (*S.* alba) [M] and are labelled with their treatment number (Table 1). Control treatments of susceptible sugar beet, sand leachate and deionised water are also included. The bars show the cumulative percentage hatch counted every seven days. Significant differences were found ($P<0.001$) between the treatments. The error bar shows LSD at 5% significance for the total percentage hatch recorded. Treatments with different letters show differences at 5% significance according to Duncan's Multiple range test.

As similar HR values were observed between almost all of the treatments (Table 2)., Radish variety 5, had the lowest HR and was significantly lower than brassica treatments 1, 2, 4, 9 and 11. However, all of the other brassica treatments had HR similar to each other. These data imply that the varieties had similar HR responses to the RLS but had different levels of maximum hatch that they could achieve (Fig 2), while all treatments appeared to inhibit hatch compared to the deionised water control.

3.2 Experiment 2 – Sugar beet root leachate hatch responses

The sugar beet root leachates caused a similar HR response in *H. schachtii* although this was significantly lower than both the water and sand controls, which themselves were different to each other ($P<0.001$) (Table 2).

As seen in Experiment 1, the greatest proportions of hatch occurred in the first three weeks of the experiment, although hatching increased each week during the first half of the experiment in most treatments (Fig 3). At week three it was clear that sugar beet treatments were stimulating greater hatch of *H. schachtii* than the control treatments. Significant differences were found between the total hatch of *H. schachtii* in response to root leachates from different varieties of sugar beet (Fig 3). Within the tolerant sugar beet varieties there was significant variation in total hatch of juveniles observed after six weeks exposure to the RLS, with SB T 18 (18.2%) being significantly lower than SB T 19 and SB T 17 (35.8 and 30.8% respectively). However, there were no significant differences between the other varieties. The resistant variety (SB R 14) showed a significantly lower hatch (21.2%) than SB T 19 and

both the light tolerant varieties (SB LT 21 and 22) but was not different to any of the other treatments (P<0.001).

The slower HR responses, despite greater levels of total hatch, show that the sugar beet treatments produced a more prolonged hatching period for *H. schachtii* than the water and sand controls.

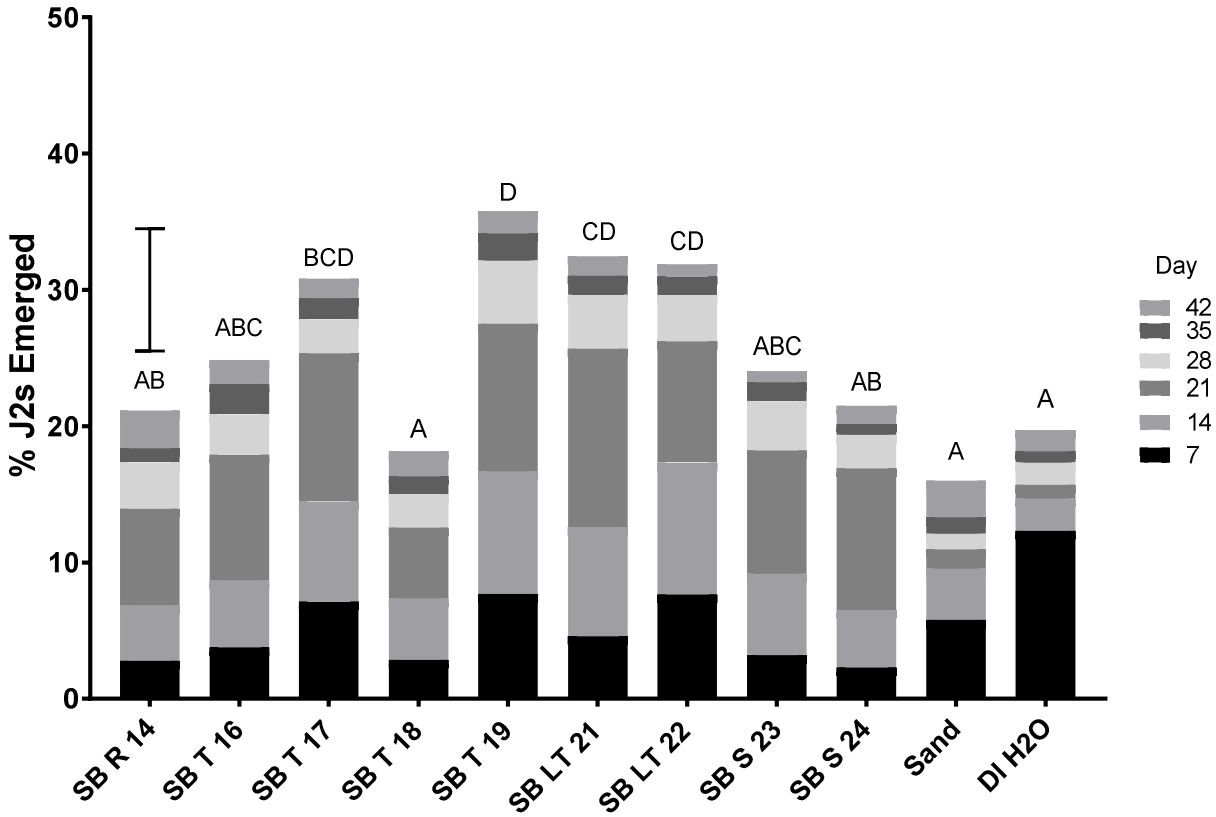


Figure 2 – Results of experiment using sugar beet treatments after six weeks of exposure to root exudate solutions. Sugar beet (*B. vulgaris*) varieties grown were wither resistant (R), Tolerant (T), Light tolerant (LT) or susceptible (S) to *H. schachtii*. Treatments are also labelled with their treatment number (Table 1). Control treatments of sand leachate and deionised water were also included. The bars show the cumulative percentage hatch counted every seven days. Significant differences were found (P<0.001) between the treatments. The error bar shows LSD at 5% significance for the total percentage hatch recorded. Treatments with different letters show differences at 5% significance according to Duncan's multiple range test.

3.3 Experiment 3 – Brassica and sugar beet hatch responses

The sugar beet varieties resulted in similar responses in terms of HR by the *H. schachtii* juveniles (Table 2), with SB LT 21, SB T 20, SB T 17 and SB R 15 resulting in similar HR to the water and sand controls. The HR response of the juveniles induced by the brassica treatments tested were also similar to one another, but resulted in much greater HR responses than sugar beet treatments SB T 16 and SB S 24 ($P=0.001$).

Different levels of cumulative hatch of *H. schachtii* J2s were found between the sugar beet varieties ($P<0.001$), but there were no significant differences between the radish varieties (Fig 4), which had a mean hatch level of 37.2%. All RLS treatments caused much greater levels of hatch than the deionised water or sand controls (mean of 5.0%), most resulting in significantly higher levels of hatch. All radish leachate treatments except R1, and all sugar beet root leachate treatments except SB R 15, resulted in significantly greater levels of juvenile hatch and emergence than the sand control and only SB R 15 did not have greater levels of hatch than the deionised water.

Within the sugar beet treatments, the root leachate of the resistant sugar beet (SB R 15) caused the lowest level of hatch (total of 24.6%). SB R 15 resulted in significantly lower hatch of juveniles than the light tolerant variety treatment (SB LT 21) ($P<0.001$), which caused the greatest level of hatch at 50%. All of the other sugar beet root leachates did not cause a significantly different hatch levels than either the resistant or light tolerant varieties tested (Figure 3).

Table 2 – Hatching rate (HR) for the responses of the hatching *H. schachtii* juveniles from cysts in response to root leachate solutions from brassicas (Experiment 1), sugar beet (Experiment 2) and a combination of brassicas and sugar beet (Experiment 3).

Treatment	Treatment name	Species	Experiment 1		Experiment 2		Experiment 3	
1	Colonel	*R. sativus*	0.08346	bc	-		0.1789	d
2	Decapo	*R. sativus*	0.07242	bc	-		-	
3	Defender	*R. sativus*	0.06282	abc	-		0.1608	cd
4	Melotop	*R. sativus*	0.07121	bc	-		-	
5	Compass	*R. sativus*	0.02839	a	-		-	
6	Baracuda	*R. sativus*	0.05631	abc	-		-	
7	Bokito	*R. sativus*	0.05675	abc	-		0.1352	bcd
8	Anaconda	*R. sativus*	0.05536	abc	-		-	
9	Romessa	*R. sativus*	0.06619	bc	-		-	
10	Siletina	*R. sativus*	0.04969	ab	-		-	
11	Silletta Nova	*R. sativus*	0.08555	bc	-		-	
12	Early Mino	*R. sativus*	0.05758	abc	-		0.1363	bcd
13	Accent	*S. alba*	0.05233	abc	-		0.1349	bcd
14	Sanetta	*B. vulgaris*	-		0.02276	a	-	
15	Coded Variety	*B. vulgaris*	-		-		0.0841	ab
16	Aurora	*B. vulgaris*	-		0.02665	a	0.0631	a
17	Thor	*B. vulgaris*	-		0.04302	a	0.0746	ab
18	Mongoose	*B. vulgaris*	-		0.02736	a	-	
19	Pamina	*B. vulgaris*	-		0.03915	a	-	
20	BTS 755	*B. vulgaris*	-		-		0.0866	ab
21	Maddox	*B. vulgaris*	-		0.03078	a	0.1025	abc
22	Sentinel	*B. vulgaris*	-		0.04697	a	-	
23	Cayman	*B. vulgaris*	-		0.02826	a	-	
24	Pasteur	*B. vulgaris*	0.08215	bc	0.02525	a	0.066	a
25	Sand		0.07272	bc	0.1751	c	0.1367	bcd
26	Deionised water		0.08844	c	0.07148	b	0.1393	bcd
LSD			0.03212		0.02123		0.05786	
P			0.012		<0.001		0.001	

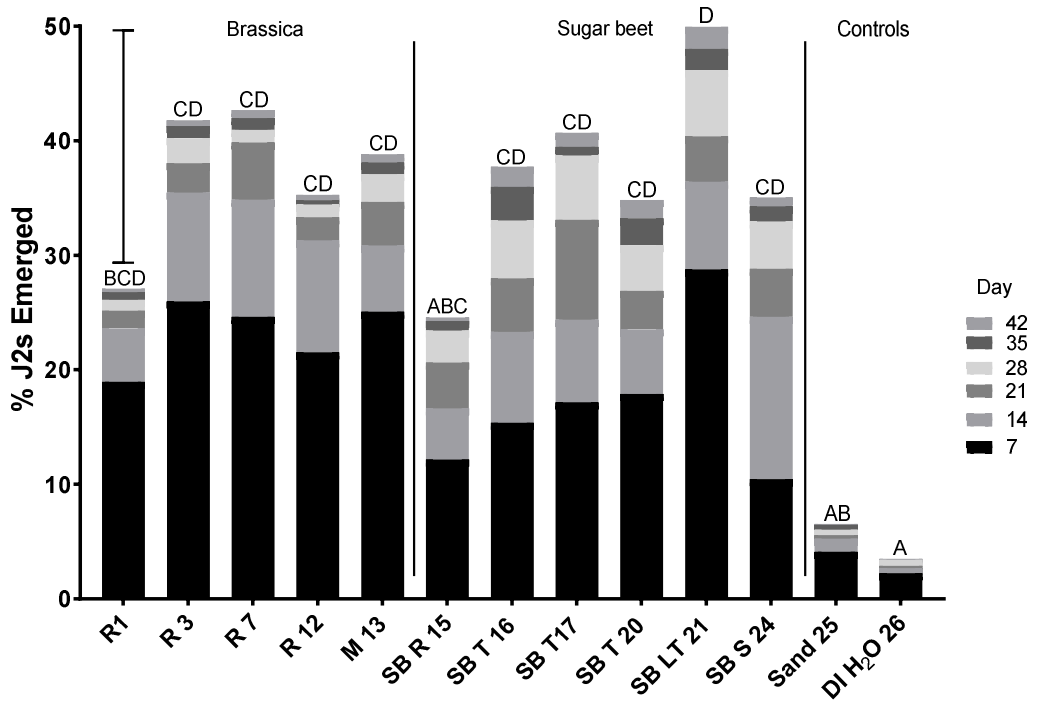


Figure 3 – Results of the combined experiment with brassica and sugar beet treatments after six weeks of exposure to root exudate solutions. The bars show the cumulative percentage hatch counted every seven days. Significant differences were found (P<0.001). The error bar shows LSD at 5% significance for the total percentage hatch recorded. Treatments are labelled R, M or SB for radish (*R. Sativus*), white mustard (*S. alba)* or sugar beet (*B. vulgaris*) respectively, and with their treatment number (Table 1). Sugar beet treatments are also labelled with their designation or Resistant (R), Tolerant (T), Light tolerant (LT) or susceptible to *H. schachtii*. Control treatments of sand leachate and deionised water were also included. Treatments with different letters show differences at 5% significance according to Duncan's Multiple range test.

3.4 Experiment 4 – Hatch responses of H. schachtii *in response to root leachates from plants of differing ages.*

In most cases, root leachates from the radish, mustard and sugar beet varieties had a stimulatory effect on the total hatch of *H. schachtii* juveniles after six weeks and a significant interaction between age and variety of plant was found (P<0.001, Figure 5).

With regard to the *H. schachtii* resistant radishes, root leachates of R1 and R3 caused the greatest total hatch of *H. schachtii* when the leachates were collected from six week old plants. In the case of the R3 treatment, there was a significant decrease in *H. schachtii* hatch

response to root leachates from the seven week old plants. For treatment R7, root leachates from the five week old plants caused the greatest level of hatch, and then this significantly reduced with root leachates from six weeks old plants. However, the response of the nematodes to RLS from the seven week old plants was not significantly different to either of the six or five week old treatments. Root leachates from radish R12, which is susceptible to *H. schachtii*, had a significant effect on stimulating juvenile emergence regardless of the age of the plant when the leachates were collected.

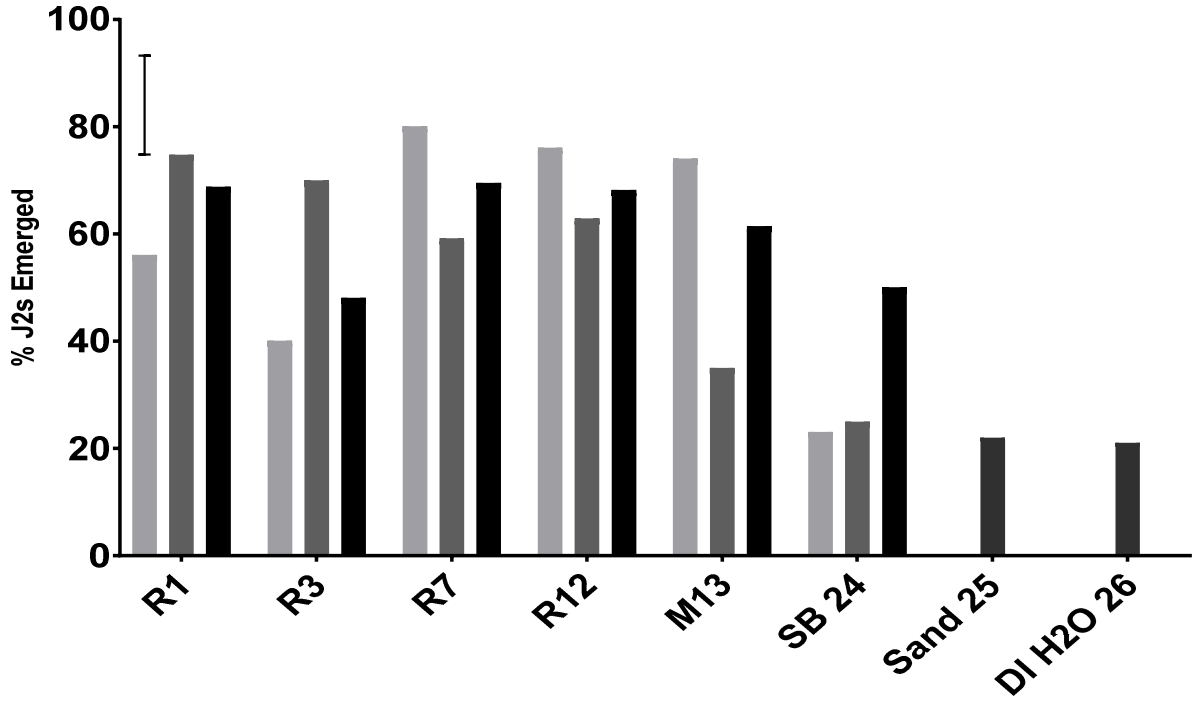


Figure 4 – Total hatch and emergence of *H. schachtii* juveniles following exposure to root exudate solutions. This experiment tested whether the age of the plant has an effect on the levels of hatch and emergence of *H. schachtii* juveniles. Root exudates were collected from radish, mustard or sugar beet plants (see table 1) at five, six or seven weeks after sowing. Sand and deionised water controls were also included. Cysts of *H. schachtii* were then exposed to the root exudate solutions for six weeks and emerged J2s were counted at weekly intervals. Significant differences were found (Age x plant type interaction $P<0.001$) and the error bar shows LSD at 5% significance for the total percentage hatch observed.

The sugar beet variety control treatment, susceptible to *H. schachtii*, was only seen to have a stimulatory effect on the hatch of *H. schachtii* when the root leachates were collected at seven weeks ($P<0.001$), with the other two ages of plant resulting in similar hatch responses to the sand and water controls.

When the cumulative weekly hatch results were analysed (Table 3), a range of responses from the various treatments were found. Significant interactions between age of the plants and plant type were found each week ($P<0.001$ for all weeks). At seven days (after the start of the experiment) clear differences were observed between the treatments. For example, leachates from the five week old R7 treatment induced very high levels of hatch (59.1%), which was higher than many of the other treatments. These distinct differences remained throughout the experiment, with the brassicas all stimulating more hatch than the water control, except M 13 at six weeks old, and R 3 at 5 weeks old.

Differences were also found between the HR of the different treatments, and again an interaction between the age of the plants and plant type were found ($P=0.037$, Fig 6). The five week old mustard (M 13) had a significantly greater HR than the six week old mustard and the seven week old R 12 caused HR greater than the other two R 12 treatments. Other varieties, such as R 1 and R 3 did not differ in their HR due to age of the plants. R 7 had the greatest HR at six weeks old and this was greater than the seven week old plants. HR was reduced at five weeks but not significantly when compared to the six week old treatment. The control treatments did not differ in their HR to each other. The sugar beet treatment (SB S 24) at seven weeks had a greater HR than the water control but the remaining SB S 24 treatments were not different to the water.

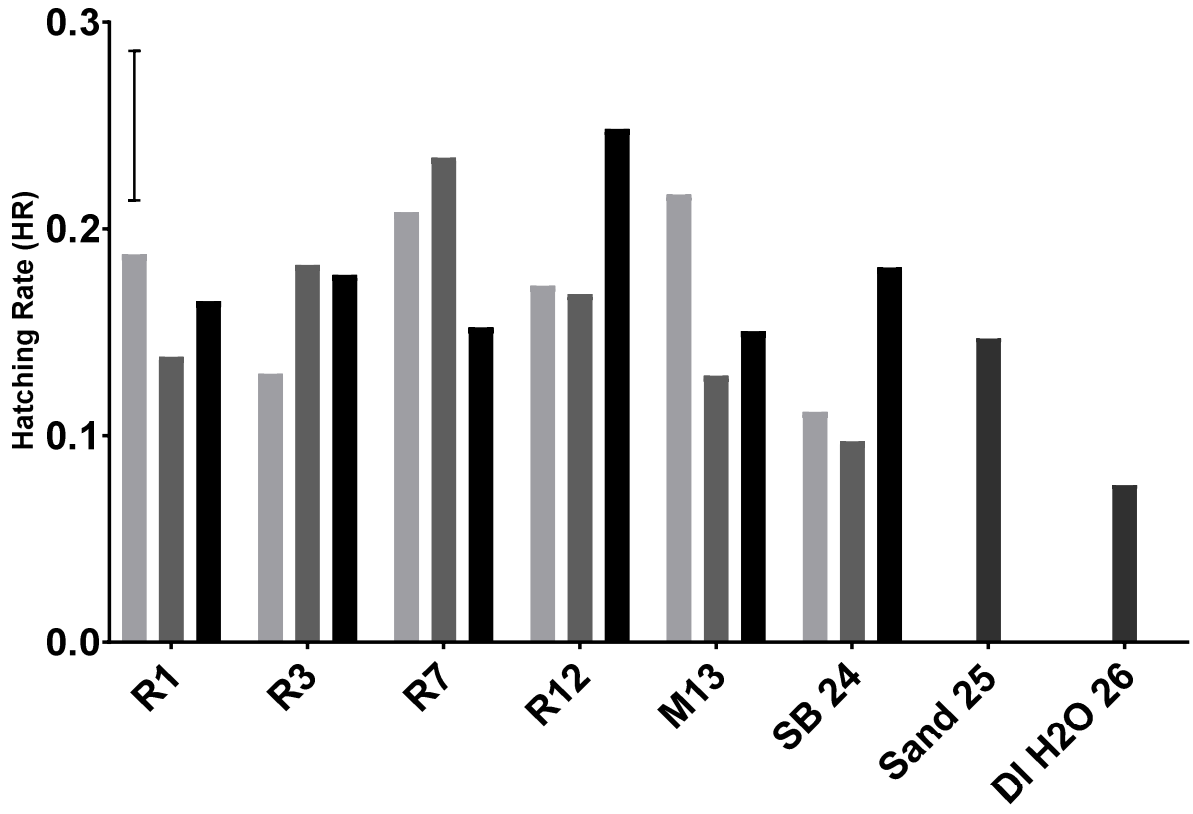


Figure 5 – Effect of brassica root leachates obtained from plants five, six or seven weeks old upon the hatching rate (HR) of *H. schachtii* Juveniles from cysts after six weeks exposure to the root leachate solutions. The error bar shows LSD at 5% significance (Age x plant type interaction P=0.037). Sand leachate and deionised water controls were also included

Biomass of the plants was found to increase with age in all of the treatments (P=0.002), except for the mustard. The mustard plants at five and seven weeks showed high levels of growth, however, the six week old plants did not show such vigorous growth (Figure 7). Root leachate from the six week old white mustard had no significant impact of juvenile emergence when compared to the control treatments, however, leachates from the five and seven week old plants did cause significant increases in *H. schachtii* emergence (P<0.001, Fig 5).

Table 3 – Cumulative weekly percentage hatch and emergence of juveniles of *H. schachtii* in response to exudates of radish, mustard and sugar beet grown for either seven, six or five weeks in a controlled environment room. The experiment also included sand and deionised water controls. Different letters adjacent to the results indicate significant differences at 5% probability according to Duncan's multiple comparison test.

		Time of cysts' exposure to root leachate solutions											
Treatment	Plant age (weeks)	7 Days		14 Days		21 Days		28 Days		35 Days		42 Days	
R1	5	35.3	cdef	51.6	defg	55.2	cde	55.7	cde	56.0	cde	56.1	cdef
	6	39.5	defg	68.8	gh	73.2	ef	74.0	ef	74.3	ef	74.7	fg
	7	46.0	efgh	63.7	efgh	67.4	def	68.5	def	68.7	def	68.7	defg
R3	5	22.6	abcd	36.3	bcd	39.4	abc	40.2	abc	40.5	abc	40.6	abc
	6	49.4	fgh	64.2	efgh	68.3	def	69.0	def	69.4	def	69.9	efg
	7	29.9	bcde	43.6	cde	47.0	bcd	47.8	bcd	47.9	bcd	48.0	bcd
R7	5	59.1	h	77.2	h	79.8	f	80.1	f	80.6	f	80.6	g
	6	44.7	efgh	55.5	defg	58.0	cdef	58.8	cdef	59.0	cdef	59.1	cdefg
	7	43.0	efgh	64.9	fgh	67.7	def	68.8	def	69.3	def	69.4	defg
R12	5	48.9	efgh	65.7	fgh	74.4	ef	76.0	ef	76.6	ef	76.7	fg
	6	42.4	efgh	59.7	efgh	62.2	def	62.6	def	62.8	def	62.9	defg
	7	54.9	gh	65.9	fgh	67.2	def	67.6	def	67.8	def	68.1	defg
M13	5	54.6	gh	68.5	gh	73.2	ef	74.4	ef	74.6	ef	74.7	fg
	6	19.6	abc	30.7	abc	34.6	ab	34.8	ab	34.9	ab	34.9	ab
	7	38.8	defg	55.0	defg	58.6	cdef	59.4	cdef	60.8	cdef	61.4	cdefg
SB S 24	5	13.8	ab	21.3	ab	22.6	a	23.4	a	23.8	a	23.9	a
	6	11.0	a	19.3	ab	22.9	a	24.0	a	24.8	a	24.9	a
	7	33.1	cdef	46.5	cdef	49.6	bcd	49.8	bcd	50.0	bcd	50.0	bcde
Sand		13.9	ab	20.6	ab	22.5	a	22.6	a	22.6	a	22.6	a
Deionised H_2O		5.6	a	13.3	a	18.9	a	20.9	a	21.6	a	21.7	a
P		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
LSD		16.31		18.05		18.37		18.46		18.43		18.46	
SED		8.15		9.01		9.17		9.22		9.20		9.22	
% CV		32.6		25.7		24.4		24.2		24.0		23.9	

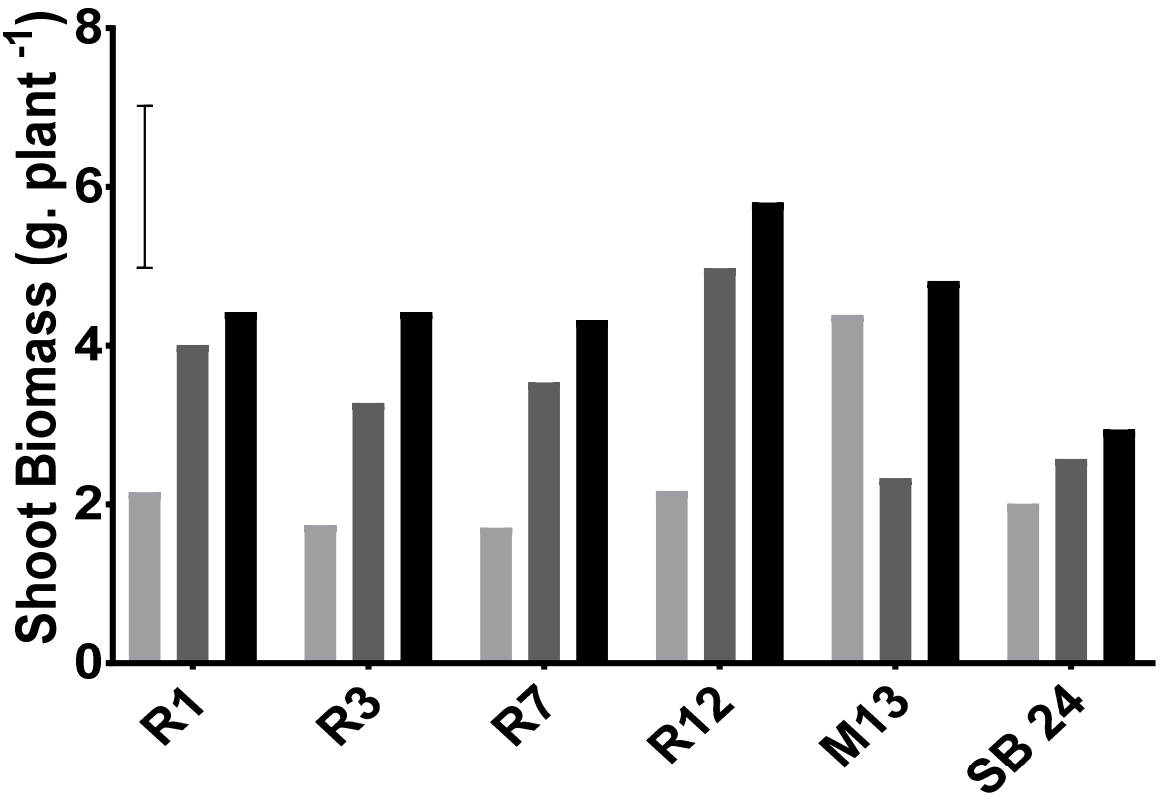


Figure 6 – Biomass measurements of shoot material harvested on the day of exudate collection. Material was dried until constant weight and then weighed. Plants were grown in a controlled environment room for either five ▒, six ▒ or seven ▒ weeks. Error bar shows LSD at 5% significance (Age x plant type interaction P=0.002).

4. Discussion

The stimulation of hatching of cyst nematode juveniles is due to a number of physical, chemical and biological factors (Sharma & Sharma, 1998). Important in the hatching process is the recognition of chemicals in host root leachates which stimulate hatch and emergence at an optimum point in the growth of the plant. These chemicals, known as hatching factors, are important as they lead to hatching of the juveniles from the eggs inside the cysts. This is due to binding of calcium to the eggshells, resulting in a change in their permeability and subsequent movement of solutes, notably trehalose, from the egg fluid and thus the J2 is able

to take up water and their hatch is stimulated (Sharma & Sharma, 1998). Hatching factors are produced in trace amounts (Masler & Perry, 2018) and the response by the nematodes can be very specific to the age of the plant. For example, *Heterodera goettingiana* has been shown to hatch in response to leachates from host pea plants between four and six weeks old (Perry et al., 1980) and *H. carotae* restricts its hatch to carrots aged between five to seven weeks old (Greco & Brandonisio, 1986). Age of brassica host plants has also been shown to influence the hatching activity of nematodes in *H. cruciferae*. Bowen (1988) showed that, despite continued growth of oilseed rape in both field and glasshouse experiments, the most stimulating point for *H. cruciferae* J2s was from plants fourteen and ten weeks old respectively. In addition to the stimulation of hatch by host plants they can also inhibit hatch (Masler & Perry, 2018). Hatch inhibiting chemicals are produced whilst young plants become established which reduces the number of invading J2s until the plant is well established and therefore better able to host the nematodes.

4.1 Brassica hatch responses

The wider understanding of responses observed in other nematode species may help elucidate our observations, especially in response to the brassica varieties evaluated. Results from our first experiment investigating the response of *H. schachtii* hatching to brassica leachates were different to Experiments 3 and 4. Experiment 1 showed clear inhibition of hatching of the juveniles in response to the root exudates compared to the water control. This effect was clear in all of the brassica treatments when compared to the water control, with the most extreme inhibition being caused by treatment R7. However, Experiments 3 and 4 found enhanced hatch of *H. schachtii* juveniles when exposed to brassica RLS. The results from the first experiment may be due to the extended storage of the leachates compared to

the other experiments, and activity root leachates of potatoes has previously been found to decline rapidly in the first two months (Widdowson, 1958), and so this may have happened with our brassica root leachates also. It is also possible that, despite being grown in the same CER for the same period of time, the plants instead produced inhibitory chemicals at the point of RLS collection. However, whilst we cannot fully explain this disparity between the experiments, the consensus of our findings in Experiments 3 and 4 show that brassica root leachates do stimulate hatch of *H. schachtii* juveniles.

Experiment 4 highlights the influence of age of the plant on hatch of *H. schachtii* juveniles. Some varieties have shown to have an optimum age at which hatching is stimulated, and the HR which is stimulated can be influenced by the age of the plant when the RLS was collected, such as radish R 3, where the peak in hatch stimulation occurred in the six week old plants compared to the five and seven week old plants. This agrees with the findings of Bowen (1988) that, regardless of the continued growth of the plants, their stimulatory effect is not increased. Other varieties, such as R7 and R 12 showed similar hatch stimulation regardless of plant age and size.

Our findings shed more light upon the hatching tactics of *H. schachtii* and future methods to control in field infestations. Understanding varieties which cause earlier and greater hatch of juveniles could allow for varieties with the most prolonged hatch period to be selected by growers. Varieties such as R3, with their limited peak hatch period may be less preferable to a variety such as R1 which shows more prolonged hatch. A variety which continues to stimulate *H. schachtii* hatch into the autumn would be of most benefit, especially in field conditions, where water availability may be limited in dry summers.

Brassica treatments R1 and M13 have recently been found to significantly lower H. schachtii populations in infested field experiments (Wright et al., 2018a). Populations were reduced by 30-40%, which is lower than reported control levels of these varieties (Bundessortenamt, 2014). Other treatments, R3 and R7, also included in the field experiments, however, did not show significant population reductions. The findings from the current *in-vitro* experiments may explain why these results occurred. The treatments that did not show control may have had limited peak hatch periods, or occurred at the wrong point in time to deliver a significant population reduction. Despite these results, it is also important to consider how a laboratory assay, using plants grown under controlled conditions and recently cultured cysts, may differ from field conditions. In the field, the conditions are less conducive to plant growth due to limited water availability, fluctuating temperatures and pest and disease pressures. The interaction between these varieties, the climate and weather conditions experienced in the field, soil type and age of the cysts in a field are far more complex than we could simulate in the laboratory. Studies using field cysts would be useful to validate our conclusions.

Variation in the responses to the varieties could be a result of the different batches of cysts used from different cultures of *H. schachtii*. If *in-vitro* tests were to be developed to further test *H. schachtii* hatching responses and evaluate future cultivars of sugar beet and brassica trap crops, the source of the cysts, and their response to the varieties, needs to be standardised to ensure repeatable results and conditions, which may inhibit hatching, as seen in our first experiment, understood and avoided.

4.2 Sugar beet hatching responses

Our experiments have also shown consistent differences between the hatch of *H. schachtii* juveniles in response to root leachates collected from different sugar beet variety types. In Experiment 2, the sand and water controls resulted in similar final levels of hatch to the majority of the varieties, indicating that most varieties did not have a stimulatory effect on the hatch of the juvenile nematodes. This was unexpected since *H. schachtii* juveniles have previously been shown to respond greatly to root leachates produced by suitable hosts (Perry, 2002). In Experiment 3 we observed clear stimulation of hatch, and therefore differences in the age of the cysts between Experiments 2 and 3, may have caused these different responses seen to the RLS, with the RLS in Experiment 2 not resulting in hatching responses by the nematodes any different to the water control, but in Experiment 3 a large response to RLS from the sugar beet was seen. Different responses in terms of HR have also been found between the experiments (table 2). For example, the same cultivars of sugar beet resulted in much lower HR in Experiment 2 than in Experiment 3, supporting the hypothesis that different batches of cysts likely influence the differences found. We have consistently observed that the light tolerant varieties stimulate significantly greater hatch of juveniles than resistant sugar beet varieties. In neither experiment did the susceptible varieties appear to be any more stimulatory than the tolerant varieties.

Assuming that the hatch responses observed in our CER experiments translate to the responses of *H. schachtii* juveniles in the field, we can deduce that the light tolerant varieties would be most likely to be at the greatest risk of damage from *H. schachtii* juveniles. This response may also be due to greater early rooting exhibited by these varieties (Wright *et al.*,

2018). Therefore, the light tolerant varieties may simply be more stimulatory as they grow roots faster and thus release a greater amount of root leachates. These findings indicate that *H. schachtii* may cause increased damage and colonisation of the root systems of light tolerant varieties, resulting in higher *H. schachtii* populations, particularly since these varieties appear to show little suppression of *H. schachtii* populations (Wright et al. 2018, submitted). As the tolerant varieties do not display any ability to reduce the hatch of the nematodes, it seems unlikely that these varieties protect themselves from damage by causing lower levels of hatch and therefore other mechanisms of tolerance must exist.

We have demonstrated that differences exist between sugar beet varieties in terms of their ability to stimulate hatch of J2s, and varieties which result in lower hatching may be preferable to grow as this would minimise the early damage to the seedlings at their most vulnerable time of growth. It would be useful to conduct field scale experiments to determine whether the varieties we have found to be less stimulatory to hatch of *H. schachtii* juveniles result in better growth and yield, also in addition to elucidating the relationship between variety, root growth and *H. schachtii* juvenile hatch and emergence.

Sugar beet growers with *H. schachtii* infested fields would usually select a tolerant variety to grow to protect yields (BBRO, 2018). With the information we have found about their stimulation of hatching, it would appear that the tolerance, and therefore maintenance of yield in *H. schachtii* infested situations, is not linked to reduced stimulation of hatching of *H. schachtii* J2s. Therefore tolerance is more likely to be more associated with a reduced nematode hosting capacity associated with such varieties as seen in other investigations (Hauer *et al.*, 2016; Reuther, Lang and Grundler, 2017, Wright et al. 2018 submitted).

Age and viability of the plants is also likely to influence the level of *H. schachtii hatch* and therefore damage to the growing plants. In Experiment 4, we observed significant differences between the responses of *H. schachtii* J2s to root leachates collected from sugar beet plants of different ages. The larger and more mature beet plants resulted in the greatest levels of hatch, whereas the five and six week old plants did not cause increased hatch over the controls. This supports our hypothesis that there is a relationship between sugar beet growth and the level of hatch of *H. schachtii* juveniles. However, future investigations into the response of sugar beet variety, age, development and subsequent stimulation of *H. schachtii* J2 hatch and emergence would be useful and may help with variety selection in the future.

5. Conclusions

We have demonstrated that the hatching responses of *H. schachtii* in response to sugar beet, radish and white mustard are related to the variety being tested and can also be influenced by the age and development of the plant too.

Our results relating to root leachates from different sugar beet varieties show significant differences in the hatching dynamics of *H. schachtii*. Age, variety type, and the vigour of different varieties all appear to influence hatch of *H. schachtii* juveniles.

Our findings also support the use of resistant trap crops to stimulate hatch of *H. schachtii* and thus reduce in field populations. We have shown that some varieties of these trap crops can maintain their stimulatory effect on hatch of *H. schachtii* J2s regardless of age of the plants, whereas other varieties have a shorter peak hatch period.

6. Acknowledgements

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7. References

BBRO (2009) Beet Cyst Nematode Technical Guide Ed British Beet Research Organisation.

BBRO (2018) *Recommended list of sugar beet varieties 2019*.

BBRO (2015) Recommended variety list 2016.

Blok V.C., Tylka G.L., Smiley R.W., de Jong W.S., Daub M. (2018) Resistance Breeding, In *Cyst Nematodes*, pp. 174–214. Eds M. Moens, R. N. Perry, and J. T. Jones. Wallingford, Oxfordshire, UK: CAB International.

Bowen S.A. (1988) *Aspects of the population biology of the cyst nematode parasites of oilseed rape*. Luton College of Higher Education.

Bundessortenamt (2014) Beschreibende Sortenliste: Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben & Zwischenfrüchte. (In German).

CABI (2018) Heterodera schachtii (beet cyst eelworm). *Invasive Species Compendium*. Available at: https://www.cabi.org/isc/datasheet/27036 [Accessed February 17, 2018].

Cooke D.A. (1987) Beet Cyst Nematode (Heterodera Schachtii Schmidt) and its Control on Sugar Beet. *Agricultural Zoology Reviews*, **2**, 135–183.

Cooke D.A. (1985) The effect of resistant cultivars of catch crops on the hatching of Heterodera schachtii. *Annals of Applied Biology*, **106**, 111–120.

Danquah W.B., Back M.A., Grove I.G., Haydock P.P.J. (2011) In vitro nematicidal activity of a

garlic extract and salicylaldehyde on the potato cyst nematode, Globodera pallida. *NEMATOLOGY*, **13**, 869–885.

Dewar A.M., Cooke D.A. (2006) Pests, In *Sugar Beet*, pp. 316–350. Ed A. P. Draycott. Oxford: Blackwell Publishing Limited.

Evans K. (1983) Hatching of potato cyst nematodes in root diffusates collected from twenty-five potato cultivars. *Crop Protection*, **2**, 97–103.

Fatemy S., Abootorabi E. (2002) Hatching activity, invasion rate and reproduction of Heterodera schachtii on oilseed rape cultivars. *Nematologia Mediterranea*, **30**, 163–166.

Greco N., Brandonisio A. (1986) The Biology of Heterodera carotae. *Nematologica*, **32**, 447–460.

Hauer M., Koch H.J., Krüssel S., Mittler S., Märländer B. (2016) Integrated control of Heterodera schachtii Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. *Applied Soil Ecology*, **99**, 62–69.

Jones F.G.W. (1956) Soil populations of Beet eelworm (Heterodera schachtii) in relation to cropping. *Annals of Applied Biology*, **44**, 25–56. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1744-7348.1956.tb06845.x.

Kakaire S., Grove I.G., Haydock P.P.J. (2012) Effect of temperature on the life cycle of Heterodera schachtii infecting oilseed rape (Brassica napus L.). *Nematology*, **14**, 855–867.

Kakaire S., Grove I.G., Haydock P.P.J. (2015) The number of generations of Heterodera schachtii completed on oilseed rape (Brassica napus L.) during the UK growing season. *NEMATOLOGY*, **17**, 557–565.

Koch D.W., Gray F.A. (1997) Nematode-Resistant Oil Radish for Control of Heterodera schachtii. *Journal of Sugar Beet Research*.

Märländer B., Hoffmann C., Koch H.J., Ladewig E., Merkes R., Petersen J., Stockfisch N. (2003) Environmental situation and yield performance of the sugar beet crop in Germany: Heading for sustainable development. *Journal of Agronomy and Crop Science*, **189**, 201–226.

Masler E.P., Perry R.N. (2018) Hatch, Survival and Sensory Perception, In *Cyst Nematodes*, pp. 44–73. Eds R. N. Perry, M. Moens, and J. T. Jones. Wallingford, Oxfordshire, UK: CAB International.

Müller J. (1999) The economic importance of Heterodera schachtii in Europe. *Helminthologia*, **36**, 205–213.

Perry R.N. (2002) Hatching, In *The Biology of Nematodes*, Ed D. L. Lee. London: Taylor & Francis.

Perry R.N., Clarke A.J., Beane J. (1980) Hatching of Heterodera goettingiana in vitro. *Nematologica*, **26**, 493–495.

Reuther M., Lang C., Grundler F. (2017) *Nematode-tolerant sugar beet varieties - Resistant or Susceptible to the Beet Cyst Nematode Heterodera schachtii?*

Sharma S.B., Sharma R. (1998) Hatch and emergence, In *The Cyst Nematodes*, pp. 191–216. Ed S. B. Sharma. London: Chapman & Hall.

Shepard A.M. (1970) Extraction and estimation of Heterodera, In *Laboratory Methods for work with Plant and Soil Nematodes*, Ed J. F. Southey. London: HMSO.

Steele A.E., Toxopeus H., Heijbroek W. (1982) A Comparison of the Hatching of Juveniles from Cysts of Heterodera schachtii and H. trifolii. *Journal of nematology*.

Widdowson E. (1958) Observations On the Collection and Storage of Potato Root Diffusate. *Nematologica*, **3**, 173–178. Available at: http://booksandjournals.brillonline.com/content/journals/10.1163/187529258x00391.

Winfield A.L., Enfield M.A., Foreman J.H. (1987) A column elutriator for extracting cyst nematodes and other small invertebrates from soil samples. *Annals of Applied Biology*, **111**, 223–231.

Wright A.J.D., Back M.A., Stevens M., Sparkes D.L. (2018a) Evaluating resistant brassica trap crops to manage Heterodera Schachtii (SCHMIDT) infestations in eastern england. *Pest Management Science*. Available at: https://doi.org/10.1002/ps.5134.

Wright A.J.D., Bussell J.S., Stevens M., Back M.A., Sparkes D.L. (2018b) Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, Heterodera schachtii (Schmidt) under uninfested conditions. *European Journal of Agronomy*, **98**, 37–45. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1161030118301084 [Accessed May 30, 2018].

Zheng L., Ferris H. (1991) Four types of dormancy exhibited by eggs of Heterodera schachtii. *Revue de Nematologie*, **14**, 419–426.

Chapter 6

Summary and Conclusions

The research described within this thesis has been undertaken to gain a better understanding of interactions between beet cyst nematode, sugar beet and brassica trap crops to investigate the following hypotheses, originally outlined in Chapter 1:

I. Tolerant varieties of sugar beet will exhibit distinctly different physiological characteristics to susceptible varieties, which explain how they can maintain yields in the presence of BCN
II. Lower yield potentials of BCN resistant sugar beet varieties can be explained by their physiological traits
III. Tolerant varieties of sugar beet allow similar levels of BCN multiplication to susceptible varieties
IV. Brassica trap crops can be used to reduce in field populations of BCN and hence provide a yield benefit to the following crop
V. Different varieties of sugar beet, radish and mustard stimulate different hatch responses of *H. schachtii*

The findings are now summarised and discussed in terms of tolerant and resistant sugar beet varieties, the appropriate use of brassica trap crops, the challenges of performing experiments with BCN and finally future research priorities. The findings are then summarised with key messages for sugar beet industry stakeholders identified and future research priorities highlighted.

1. Sugar beet varieties

Much of the research undertaken has been to understand more about varieties of sugar beet, how they differ to each other and how they interact with *Heterodera schachtii*. The discussion has been structured into sections to address the findings relating to tolerant, light tolerant and resistant varieties of sugar beet to *H. schachtii*.

1.1 Tolerant sugar beet varieties

Since their introduction to the UK market in 2009 (Wright et al., 2018), growers affected by infestations of BCN have embraced the use of tolerant varieties to protect crop yields, and the market share of these varieties has been increasing each year. The continual development of tolerant varieties has led to recently introduced varieties yielding as well as their susceptible counterparts in recommended list (RL) trials (BBRO, 2018).

What remained largely unknown at the start of this study was how tolerant varieties respond to an infestation with BCN, whether the responses of different tolerant varieties differ and the ways in which tolerant mechanisms operate. We sought to address Hypothesis I through a range of experiments to understand more about the current tolerant varieties of sugar beet.

The initial experiments in the glasshouse, outlined in Chapter 2, were conducted to identify differences in physiology between a range of varieties (susceptible, tolerant or resistant to BCN) in the absence of BCN infestation. Our results showed that no distinct differences could be measured in any of the measurements taken when comparing the tolerant varieties with the susceptible varieties. Canopy development of the tolerant varieties showed no distinct differences to the susceptible control varieties and the photosynthetic activity of the varieties

was also similar to the controls. Other measurements that were taken but not described in the paper, such as SPAD, also showed no clear differences between the variety types. Differences between the tolerant varieties which did exist, such as impurity levels, were not consistently associated with all of the tolerant varieties and may therefore be instead associated with individual varieties or the original germplasm from the different breeders.

One hypothesis was that tolerant varieties develop their root systems faster than a susceptible variety in an attempt to grow away from areas with BCN infestation. This hypothesis was not supported in the hydroponic tank experiment, where we found that the tolerant varieties had similar rooting properties to the susceptible varieties. Experiments to investigate the responses of these varieties grown in soil and infested with BCN would be useful to support or reject our conclusion.

In the box experiments conducted in 2016 and 2017, where sugar beet plants were uniformly inoculated with BCN J2s, we were able to gain a better insight into how the tolerant varieties respond to BCN infestation and found physiological differences of chlorophyll levels and stomatal conductance between the varieties were expressed only in the presence of the nematodes. The tolerant varieties responded differently to each other in this case, and this may be due to the fact that each tolerant variety assessed was sourced from a different breeder and they therefore may have introduced different traits into their tolerant varieties. We can, however, conclude that all of the tolerant varieties tested produced similar final BCN populations to each other and that these populations were approximately ten times lower than the susceptible control variety. This is likely to be associated with the common source of tolerant genes which were utilised to develop tolerant varieties (Lange and De Bock, 1994)

which were introgressed from wild sea beet (*B. vulgaris* ssp. *maritima*). In fact, we postulate that these tolerance genes actually infer partial resistance against BCN to the tolerant varieties in these studies.

The partial resistance to BCN by the tolerant varieties results in fewer females developing on these plants and therefore the nematodes have a lower total nutrient demand on the plants since females require nearly 30 times the nutrition of a male (Müller et al., 1981). Therefore, the tolerance trait is likely associated with a reduction in the number of successfully established and maintained syncytia by the juvenile nematodes inside the roots of the sugar beet. The differences we then measured, such as leaf chlorophyll levels and soil tare, are associated with the plants being less stressed by the nematodes than the susceptible counterparts. However, it does seem that the newer tolerant varieties appear better suited to tolerate the infestations, since the oldest tolerant variety investigated had high soil tares similar to the susceptible variety.

The upward trend for better yield in the tolerant varieties which has been recorded in RL trials over the last decade is undoubtedly important for sugar beet growers in managing BCN infestations, since the yield penalty against these varieties is much smaller than it used to be, and some BCN tolerant varieties now yield as well as elite susceptible varieties of sugar beet when grown in the absence of BCN (BBRO, 2018). However, we must reject our initial hypothesis (Hypothesis III) since our data support instead that BCN tolerant varieties have a lower fecundity than susceptible sugar beet varieties. However, they cannot be considered a method of control since they do not lower BCN populations in the soil. Instead, tolerant varieties are useful to manage populations and reduce the fecundity of an infestation of BCN

when compared to a susceptible variety, findings which are also supported by other recent publications (Hauer et al., 2016; Kenter et al., 2014; Reuther et al., 2017). By using the box method, knowledge of the ability of tolerant sugar beet varieties to host BCN over entire growing seasons, where multiple generations can develop, and then subsequently closely monitor the infested plants, has been of a large benefit and this method has potential to be developed into a screen for commercial variety testing. This would then add to the information currently given about the tolerant varieties by the breeders and also allow for them to be compared using a common method. It will also enable the very highest yielding tolerant varieties to be assessed which were not commercially available until recently. This will allow for assessments to be made to ensure that the tolerance is equally effective in managing BCN population build-up even on the very high yielding varieties. However, it is also important to stress that the method requires both in field validation of the results using infested sites and also refinement of the dosage and timing of inoculation to the boxes to clearly see yield impact upon susceptible control varieties and thus validate the yield tolerance.

1.2 Light tolerant sugar beet varieties

Light tolerant varieties were last listed on the BBRO RL in 2015 for cultivation in 2016 (BBRO, 2015), so whilst they are not currently available to the market, they appear to be distinctly different the other variety types studied.

From our findings in the glasshouse and hydroponic tank experiments (Chapter 2), the light tolerant varieties appear more vigorous than any of the other varieties studied, developing larger canopies faster and with the greatest root length measured in the tank experiments.

In the field experiments described in Chapter 2, early canopy development was observed in both years, and this variety type also benefitted the most from the highest plant population density established in 2016. However, these benefits relating to the light tolerant varieties are outweighed by the negative traits measured in the box experiments. The light tolerant variety resulted in either similar or even greater final BCN populations (*Pf*) than the susceptible variety in both years of the box experiments. The light tolerant variety also displayed large soil tares in both years of the experiments.

The hatch experiments also revealed that the light tolerant varieties consistently stimulated the greatest BCN J2 hatch and emergence, supporting Hypothesis V relating to hatching stimulation by sugar beet varieties. Combining this knowledge with the greater BCN populations supported on the light tolerant varieties and also their rooting traits, the light tolerant varieties have the potential to severely increase BCN populations, compared to the tolerant varieties. These varieties will also be exposed to a greater volume of soil faster, due to more vigorous rooting and in turn stimulate more J2 invasion, thus putting such plants at more risk from infestation than a tolerant variety. Therefore if ever new varieties of this type are approved for cultivation, their use should be carefully evaluated.

1.3 Resistant sugar beet varieties

We initially hypothesised that resistant varieties possess physiological traits which explain their lower yield potentials compared to 'elite' varieties (Hypothesis II) and this prevents them being widely commercially adopted. The canopy expansion in the glasshouse and field experiments was slower than the other varieties tested and this may explain why they yield less than the other varieties. However, in 2017 the resistant variety was the second highest

yielding variety and yielded greater than the susceptible and light tolerant varieties. The varieties tested did differ between the field experiments and the experiment in the glasshouse. In the glasshouse a much older BCN resistant variety 'Sanetta' was used. This variety was initially first approved for cultivation in Germany in 2007 (Bundessortenamt, 2014), whereas, in the field, a variety under development from the same breeder was used. This may explain why it was able to yield better, due to approximately ten years of extra breeding to reduce the negative traits associated with these variety types. Other negative quality traits were also observed, for example, In the field experiments at Sutton Bonington, the resistant variety consistently had greater sodium impurities than the susceptible variety, however, this difference was not clearly significantly greater than either the tolerant or light tolerant varieties tested.

An interesting finding from the research, which may have important commercial implications, is that resistant varieties may benefit from a higher population density (than other variety types) to counteract the less vigorous canopy. The slower developing canopy was clearly seen in the glasshouse experiment and also in the 2016 field experiment, where the higher PPD also increased yield in the resistant variety. However, in 2017 this benefit was not as clear, but planting at the higher PPDs did not have a negative impact upon final sugar yield of the resistant variety.

One benefit of growing BCN resistant varieties was demonstrated in the box experiments as they produced the lowest final population of BCN in both years. The resistant varieties also consistently showed the lowest hatch stimulation of any sugar beet variety tested in the BCN hatch experiments (Chapter 5), and this may be due resistant varieties producing fewer

hatching factors for the juveniles to detect and then respond to. The yield of the resistant beet was the lowest from the box experiments in both years. However, they did maintain their quality in terms of alkalinity ratio in both years. Infestation of the resistant variety led to reduced transpiration and this may be due to the damage caused to the roots after the detection of a feeding nematode and the subsequent death of the syncytium following a hypersensitive response.

Should the development of yield of resistant varieties follow the yield increases already seen in BCN tolerant varieties in recent years, and the negative canopy traits can be bred out by the breeders, then the additional seed rate may not be required, and they may become a popular option to cultivate in BCN infested conditions. Therefore, it may be useful for the industry to approve the addition of one resistant variety to the RL, much in the way a variety of beet is listed for resistance to the AYPR strain of rhizomania, but is not recommended for widespread cultivation (BBRO, 2017). If this were to happen, an assessment of canopy vigour of these varieties would need to be undertaken and the optimum plant population identified. In field tests to validate BCN population reductions achieved by resistant varieties would also be of benefit to support their use.

1.4 Variety terminology

Whilst the sugar beet varieties have been referred to as tolerant, light tolerant, resistant or susceptible throughout this thesis it seems that the tolerant varieties may need to be reclassified as partially resistant instead. In addition, the light tolerant varieties should not be considered for cultivation in the UK anymore due to their negative traits. This would leave growers with a choice of either resistant varieties, partially resistant & tolerant varieties

(Since the two traits are separate) or susceptible varieties. Our results from the box study support this notion, as well as findings from the use of similar varieties in Germany (Hauer et al., 2016; Reuther et al., 2017). Tolerant varieties have been found to consistently limit the multiplication of BCN compared to susceptible controls, but cannot achieve reproductive factors (*Rfs*) as low as those of the resistant varieties, indicating that tolerant varieties are partially resistant to BCN. This partial resistance mechanism, and the subsequent reduced stress to the plant appears to underpin the yield tolerance of these varieties. Consequently, the lower fecundity of tolerant varieties supports the future reclassification of such varieties as partially resistant. Reclassification may however lead to greater confusion with BCN varieties, especially since many growers in the UK believe that they are already using resistant varieties (Wright, 2017), when in fact only tolerant varieties are available currently. Instead, a focus could be placed upon scoring the partial resistance of the tolerant varieties and including these data as part of the RL results. This should be done in line with other resistance scoring for plant diseases, on a 1-9 scale, where a number 1 score indicates a high susceptibility to a pest or disease and a 9 indicates high resistance to a pest or disease. Scoring such as this is currently performed on potato varieties for their resistance against a range of pests and diseases, including both potato cyst nematode (PCN) species *Globodera pallida* and *G. rostockiensis*. These trials are currently commissioned by the Agricultural and Horticultural Development Board (AHDB) Potatoes division (Campbell et al., 2017) and similar methods could be used in future BBRO/BSPB variety trials. Guidelines (EPPO Standard 2006-09) exist for PCN variety testing and could be suitably adjusted for use on sugar beet (EPPO, 2006).

Quantifying differences between tolerant varieties in terms of their BCN resistance may allow growers to select a variety with the lowest *Rf* should they have a high population of BCN, or

instead choose a variety with a higher *Rf* but greater yield potential, especially if they are able to use a long rotation as part of a BCN IPM strategy. Such a method to test the tolerant varieties against each other could also encourage breeders to develop varieties with lower *Rf*, with the aim to achieve *Rfs* as close as possible to 1.0 and therefore achieve complete resistance.

2. Brassica trap crops

Research into the use of brassica trap crops in the field experiments has produced mixed results which may be explained by the findings of the hatch experiments. Such results are important to aid growers when selecting varieties of brassicas to grow as a cover crop on fields with BCN infestations. Our field experiments were the first in the UK in many years to assess brassica trap crops, and our findings do agree with those previously published by Cooke (1985) who also found that only some of the trap crop varieties trialled resulted in significant population reductions of BCN. As discussed in Chapter 4, our results also agree with more recent studies conducted in the USA and Germany (Hauer et al., 2016; Kenter et al., 2014; Koch and Gray, 1997), whereby population reductions can be found using brassica trap crops, but the results obtained are not always consistent across field sites or between years due to different climatic and soil conditions.

We aimed to test the hypothesis (Hypothesis IV) that brassica trap crops can be used to reduce in field populations of BCN and provide a yield benefit to the following crop through a range of field experiments. As we demonstrated in Chapter 4, two varieties of brassica trap crop (the class one radish and class two mustard) led to significant population reductions of BCN, whereas the two class two radishes did not lead to significant population reductions

compared to the susceptible radish or fallow controls. It appears that at least some brassica trap crop varieties can reduce BCN field populations, supporting part of the original hypothesis. When the hatching stimulation of the same variety of trap crop was assessed in the laboratory, one experiment showed inhibition of hatch, another no differences between the varieties and the final experiment suggested that the age of the plant can influence the hatch of the juveniles leading to peaks of hatch when the plants are of a certain age.

These peaks in hatch may also occur in the field, thus limiting the amount of hatch that can be stimulated by a trap crop. However, only some of the varieties showed this peak in age-related hatch, whereas other varieties showed more sustained hatch of the nematodes and supports the latter part of Hypothesis V. Therefore, different varieties can result in different hatching responses from the nematodes. The first hatch experiment also demonstrated that the root leachates have the potential to inhibit hatch and in this case water caused significantly greater amounts of hatch. It is possible that these plants produced hatching inhibition factors (Masler and Perry, 2018) and this may be due to the extra length of time the root leachates were stored.

The use of brassica trap crops requires further investigation. Our field experiments were established in early September, only three weeks before the autumnal equinox, so day length was shortening and soil temperatures declining. Earlier sowing, to take advantage of greater temperatures and photoperiods, may encourage more growth of the trap crops and consequently their ability to stimulate hatch of the nematodes, a conclusion supported by recent work in Germany (Hauer et al., 2016; Kenter et al., 2014; Koch et al., 2017).

Another consideration is their cost and other benefits to the farming system, as the use of a trap crop needs to be appropriate and suitable for the rotation (Cooke, 1985). As sugar beet is spring sown it often makes rotational sense to plant a cover crop after the preceding combinable crop is harvested. There is then a cost to plant, fertilise and destroy the cover crop which could exceed £120 per hectare, as discussed in Chapter 4. It is therefore unlikely that cost could be returned immediately in the following sugar beet crop, especially as a tolerant sugar beet variety should also be chosen to minimise yield. However, soil health benefits, such as prevention of soil erosion (and possible BCN spread), or soil structural improvements may, in the long term, benefit not just the following sugar beet crop but also other crops in a growers' rotation and therefore should be taken into consideration.

Our trap crop studies were extended by planting sugar beet on the same plots in 2017 in order to test the second part of Hypothesis IV. The variety 'Pasteur' was chosen as this had been used as the susceptible control variety throughout all previous experiments. Unfortunately, the experiment was heavily grazed by rabbits in the spring of 2017 and required re-drilling in June. As a result, the plants never established well and yielded poorly. However, a significant relationship was found between the final population of BCN and sugar yield but no significant effect of trap crop treatment in the preceding autumn was found. Neither was nematode population after the trap crops found to be significantly related to yield which could be due to the poor initial establishment of the experiment. Appendix 1 provides more information about this experiment and the significant Pf-yield relationship found.

As no relationship was detected between trap crop treatment and the yield of the following beet crop, it could be that there is no residual benefit to the following crop. Although, as we only have one year's worth of data and the fact that this experiment was re-drilled very late we cannot be certain and requires more in-depth investigation in the future. Additional studies using trap crops over a range of soil types and infestations over more seasons would be needed to support or dispute this conclusion.

If trap crops become more popular in the future, a standardised test into their hatching stimulation over the age of the plant would be useful and provide more information to a grower than the simple and current class one or class two score. This would be important to choose more accurately the best varieties of trap crops. Crucially, this would test the hatching of the different varieties and species of brassica trap crops, which is the trait they are grown for, rather than just their resistance to BCN which is quantified in bioassays (Bundessortenamt, 2014).

3. Challenges and limitations of working with *Heterodera schachtii*

Working with BCN resulted in many challenges throughout the course of this research, regardless of the scale of the experiment.

In the hatch experiments, despite cysts being cultured under similar and controlled conditions, different batches of cysts produced different hatch responses; this was noticeably different between experiment three and the others (Chapter 5). Optimal conditions need to be identified for future BCN research in an attempt to standardise the response of the juveniles inside the cysts.

In the box experiments, despite detecting population differences in both years, and measuring physiological differences between the infested and non-infested plants, the inoculation appeared to be too late to induce significant yield differences. The inoculation was delayed until the plants were at the six-leaf stage to ensure that good root development had occurred for the juveniles to invade. However, this may have been too late to cause sufficient damage to the plants to affect yield, especially as in the field plants would be infested very soon after germination of the sugar beet seedling, particularly in later drilled crops which are drilled into warm soils (>10°C, where BCN hatching begins (Dewar and Cooke, 2006)). Despite similar accumulated GDD (Growing degree days) were measured in the boxes and field, the boxes were likely to warm up and cool down faster than the field soil and therefore may have had a beneficial impact on the plant, since the nematodes may have been less active in the boxes than in fields due to this. Further refinement of the box method, which shows promise as being a useful variety screening tool, likely requires earlier and more prolonged infestation of the plants to elicit significant yield differences between the varieties.

With regard to the brassica trap crop field experiments, our field sampling also produced some seemingly erroneous results relating to BCN population changes. Whilst this is discussed in chapter 4, it is worth considering whether better methods should be developed for BCN studies in the future, and look to methods used in other countries and for other species of cyst nematode for ideas. Undoubtedly smaller, squarer plots may reduce the intra-plot variation of BCN populations and also the number of replicates for each treatment could be increased to reduce inter-plot variation. However, our experiments were limited by the size of the seed drills available on the host farm. A mechanised soil sampler may also reduce the variation, as especially if it were GPS RTK guided, sampling across a plot could be made accurate to ± 2cm on repeated passes and ensure the same part of each plot were sampled each time. Recent findings also suggest that increasing the number of soil cores, but decreasing the diameter of the sampler can improve sampling accuracy (Davie et al., 2016).

Only one population of BCN was used for all of the experiments, obtained from the host farm used for the trap crop experiments. Populations from other locations in the UK, or from overseas, may respond differently to the tolerant sugar beet varieties or the brassica trap crops. Further research into identifying how diverse British BCN populations are, and if this leads to differences in their response to control measures would be useful, especially since some traits, such as resistance to BCN in sugar beet, are inferred by a single gene and thus this resistance could be overcome by a population with a suitable mutation.

4. Key messages for sugar beet industry stakeholders

Tolerant varieties:

Use of tolerant varieties should continue to be encouraged in BCN infested fields. Such varieties now yield as well as elite susceptible varieties and also can manage BCN population build-up, due to being partially resistant. The use of tolerant varieties will minimise future BCN problems in sugar beet and other host crops too. Differences in quality parameters measured in the box experiments (soil tare and alkalinity ratio) demonstrate that some tolerant varieties would be more preferable to cultivate commercially than others. Refinement and improvements to the box method and field validation of the results will allow for tolerant varieties to be assessed fully and compared more robustly in the future.

Light tolerant varieties:

Light tolerant varieties do not show any suppression of BCN population multiplication so would be less beneficial to grow than truly tolerant varieties. As tolerant varieties now yield as well as susceptible sugar beet varieties, there would appear to be no future need to consider listing light tolerant varieties on the RL.

Resistant varieties:

Resistant varieties may be of interest to British sugar beet growers with short rotations and high BCN population pressures. The occasional use of a resistant variety to actively lower BCN populations every second or third time beet is cultivated in a field would prevent further build-up of populations and also be of benefit to any following host crops, such as oilseed rape, and this control method would be as simple as changing the sugar beet variety being

sown. As the yield potential of the newer (but currently under development)resistant variety appeared to be greater than the older resistant variety 'Sanetta' in our field experiments, resistant varieties may soon become more favourable to grow, especially as this increase in yield potential has also been found in other studies (Hauer et al., 2016). However, as we have found, a higher PPD is likely to be needed to optimise the yield of resistant varieties and this would lead to additional costs to growers. There would likely be a premium charged to purchase a resistant variety and then also the additional cost for the seed needed to reach a higher PPD. Finally, since resistant varieties are not widely cultivated in countries with more widespread BCN populations (Märländer et al., 2003) ,the justification for their use in the UK may be limited.

Brassica trap crops:

The use of brassica trap crops should be encouraged with caution. Our results appear to show that only some varieties of these trap crops produce significant BCN population reductions. However, we do not yet have any data from our own experiments to consider if these trap crops have a beneficial impact on the following sugar beet crop and if they can justify their establishment costs. Growers with BCN infestations who wish to plant a cover crop for other reasons, such as soil structural benefits, nutrient retention and preventing soil erosion, would benefit from planting a resistant brassica trap crop to prevent BCN population increases.

The importance of sampling for BCN:

Sampling for fields on the host farm used for the trap crop experiments to find fields without BCN infestations proved difficult. The host grower believed that only three of the fields were infested, but in actual fact when trying to find control fields, over ten fields were identified

with infestations. It would therefore seem apparent that sub-clinical infestations are spread across this farm (which exceeds 1200 ha in size) and the problem has only been identified in a select few fields where infestations have harmed susceptible crops in the past. If this is the case across the entire sugar beet growing region of the UK, the estimated 6% of area infested with BCN may be far higher and the problem may be building, especially since rotational frequency of sugar beet cultivation has generally increased in the region in the recent past. Our host grower has now switched to cultivating almost exclusively tolerant sugar beet varieties, and other growers should be advised to do the same if field sampling finds cysts in 'uninfested' fields and a quantification of BCN infestation across the British sugar beet growing area would be of use to the industry as a whole.

5. Future research priorities

This research has generated data which answer the hypotheses devised and outlined at the beginning of this chapter. In addition, the findings have identified a range of new research ideas which, if followed up, could be of benefit to those involved with BCN management globally, but especially in the UK.

5.1 Up-to-date survey of BCN distribution in the UK

Firstly, a survey needs to be conducted across the entire sugar beet growing area in the UK to identify the current spread of BCN and levels of infestation. A survey could also identify if different populations of BCN exist within the UK. Comprehensive surveys were last conducted during the 1980s and 1990s (Cooke, 1984; Cotten et al., 1992), with a survey in 2010 confirming infestation is still largely confined to the area where it has been found historically (Burks et al., 2011). An up-to-date and thorough survey, using modern identification methods

could then be used to more accurately ascertain the area infested with BCN and whether recent changes to the area of the UK cropped with sugar beet, and also the rotational frequency at which sugar beet is grown, has changed distribution and intensity of BCN infestations. If different populations of BCN are found, further research will be required to understand if they all respond similarly to tolerant sugar beet varieties, brassica trap crops or any other management methods which may be introduced in the future, such as biological control or biofumigation. The results of this survey can then inform future BCN research in the UK.

5.2 Sugar beet variety screening

Developing the box screening method outlined in Chapter 3 into a commercial variety screen would be of a benefit to the entire UK sugar beet industry. The method is relatively easy to establish and could easily be scaled up to include more varieties than we assessed. This avoids the need to establish field sites with relatively homogenous BCN infestations which would be more time consuming and also costly. Since the presence of BCN in the UK may be greatly underestimated, taking a proactive approach to understand more about the tolerant varieties, and then arm growers with this information, will allow for more understanding of the threat that BCN poses to their crops and how it can be managed. This method requires further refinement, especially the timing and quantity of inoculum applied to the sugar beet plants to stimulate significant yield differences in the susceptible control varieties. However, the repeatable differences in *Pf* demonstrated by the technique does show that it offers a promising method to assess future tolerant sugar beet varieties' differing fecundity to BCN.

5.3 Brassica trap crops

As we have identified a benefit to using the trap crops at a relatively late sowing date, further research into their use may be beneficial to understand how earlier sowing may make them more effective. Repeating the hatch assays using cysts from infested fields may then produce results which are more true to the conditions in the field caused by the trap crops. More experiments with the trap crops in the field may also reveal benefits to the farming rotation for following sugar beet crops and other crops.

5.4 Resistant sugar beet varieties

Resistant varieties undoubtedly have benefits which are not currently utilised by growers as they are deterred by their current lower yield potential. More needs to be done to test their canopy development, influence on nematode populations when grown in a field, and whether modern varieties, which may yield better than their older counterparts, are suited to cultivation in the UK. More investigation into rotational benefits of using resistant sugar beet varieties are needed and whether they are required in the UK.

6. Conclusions

The major conclusions from the works reported in this thesis can be summarised as follows:

1. Beet cyst nematode remains a major threat to sugar beet crops. Successful control requires an ongoing understanding of the nematode and appropriate ways in which to manage BCN infestations.

2. The use of resistant varieties of sugar beet may be more favourable in the future since they appear to yield better than previously and their yield can be manipulated by increasing the plant population density.

3. Tolerant varieties of sugar beet should be considered partially resistant following the reproduction data generated in this research and other recent studies. They now yield similarly to susceptible varieties but do not appear to exhibit clear differences in their physiology when uninfested. Future work should focus on tolerant varieties and ways to use them to ensure the best yield whilst minimising BCN population increases.

4. Certain types of brassica trap crops can reduce populations of BCN, but the effect differs between the variety and species selected. Earlier sowing of trap crops may increase their benefit further. Their use may not be of major benefit as long as growers with infestations are using BCN tolerant varieties.

5. A UK survey of current BCN distribution is needed. This can then be used to investigate if different populations exist and whether they respond differently to management

strategies. The survey findings would support our findings relating to tolerant sugar beet varieties and brassica trap crops or outline if further work is needed to expand on our other conclusions.

6. Finally, long rotations without a host species (five or more years), good farm hygiene and appropriate selection of sugar beet varieties will ensure BCN infestations can be well managed into the future.

7. References

BBRO, 2018. Recommended list of sugar beet varieties 2019.

BBRO, 2017. Sugar Beet Recommended Variety List for 2018 [WWW Document]. URL https://bbro.co.uk/media/1251/2018-sbt-rl-varieties-tables-final-27mar2017.pdf

BBRO, 2015. Recommended variety list 2016.

Bundessortenamt, 2014. Beschreibende Sortenliste: Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben & Zwischenfrüchte.

Burks, E., Morris, N., Stevens, M., 2011. Beating Cyst Nematode. Br. Sugar Beet Rev. 79, 26–29.

Campbell, H., Pickup, J., Fenton, B., Roberts, A., Kirkwood, M., Nevison, I., Lees, A., 2017. AHDB Potatoes Independent Variety Trials.

Cooke, D.A., 1985. The effect of resistant cultivars of catch crops on the hatching of Heterodera schachtii. Ann. Appl. Biol. 106, 111–120. https://doi.org/10.1111/j.1744-7348.1985.tb03100.x

Cooke, D.A., 1984. The relationship between numbers of Heterodera schachtii and sugar beet yields on a mineral soil, 1978-81. Ann. Appl. Biol. 104, 121–129. https://doi.org/10.1111/j.1744-7348.1984.tb05594.x

Cotten, J., Cooke, D.A., Darlington, P., Hancock, M., 1992. Surveys of Beet Cyst Nematode (Heterodera-Schachtii-Schmidt) in England. Ann. Appl. Biol. 120, 95–103.

Davie, K., Reid, A., Cole, Y., Pickup, J., 2016. Potato cyst nematode threshold values and quantification of field populations using egg counts and real-time PCR, in: Advances in Nematology. Association of Applied Biologists, London, United Kingdom.

Dewar, A.M., Cooke, D.A., 2006. Pests, in: Draycott, A.P. (Ed.), Sugar Beet. Blackwell Publishing Limited, Oxford, pp. 316–350.

EPPO, 2006. Testing of potato varieties to assess resistance to Globodera rostochiensis and Globodera pallida. EPPO Bull. 36, 419–420. https://doi.org/10.1111/j.1365-2338.2006.01032.x

Hauer, M., Koch, H.J., Krüssel, S., Mittler, S., Märländer, B., 2016. Integrated control of Heterodera schachtii Schmidt in Central Europe by trap crop cultivation, sugar beet variety choice and nematicide application. Appl. Soil Ecol. 99, 62–69. https://doi.org/10.1016/j.apsoil.2015.11.017

Kenter, C., Lukashyk, P., Lukashyk, P., Daub, M., Ladewig, E., 2014. Population dynamics of heterodera schachtii schm. and yield response of susceptible and resistant sugar beet (beta vulgaris L.) after cultivation of susceptible and resistant oilseed radish (raphanus sativus L.) | Populationsdynamik von heterodera schacht. J. Cultiv. Plants 66, 289–299. https://doi.org/10.5073/JFK.2014.09.01

Koch, D.W., Gray, F.A., 1997. Nematode-resistant oil radish for Heterodera schachtii control in sugar beet-barley rotations. J. Sugar Beet Res. 34, 31–43.

Koch, H.J., Windt, A., Mittler, S., Hauer, M., 2017. Effect of weather variables on biomass production and N uptake of catch crops,and their influence on soil water and Nmin content in Northern Germany. J. Cultiv. Plants 69, 361–372. https://doi.org/10.1399/JfK.2017.11.01

Lange, W., De Bock, T.S.M., 1994. Pre-breeding for Nematode Resistance in Beet. J. Sugar Beet Res. 31, 13–26.

Märländer, B., Hoffmann, C., Koch, H.J., Ladewig, E., Merkes, R., Petersen, J., Stockfisch, N., 2003. Environmental situation and yield performance of the sugar beet crop in Germany: Heading for sustainable development. J. Agron. Crop Sci. https://doi.org/10.1046/j.1439-037X.2003.00035.x

Masler, E.P., Perry, R.N., 2018. Hatch, Survival and Sensory Perception, in: Perry, R.N., Moens, M., Jones, J.T. (Eds.), Cyst Nematodes. CAB International, Wallingford, Oxfordshire, UK, pp. 44–73.

Müller, J., Wyss, U., Rehbock, K., Wyss, U., Rehbock, K., 1981. Growth of Heterodera schachtii with remarks on amounts of food consumed. Rev. Nematol. 4, 227–234.

Reuther, M., Lang, C., Grundler, F., 2017. Nematode-tolerant sugar beet varieties - Resistant or Susceptible to the Beet Cyst Nematode Heterodera schachtii?, Sugar industry.

Wright, A.J.D., 2017. PhD Update- Beet Cyst Nematode. Br. Sugar Beet Rev. 85, 50–53.

Wright, A.J.D., Bussell, J.S., Stevens, M., Back, M.A., Sparkes, D.L., 2018. Physiological differences between sugar beet varieties susceptible, tolerant or resistant to the beet cyst nematode, Heterodera schachtii (Schmidt) under uninfested conditions. Eur. J. Agron. 98, 37–45. https://doi.org/10.1016/j.eja.2018.05.005

Appendix 1

Yield - BCN population relationship from sugar beet trials 2017/2018

Sugar beet was planted over the infested field site used to investigate control of BCN using brassica trap crops in 2016/2017. Susceptible beet variety 'Pasteur' was sown at 125,000 seeds per hectare initially on 28 March 2017. Severe rabbit grazing on the site lead to re-drilling on the 5 June. The result of this was that the beet never established well, likely as a result of the enhanced BCN activity in the warm soil in June and also the fact that the plants missed out on the majority of incoming light since they were sown so close to the longest day of the year. The beet were harvested on 16 January 2018 and analysed for yield at the BBRO Tare house using the same methods described in Chapter 3. Plots were then sampled for BCN populations as described in Chapter 4.

No significant yield responses to either *Pi* or brassica trap crop treatment were found. The relationship between *Pi* and yield did however show a trend (P=0.11) for yield to decrease with increasing *Pi*. However, a relationship between *Pf* and Yield was found as expected. The plants which yielded best had lower final populations of the nematode, whereas areas which resulted in higher populations of BCN yielded worst (Fig 1).

Whilst these findings are as expected, and no relationship between the brassica trap crop treatments and sugar yield was found, future repeats of these experiments, with better and earlier plant establishment, may produce better results and warrant further investigation. Unfortunately our findings highlight the difficulties of conducting on farm trials with BCN.

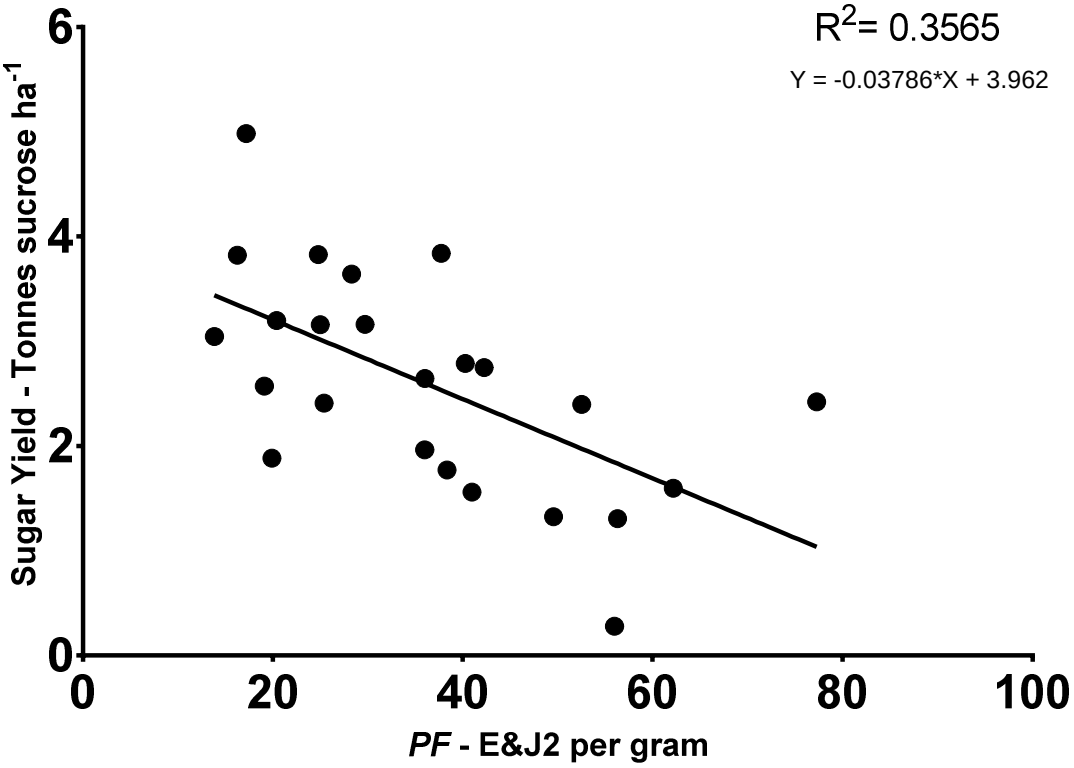


Figure 1 – Relationship found between final population of BCN in field plots and subsequent sugar yield of sugar beet from the same plots in 2017/18 following the planting of brassica trap crops in 2016 (P=0.002).

In addition, a control site, believed to be free of BCN was established with the same brassica trap crops in September 2016 with the aim of investigating if the brassica trap crops had any benefits to following sugar beet yield that weren't related to BCN control. The trap crops were grown for the same length of time as the infested site, with all treatments being the same (except that this site had a different cropping history, and was ploughed before planting the trap crops since the previous crop had been a two year grass crop).

Sugar beet were also sown on the infested site on 28 March 2017. As the sugar beet crop grew it became apparent that unfortunately this site was also infested with BCN. As a result after the beet were harvested these samples were also sampled for Pf (Fig 2) and significant relationships between yield and Pf were also found (P=0.0002).

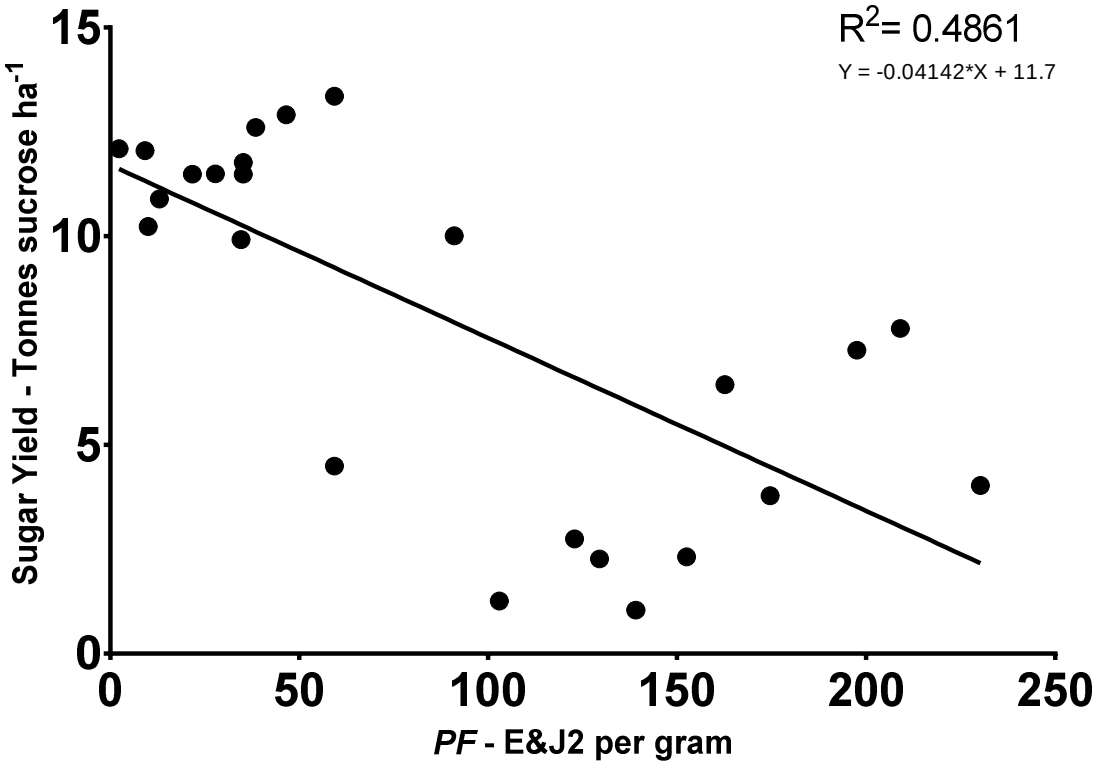


Figure 2 – Relationship between *Pf* and sugar yield at the control site which had a previously unknown infestation of BCN (P=0.0002).

This field was sampled for BCN prior to being ploughed and no viable cysts were found in the sample and the host grower was not aware that BCN was present at this field (One very old and empty cyst was found in the sample). Our findings therefore highlight the importance of thorough BCN testing, how sugar beet yields can be severely reduced by high BCN populations and how BCN populations can build up from sub-clinical and undetected to severe in just one sugar beet cropping season.