

A new method to validate and compare varietal resistance and yield tolerance of sugar beet (*Beta vulgaris*) against the beet cyst nematode, *Heterodera schachtii* Schmidt

Alistair JD Wright,^{a,b} Mark Stevens,^b Matthew A Back^c  and Debbie L Sparkes^{a*}

Abstract

BACKGROUND: Beet cyst nematode, *Heterodera schachtii* Schmidt is a global threat to sugar beet crops, and is found in every major sugar beet growing region. Annual losses due to this nematode can be severe, being estimated at €90 m in Europe alone in the 1990s. Fortunately tolerant, resistant and partially resistant varieties have since been introduced which help to limit yield loss and are now widely being deployed in infested fields. However, understanding yield performance of these varieties has been difficult, especially when variety testing programmes usually require uninfested fields.

RESULTS: For the first time, and in a standardised manner, we can now assess simultaneously the resistance of different varieties to BCN and their actual yield tolerance, by comparing them to varieties grown in uninfested micro-plots alongside those which are infested. This method provides new insights on variety yield performance and nematode reproduction over an entire growing season. In addition, the investigations are also been able to detect significant physiological differences in the development and growth of the tolerant varieties' canopies and leaf chlorophyll levels.

CONCLUSIONS: Our findings are of direct benefit to sugar beet growers challenged by BCN. The standardised testing provides new information on predicted variety performance. We found that these tests are justified, as not all tolerant varieties respond in the same manner to nematode infestation. Therefore, these assessments will become a vital part of variety testing for sugar beet growers, allowing for tailored deployment of variety types and more informed decision making on-farm, helping to maximise yields whilst minimising nematode damage.

© 2022 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Keywords: IPM; variety tolerance; nematode resistance; sugar beet; root crop

1 INTRODUCTION

It is estimated that nematodes annually cause over US\$80 billion of losses to crops around the world.¹ 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,² and in all major sugar beet growing regions.³ In Europe, it has been estimated to cause annual sugar beet losses in excess of €90 million,⁴ although with new tolerant and resistant varieties the degree of loss may be much lower.⁵ 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 where there are severe infestations combined with drought.⁶

Since the withdrawal of licenced nematicides for use against BCN,³ there are limited control options available. Long rotations without suitable host species, which allow for natural population decline over time,⁶ were previously enforced. For example, in Britain, infested fields were limited by law to cultivation of a BCN host

species in no more than one in 5 years.⁷ However, such orders have been revoked and rotations often include BCN host crops planted as frequently as one in every 3 years. Therefore, other methods to manage BCN infestations are required to prevent future population build-up. Some methods, such as resistant brassica trap cropping^{8–10} or biological control¹¹ may be used, but

* Correspondence to: DL Sparkes, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK, E-mail: debbie.sparkes@nottingham.ac.uk

a School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, UK

b British Beet Research Organisation, Norwich Research Park, Norwich, UK

c Centre for Integrated Pest Management, Harper Adams University, Newport, UK

increasingly, sugar beet variety choice is the preferred way to manage BCN and limit yield losses.

Sugar beet varieties which are resistant, and unable to host BCN successfully, have been in use in Europe since the late 1990s⁴ when the *HS1^{pro1}* gene was successfully introgressed into sugar beet from wild beet relative, *Patellifolia procumbens*.¹² They actively kill invading juvenile nematodes by restricting development of their syncytium (feeding cell), which prevents nematode reproduction¹³ and therefore reduces soil populations. However, a yield penalty associated with these resistant varieties exists,^{3,14} and they are not widely cultivated.¹⁵ Recent publications state that modern resistant varieties may have improved yield potentials,^{8,16} which means that they could be more widely used in the future. However, since 2009, varieties which claim to have yield tolerance, and therefore are marketed within the industry as 'Tolerant', under BCN infestation 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*.^{17,18} 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 (Additionally, For a limited period (2015 to 2017), varieties with an additional trait claim "Light tolerance" were also available. These varieties claimed to have greater yield potentials in the absence of BCN than a 'tolerant' variety, but suffered more yield damage under widespread or severe infestations.³⁸). Adoption of tolerant varieties has been successful and they now make up around 6% of the UK sugar beet area¹⁶ which is the same area estimated to be infested with BCN¹⁹ indicating that growers with known BCN infestations are likely selecting tolerant varieties. Recent sampling, however, anticipates that the actual area infested with BCN is far greater than 6%,²⁰ but populations are currently at sub-clinical levels and symptoms are harder to detect. 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.²¹ 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 any differences which exist between the tolerant varieties available.

In the UK, tolerant varieties are submitted into recommended list trials operated by British Beet Research Organisation 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 area 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 determine their effect on BCN fecundity, would provide a novel solution for variety trait validation.

Work to develop such a method was initially undertaken at Broom's Barn Research Station near to Bury St Edmunds, Suffolk, UK.²² These studies were conducted between 2009 and 2013 on the very first 'Tolerant' varieties. To do this, fully randomised microplots were established using 1 tonne heavy duty plastic storage boxes filled with BCN-free soil that was then overlain with

either a 5 cm layer of BCN-infested soil or further BCN-free soil. The infested soil was collected from a commercial sugar beet field and assessed, prior to use, by extensive sampling and analysis of BCN cysts. Initial results showed the method could differentiate between the first commercially available varieties in terms of their differential BCN replication characteristics and yield performance with and without the presence of BCN. However, lack of BCN population uniformity across the soil profile (and/or cyst viability) plus the challenges of either the boxes flooding during heavy rain events or, in some years, soil drying through lack of rain, affecting BCN population dynamics, led to the method being re-evaluated. Work has now continued to further refine a method to validate varietal traits in response to BCN infestation. This paper documents how this method, now in a third iteration, has been developed to assist sugar beet growers in selecting varieties based on the level of yield tolerance, physiological responses by the plants to BCN infestation and also resistance to nematode multiplication.

2 MATERIALS AND METHODS

2.1 2016 and 2017 experiments

In 2016 and 2017, micro-plot experiments were established at the University of Nottingham's Sutton Bonington Campus, Nottinghamshire, UK. 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 10 boxes (increased to 12 in 2017) which were filled with 605 L 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. In 2016, the boxes were filled on 24 March and subsequently allowed to settle for 2 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 16 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 16 plants were still sown. Each block had two boxes of each variety, one of which was then infested with BCN 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.

All boxes received the equivalent of 120 kg of N.ha⁻¹ in 2016 using ammonium sulphate [(NH₄)₂SO₃], 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 [NH₄NO₃] and applied at a rate of 80 kg of N.ha⁻¹: 50% applied pre emergence 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.

In 2016, the boxes were hand watered to prevent drought stress. 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 m pipe running between the rows of the sugar beet plants to deliver an even amount of water to each box when needed. Irrigation was controlled using an automatic timer, with the duration of watering changed when the

Table 1. Descriptions of the varieties used in the investigations, detailing their declared traits to BCN, years available to grow in the UK and their respective breeding houses

	Variety code	Variety name	Breeder	Trait description†	Years listed in UK RL
2016/17 Experiments	Sus A	Pasteur	Strube	Susceptible	2011–2018
	L. Tol	Maddox	Syngenta Seeds‡	Light Tolerant	2014–2016
	Tol A	Aurora	SESVanderHave	Tolerant	2015–2019
	Tol B	BTS 755	Betaseed	Tolerant	2016
	Tol C	Thor	Strube	Tolerant	2012–2018
	Res A	-	Syngenta Seeds‡	Resistant	N/A
2019 Experiment	Sus B	Sabatina	KWS	Susceptible	2015–2021
	Sus C	BTS 860	Betaseed	Susceptible	2016–2020
	Tol D	Flixter	MariboHilleshög‡	Tolerant	2019–2020
	Tol E	Gauguin	Strube	Tolerant	2018–2020
	Tol F	Cantona	KWS	Tolerant	2016–2021
	Tol G	Daphna	KWS	Tolerant	2017
	Res B	-	MariboHilleshög‡	Resistant	N/A
	Unk	-	SESVanderHave	Unknown	N/A

† As described by breeder when submitted for testing in UK national and recommended list trials.

‡ Now DLF Beet Seed.

Traits are declared as follows:

Resistant [Res]: Plant able to inhibit BCN reproduction relative to a susceptible variety.³⁷

Susceptible [Sus]: Supports a very high level of reproduction of BCN, the counterpart of resistance, and as a result, yields poorly when grown in infested conditions.³⁷

Light Tolerant [L. Tol]: Able to yield well under a low infestations of BCN, but suffer significant yield losses at high nematode infestation levels.³⁸

Tolerant [Tol]: Plant able to sustain growth and yield successfully in the presence of BCN. Trait is not necessarily related to resistance.³⁷

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

uninfested susceptible variety boxes showed symptoms of wilting, allowing for watering to respond to limited water availability and not nematode damage symptoms.

2.2 Nematode inoculation

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 1000 µL 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.

Juveniles were hatched from cultured cysts grown in a controlled environment room. Cysts were cultured on oilseed rape (*Brassica napus*) and extracted from the soil using a Wye Washer.²³ 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 3 days, the water was then poured onto a single Hygia favoritII filter. Over a four-hour period the juveniles were allowed to actively migrate through the filter so only viable juveniles were collected. The density of the juveniles was adjusted so sufficient volume was available to provide 4 mL per plant, i.e. 64 mL per box per inoculation, and quantified using a microscope. 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 50 mL combitip, Eppendorf, Hamburg, Germany) and dispensed in 1 mL doses into each well. Table 2 details the densities and timings of juveniles dispensed. Plants were inoculated when at the six-leaf stage to avoid overwhelming young plants with simultaneous invasion by large amounts of nematodes.

2.3 Physiological measurements

In 2017 a SPAD 502plus (Konica Minolta Inc. Tokyo, Japan) was used during July and August to measure leaf greenness which can be used to estimate leaf chlorophyll content.²⁴ 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.

2.4 Harvest analysis

Beet were harvested on 15 November 2016 and 8 November 2017. The roots were lifted by hand and bagged prior to being sent for sugar and impurity analysis at the BBRO tarehouse at the Wissington Beet Sugar factory (Norfolk, UK). Each sample was weighed dirty and then washed and reweighed which allowed the soil tare, the amount of soil which adheres to the storage root at harvest, to be calculated. Calculation of a soil tare percentage was then made to express the proportion of the dirty sample which was soil as follows:

$$\text{Soil Tare\%} = \left(\frac{\text{Dirty Sample weight} - \text{clean sample weight}}{\text{Dirty sample weight}} \right) \times 100.$$

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.²⁵ Sugar (Sucrose) yield of the entire sample was calculated multiplying the sugar % of the sample by the clean weight.

2.5 BCN population determination

The soil in the centre of each infested box (approximately central 50 x 50 cm) was sampled for BCN 2 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. 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.²³ Cysts were then counted, crushed,²⁶ diluted in 50 mL of water and the mean egg and juvenile densities in 1 mL of the solution determined using a stereo microscope (Leica M80, Leica, Wetzlar, Germany).

2.6 2019 experiment

To overcome limitations identified in 2016 and 2017 a third iteration of the experimental design was introduced in 2019. Instead of using either a layer of infested soil, or hatched juveniles, this new method used completely infested soil in the microplot.

Another change was the type of microplot used. Instead of using the plastic boxes for the microplots, wooden frames were used. Each microplot was comprised of two wooden pallet collars stacked together and measured 1.5 x 2.0 x 0.4 m when assembled (Bageta, Meškuičiai, Lithuania). These were placed in a field without BCN infestation, directly onto the field soil below. Prior to placement the ground was thoroughly cultivated to 20 cm depth. The trial location was at Bridgham, Nr Thetford, Norfolk, UK at 52.427987 N, 0.863119E.

Into the frames was placed either uninfested soil or BCN infested soil, both of the same loamy sand soil type and locally sourced either from an infested field 400 m north-east from the site or clean soil from the experiment site itself. The experimental design comprised of four blocks and utilised a split-plot design. BCN infestation was the main factor of the design and each main plot (+/- BCN) was divided up into sub-plots (comprising one frame set) into which different varieties were randomly assigned. The main plots were isolated from each other through the use of discard frames to prevent BCN spread into uninoculated plots via the field soil. Each block therefore contained 16 experimental

frames along with additional discard frames at the end and in the middle. A range of variety types were grown, including one resistant variety, five tolerant varieties, two susceptible varieties and a variety for which the BCN status was unknown (Table 1).

Prior to sowing, the infested boxes were assessed for their initial nematode population (*Pi*). 25 soil cores were sampled in a grid pattern evenly across the boxes to create a bulk sample, from which 200 mL was extracted and the nematode population of each box estimated as previously described. ANOVA was then used to confirm that there was an even distribution of the varieties across the range of populations measured. The mean infestation level was 6.55 eggs mL⁻¹ soil. (Range 1.75–12.25 eggs mL⁻¹ soil), exceeding historic UK recommended threshold (prior to the introduction of tolerant varieties) for cultivation of sugar beet under BCN infestation of 4 eggs.mL⁻¹ soil.⁶

The frames were filled with soil between 2 and 4 April, sampled for BCN on 5 April and sown on 24 April. Four rows, each 1.5 m long were sown into each frame (inter-row spacing 50 cm). In each row, eight plants were sown, at a 15 cm intra-row spacing. Each box received the equivalent of 120 kg N ha⁻¹ (since the soil type was naturally low in fertility). 40 kg N ha⁻¹ equivalent was achieved by using Universol Blue 18–11–18 (ICL Speciality fertilisers, Ipswich, Suffolk, UK). 67 g of product was diluted in 10 L of water in a watering can and applied evenly across each box. The remaining 80Kg.ha⁻¹ was applied in the form of liquid fertiliser (Yara Chafer Nuram 35 + S, Yara International, Oslo, Norway) applied by a boom sprayer. All fertiliser was applied before the plants reached two fully expanded true leaves. Good agronomic practise was deployed to keep the boxes free of pests, disease and weeds, with supplementary hand weeding where needed. Due to the free-draining nature of the soil, an irrigator (Briggs 63–130 with 18 m boom, Briggs Irrigation, Corby, UK) was used to ensure adequate water was available to the plants and allow for movement of the nematodes towards the roots.

Development of the plants was assessed using a drone (DJI Matrice 210 V2, SZ DJI Technology Co., Ltd, Shenzhen, China) equipped with high resolution colour camera (DJI Zenmuse X5S) and also a multispectral/thermal camera (Micasense Altum, Micasense Inc. WA, USA). Drone flights were conducted on 30 June, 6 August, and 10 October 2019. The imagery was then processed using Pix4D Mapper photogrammetry software (Version 4.5.2 – Professor License, Pix4D S.A. Prilly, Switzerland) to create orthomosaic images of the datasets. These were then imported into QGIS (Version 2.8) to allow for extraction of data for calculation of green area cover using ImageJ (Version 1.52) according to Wright *et al.*¹⁶ and multispectral reflectance for each plot. From this data, a spectral reflectance index, *mND_{blue}*, was calculated. This index which has been specifically developed for canopy chlorophyll estimation in sugar beet²⁷ and as follows using the reflectance values for each microplot from wavelengths which most closely aligned to those described by Jay *et al.*²⁷:

$$mND_{blue} = \frac{\text{blue [475nm]} - \text{Red edge [717nm]}}{\text{blue [475nm]} + \text{Near infrared [842nm]}}$$

Harvest was conducted on 8 November 2019, with all roots in each box being hand harvested and analysed for yield as previously described. Yield of each box was then upscaled to values per hectare. After harvest, the BCN populations in the soil were again assessed and the reproduction ratio (Pf/Pi) calculated.⁹

2.7 Data analysis

Data were analysed using GenStat 20th edition (VSN international, Hemel Hempstead, UK) using two-way ANOVA and calculation of the least significant difference. Tukey's multiple comparison test was used to compare the results of one-way ANOVAs. SPAD data (leaf chlorophyll content) were analysed using a repeated measures ANOVA. GenStat was also used to generate linear regression of the canopy data. Figures were produced using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and GraphPad Prism Version 9 (GraphPad Software, San Diego, CA, USA).

3 RESULTS

Experiments in 2016 and 2017 partially allowed measurement of the desired traits of the sugar beet varieties. For example, by using hatched J2s we successfully assessed resistance of the varieties to BCN (Fig. 1). In both years the tolerant varieties did not produce significantly greater final populations than the resistant variety (Res A). The tolerant varieties also always had significantly lower Pf's than the susceptible and light tolerant varieties.

However, in neither year did this method allow for significantly reduced sugar yields to be measured (Table 3). Yields of varieties were also inconsistent between both years and no assessment of tolerance under nematode infestation could be made. However, the method was able to identify significant differences in yield potential of the varieties, and also traits associated with the quality of the sugar beet, such as soil tare (Figs 2 and 3).

In 2017, some varieties (Susceptible A and Tolerant B) sustained a significantly reduced level of chlorophyll due to infestation with BCN at all measurement times whereas the other varieties still showed a reduction in chlorophyll by late July, but then recovered in August, such as Tolerant A, C and Resistant A. The Light Tolerant variety showed lower chlorophyll levels on the infested plants only at the later measurement times (Fig. 4; $P = 0.014$).

Soil tare in both years was affected by infestation, with significant variety \times infestation interactions being found (Figs 2 and 3). In both years, the resistant and susceptible varieties had significant increases in soil tare when inoculated with BCN J2 whilst

tolerant varieties A and B did not show an increase in either year. The light tolerant variety only had an increase in 2017 when also the additional variety (Tolerant C) had a significant increase in soil tare due to BCN.

The upgraded method (Fig. 5) used in 2019, found significant differences in canopy expansion during the exponential canopy expansion phase. On 30 June, there were clear differences in canopy cover between the microplots (Fig. 6). Linear regression with groups (Fig. 7) showed that canopy cover was negatively related to Pi and that the slope of the response differed between varieties ($P < 0.001$, $R^2 = 54.1$).

For example, Susceptible B had the most stunted canopy growth as indicated by the steeply sloping line on Fig. 6 whereas Tolerant varieties D and G had the least steep responses, indicating their canopy expansion was least affected by BCN.

By August, canopy cover differences were no longer detectable, and all plots had reached canopy closure. However, varieties Susceptible B, Tolerant D and Unknown had significantly more negative mND_{blue} values (Fig. 8), indicating significant reductions in canopy chlorophyll levels when infested with BCN, whereas the other varieties did not show a reduction when infested ($P = 0.029$).

Using the modified methodology in 2019, we were able to detect that the susceptible control varieties and two of the tolerant varieties (Tolerant D and F) suffered significant yield loss under BCN infestation (Fig. 9; $P = 0.007$). The unknown variety was also found to suffer significant yield loss due to BCN infestation in addition to possessing a low yield potential, having the second lowest yield of all of the tested varieties, the lowest yielding variety being the resistant variety.

Again, soil tare was found to be significantly affected by BCN infestation (Fig. 10; $P = 0.029$). Both susceptible varieties (B and C) had significantly increased soil tares due to BCN, along with one of the tolerant varieties (Tolerant F) and the Unknown variety.

Finally, the populations of BCN in the soil after harvest were significantly different between varieties ($P < 0.001$). Both susceptible varieties had more than 20-fold increase in BCN populations. The unknown variety was statistically the same as the susceptible

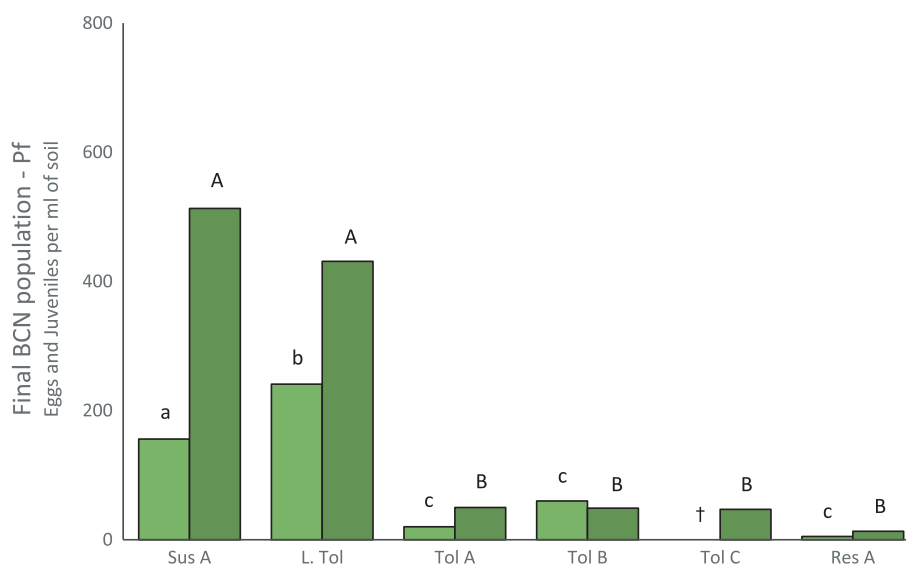


Figure 1. Final populations (Pf) of BCN from soil in the infested boxes in 2016 (light green) and 2017 (dark green). Soil was sampled from the central area of the box 2 weeks after harvest of the sugar beet plants. $P < 0.001$ for both years. Letters which are different indicate a significant difference at $P = 0.05$ (2016 Lowercase, 2017 Uppercase letters). $LSD_{5\%}$: 2016 = 53.9, 2017 = 121. † There are no data for this variety in 2016 as it was not grown.

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. Refer to Table 1 for variety details

Variety	2016			2017		
	Non infested	Infested	Mean	Non infested	Infested	Mean
Sus A	2.59	2.46	2.52	2.68	2.67	2.67
L. Tol	2.62	2.61	2.62	2.72	2.65	2.69
Tol A	2.37	2.25	2.31	2.56	2.62	2.59
Tol B	2.59	2.62	2.61	2.89	3.00	2.94
Tol C†				2.53	2.57	2.55
Res A	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.

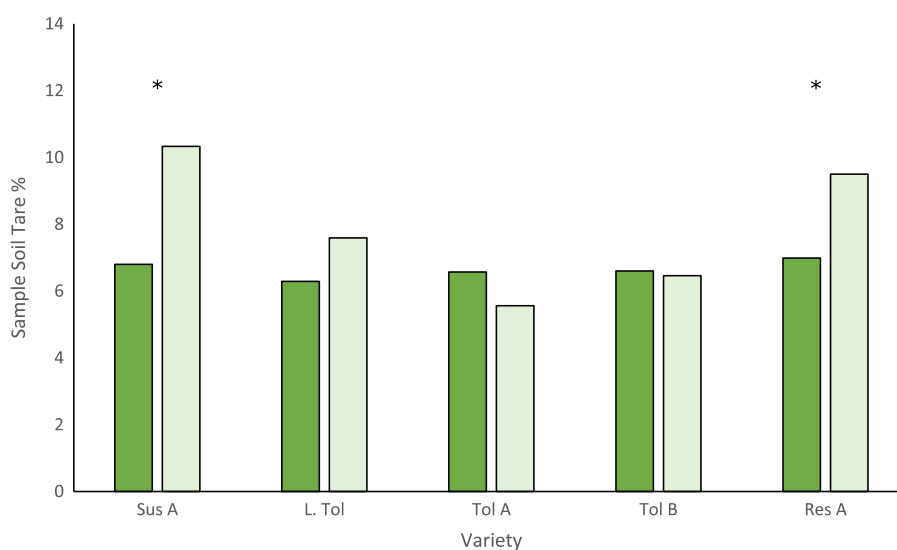


Figure 2. Soil tare, expressed as the percentage of the uncleaned sample weight which was soil, from the experiment in 2016. Non-infested samples (dark green) Infested samples (light green) ($P = 0.023$ variety \times infestation. $LSD_{5\%} = 2.25$). Asterisks denote varieties which show a significant difference when infested with *H. schachtii* according to the LSD.

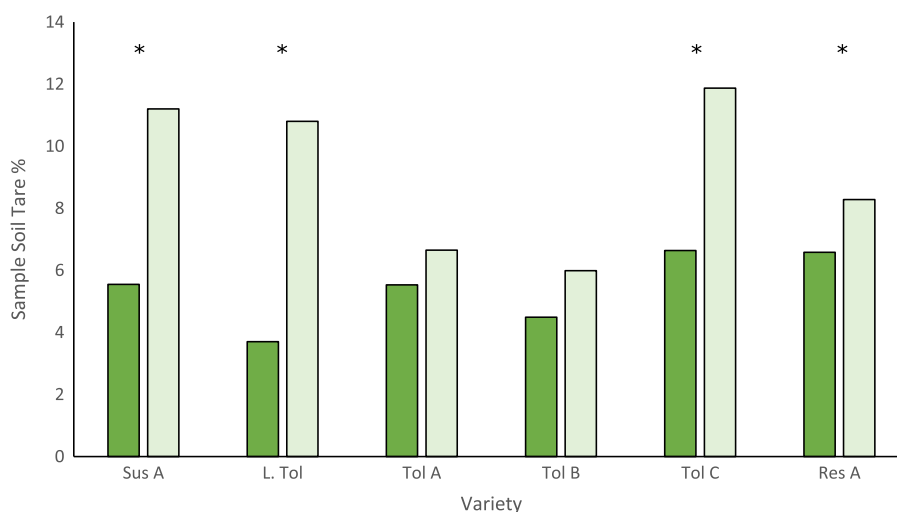


Figure 3. Soil tare, expressed as the percentage of the uncleaned sample weight which was soil, from the experiment in 2017. Non-infested samples (dark green) Infested samples (light green) ($P < 0.001$ variety \times infestation. $LSD_{5\%} = 1.54$). Asterisks denote varieties which show a significant difference when infested with *H. schachtii* according to the LSD.

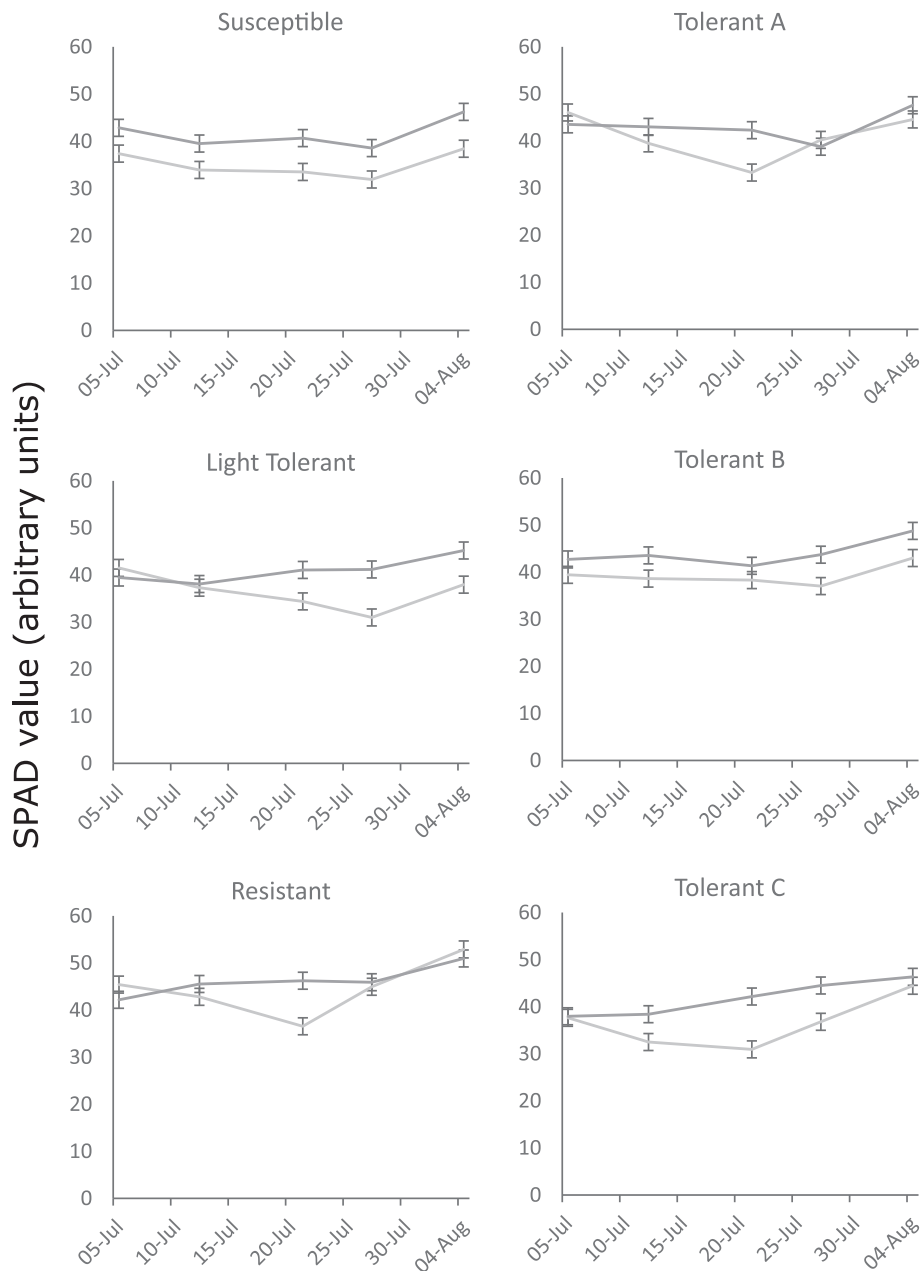


Figure 4. Chlorophyll measurements (SPAD) taken throughout July and August 2017 on the six varieties of sugar beet being grown (Infested Non infested). Variety x Infestation x Time interaction $P = 0.014$, Error bars show $LSD_{5\%}$.

controls, with large population multiplication. All of the tolerant varieties had reduced population density increases in comparison, all of which were lower than a mean doubling of population density and were not significantly different to the resistant variety (Fig. 11).

4 DISCUSSION

After 10 years of evolution and development, we have identified a method that optimises both sugar beet growth and nematode reproduction, allowing for both traits to be assessed on a range of varieties simultaneously.

Whilst the initial methodologies used at Broom's Barn and the University of Nottingham did not allow for sufficient growth of

the sugar beet, this was overcome with the latest method of using the large wooden frames in lieu of the plastic boxes. The modified method also allows for canopy growth and development to be monitored more easily since the spacing of the plants are the same as those used in commercial fields. This could not be achieved in the plastic boxes due to their much smaller size. Therefore, we have far greater confidence in the yield data obtained in 2019 than in any of the previous iterations of the method. Additionally, assessments of yield characteristics are more likely to align to results seen in field conditions and we detected large yield differences between the different variety types (Fig. 9).

Growing the sugar beet for an entire season allows for a true impact on yield to be measured and also for multiple generations



Figure 5. Evolution of the variety testing method. (A)–The system used in 2016 and 2017 at The University of Nottingham. Large plastic boxes (1.0 x 1.1 x 0.6 m) were filled with soil into which hatched *H. schachtii* juveniles were dispensed to growing sugar beet plants. (B) Revised method deployed in 2019 which utilises much larger wooden frames (2.0 x 1.5 x 0.4 m) were filled with either uninfested soil or soil inoculated with *H. schachtii* cysts and placed on an uninfested arable field.

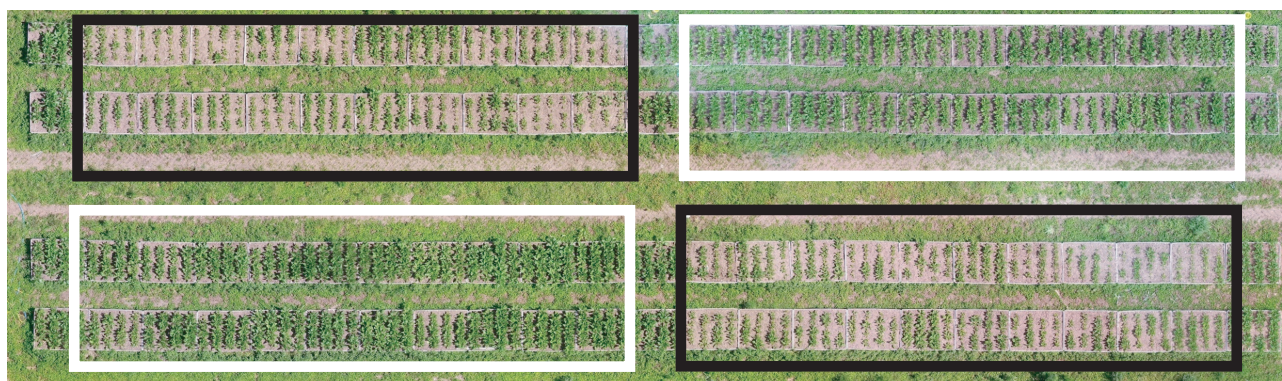


Figure 6. Canopy cover of the 2019 experiment on 30 June 2019, 67 days after sowing. Clear differences can be seen between the uninfested microplots, bounded by a white border, and the BCN infested microplots, bounded by a black border.

of BCN to occur, allowing for more accurate predictions of responses in field conditions to be made. Recent studies have demonstrated clear evidence of reduced fecundity of tolerant varieties of sugar beet when compared to susceptible varieties.^{8,28}

The 'Light tolerant' variety assessed in 2016 and 2017 had a phenotype more similar to the susceptible variety. Likewise, in 2019 the unknown variety, which was expected to be phenotypically tolerant, instead, appears to be BCN susceptible. The modified method allows for accurate quantification of the reproduction rates of BCN and is more representative of a field situation than the plastic box approach. The use of cysts, in infested soil, mimics natural influences on hatching, which was not possible using the hatched J2. Additionally, the prolonged period of hatch, and the greater number of eggs and juveniles, is also more likely to cause significant levels of yield loss, which was clearly evident in 2019

Our results relating to the final population densities of BCN, which developed on the sugar beet plants agree with findings of Hauer *et al.*⁸ They found that tolerant sugar beet allowed for 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 allowed for population reduction. Westphal²⁹ 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 was previously shown that tolerant varieties vary in nematode reproduction,²⁸ as seen in our results with the light tolerant variety producing higher BCN population densities than the tolerant varieties.

Understanding the dynamics of both yield responses and also BCN fecundity on different varieties will allow sugar beet growers to decide which varieties may be better for long term BCN management. From our results, it appears that BCN tolerant varieties should be classified as partially resistant as their fecundity is reduced when compared to 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*¹⁸ and then the lines with the highest levels of resistance were crossed with

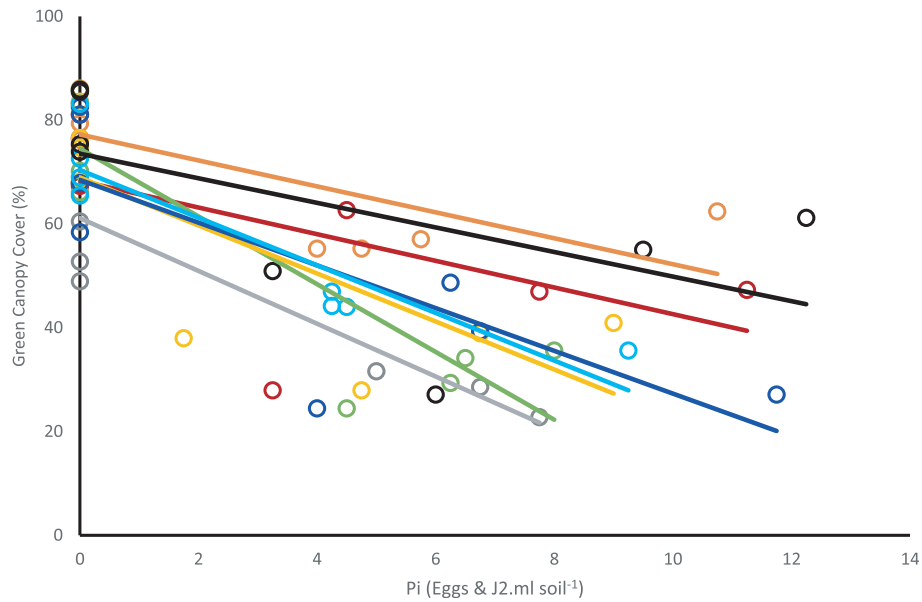


Figure 7. Significant regression of percentage green area cover of the sugar beet varieties [Sus B—, Sus C—, Tol D—, Tol E—, Tol F—, Tol G—, Res B—, Unk—] on 30 June 2019, 67 days after sowing, and the level of initial nematode infection (Pi) measured in each microplot. ($P < 0.001$ [Variety \times infection], $R^2 = 54.1$).

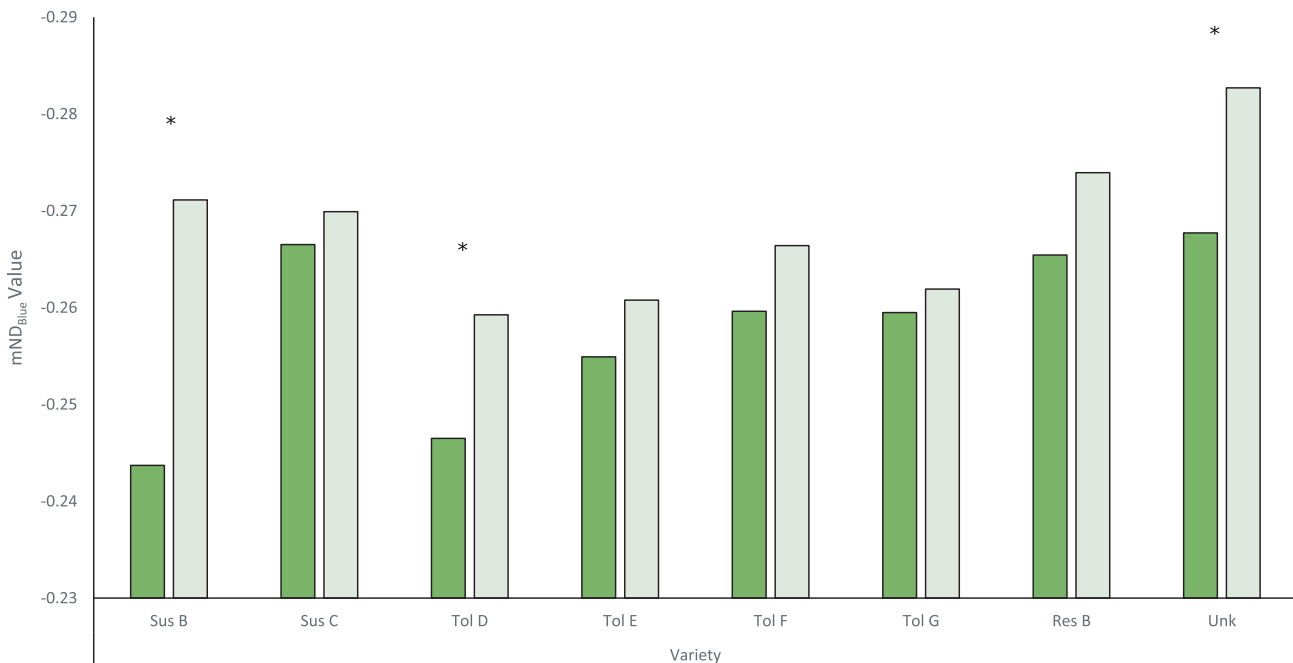


Figure 8. mND_{blue} values of the microplots measured on 6 August 2019 from different varieties of sugar beet grown either in unfested or BCN infested conditions. The more negative a value of mND_{blue} demonstrates a lower level of chlorophyll in the canopy. Asterisks show varieties which had a significant reduction in chlorophyll levels when infested with *H. schachtii* according to the $LSD_{5\%}$. ($P = 0.029$ variety \times infestation interaction, $LSD_{5\%} = 0.01$).

high yielding lines to develop the tolerant varieties. This conclusion is supported by our data and also data from Reuther *et al.*²⁸ who proposed that tolerant varieties fall into a spectrum of resistance. We seem able to support this finding too, with reported tolerance on some of our varieties not demonstrating the reduced fecundity of the majority of the tolerant varieties assessed. Reductions in chlorophyll levels seen in 2017 may not have resulted in a negative impact on yield, which supports our finding that the

inoculation level was insufficient to cause clinical levels of damage to the plants. It has been shown that a decrease in nitrogen in a crop does not change the light conversion coefficient of sugar beet³⁰ and may explain why a significant decrease in yield in the susceptible variety was not detected. However, the greater and earlier infestation of the plants provided by the encysted eggs and J2 in 2019, resulted in delayed canopy formation when infested with BCN which was followed by measurable changes

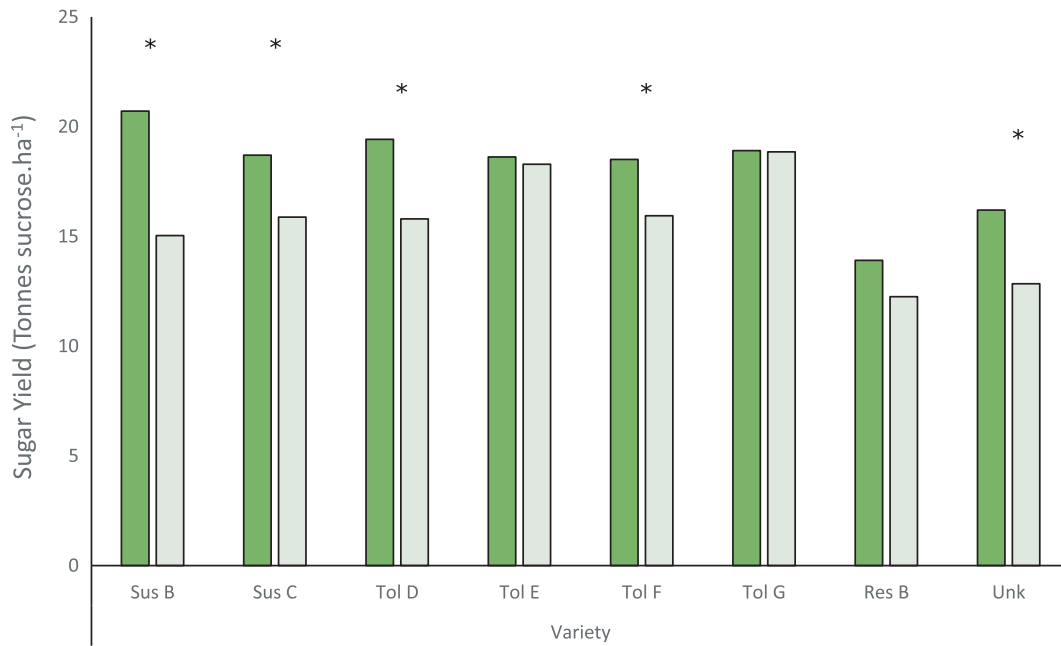


Figure 9. Yields of the sugar beet grown in unfested ■ or infested microplots □ in 2019. Significant differences were found in response of the varieties of sugar beet to nematode infection. ($P = 0.007$ Variety \times Infestation interactions, $LSD_{5\%} = 2.1$). Asterisks denote varieties, according to the $LSD_{5\%}$ which had a yield reduction when infested with *H. schachtii*.

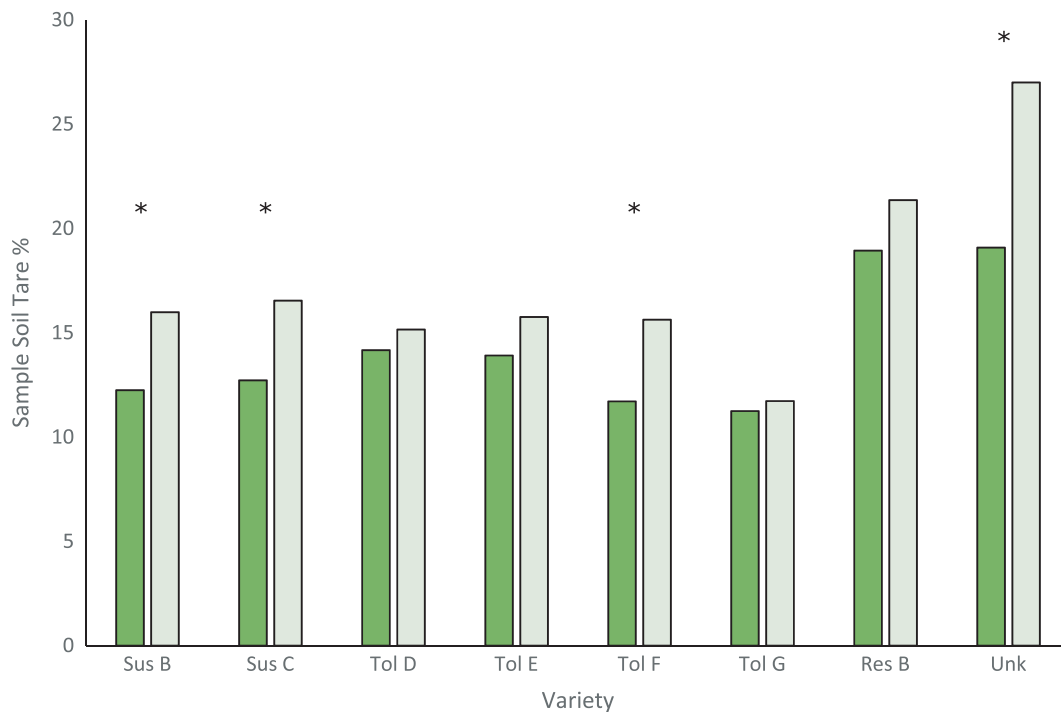


Figure 10. Soil tare, expressed as the percentage of the uncleaned sample weight which was soil, from the experiment in 2019. Non-infested samples - ■ Infested samples - □ ($P = 0.029$ variety \times infection interaction, $LSD_{5\%} = 3.1$). Asterisks denote varieties, according to the $LSD_{5\%}$ which had a significant soil tare increase when infested with *H. schachtii*.

in mND_{blue} later in the season support our yield data and the conclusion that we were able to achieve levels of damage to differentiate varieties with differing levels of tolerance to BCN.

One major drawback of the plastic boxes was the need to provide adequate irrigation and this proved to be a difficult challenge

during hot and sunny spells in the summer. Therefore, the movement of juveniles and recolonization of roots by BCN may have been reduced despite additional hand watering and drip-irrigation being regularly administered. The boxes dried out very rapidly on hot days and, when watered in the mornings, the

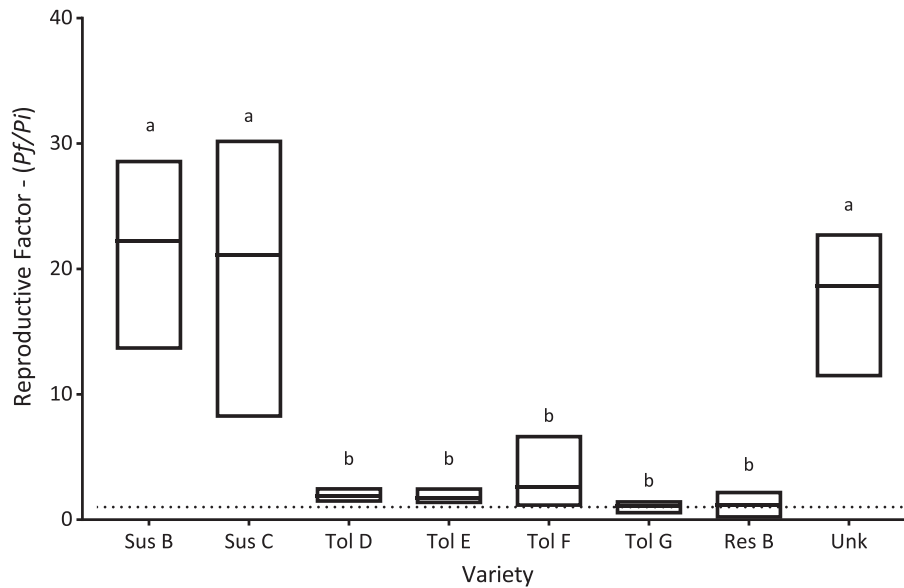


Figure 11. Box plot of population multiplication of BCN on the different sugar beet varieties tested in 2019. Central line in the box shows the mean value and the box shows the complete range of values assessed. Highly significant differences were observed between the different varieties ($P < 0.001$, $LSD_{5\%} = 7.28$) Letters which are different indicate a significant difference at $P = 0.05$. The dashed line indicates a Pf/Pi of 1.0, i.e. no net population change.

plants would become stressed by the afternoon and therefore differed from plants grown in the field, where deeper root systems would have had access to greater amounts of water. This was overcome by using the larger boxes with open access to the field soil below in 2019 and the application of irrigation using an overhead boom irrigator. However, the change to our approach required a field site which had no prior BCN infestation.

The most challenging aspect of developing a method was to enable the plants to express their yield both with and without BCN infestation. In 2016 and 2017 the soil used was obtained from British Sugar and was comprised of soil collected at the processing factories when the beet are washed prior to being processed. Therefore, trace amounts of BCN were found in the soil despite the soil-recovery process not being conducive to nematode survival (due to prolonged periods where the soil is kept in anaerobic conditions). Therefore, the lack of yield differences seen in 2016 and 2017 may partly be down to this drawback of using factory-recovered soil. It therefore became paramount to use soil free of BCN infestation in 2019 for the uninfested plots. This was successfully achieved since the field soil was checked for infestation prior to use. All implements used between the infested and clean boxes were also well cleaned to keep nematodes away from the 'healthy' plants and the use of discard boxes buffered the infested boxes from the clean boxes. More labour was needed to set-up the wooden frames than the plastic boxes, especially when it came to measuring the initial BCN populations (P_i), but the clear relationships with the measured responses, such as canopy development and final yield demonstrates that this additional time is not only worthwhile but essential to the success of the experiment.

Another important factor to measure at harvest is the soil tare, which is increased by excessive lateral rooting caused by disruption of auxin transport by the developing syncytia of BCN.³¹ Our findings relating to soil tare are important to consider in terms of sugar beet quality 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 the tolerant varieties may be explained by differences

in the development of roots of tolerant sugar beet varieties, along with the reduced impact of feeding, reproduction and subsequent reinfection of BCN. However, we were able to measure that one of the tolerant varieties assessed in 2019 (Tolerant F) showed an increased soil tare when infested and therefore might be less appealing to sugar beet growers and processors alike.

We can now demonstrate clear phenotypic differences, in terms of root yield under nematode infestation, canopy development, and nematode fecundity, between various tolerant varieties. These findings will support more targeted variety choices and the findings will complement the data already published to growers.³² Additionally, This methodology of using frames filled with soil and nematodes could easily be modified for a range of other crops and is relatively straightforward to implement. Soybean cyst nematode, *Heterodera glycines*, found widespread across the USA and China, should work equally effectively since it is a close relative of *H. schachtii*.⁴² This method should also be applicable to potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) and potato variety testing to help address the £50 m worth of damage caused by these species every year in the UK,³³ let alone greater losses across the world. Non-cyst forming species, such as root knot nematodes (*Meloidogyne* spp.) could also be introduced to a similar system and work on a range of either temperate and tropical crop species that suffer damaging levels of crop losses, such as carrots, wheat, potatoes and maize.³⁴ There is also scope to substitute the soil-based pathogens for others of sugar beet, such as Beet necrotic yellow vein virus, commonly referred to as rhizomania,³⁵ or root fungi e.g. violet root rot caused by *Rhizoctonia crocorum*.³⁶

5 CONCLUSIONS

After a long period of development and refinement, our methodology is now deployed to simultaneously assess BCN fecundity, its impacts on the physiological development of sugar beet plants, and their final yield over an entire growing season. We can demonstrate differences in yield, and therefore tolerance to BCN, between different varieties and screen unknown varieties to derive their BCN

status. Data such as these have never been available to growers in the UK before. Therefore, our methodology and results obtained can allow for more informed variety selection and help to optimise productivity of their sugar beet crops in BCN infested fields.

ACKNOWLEDGEMENTS

The experiments conducted in 2016 and 2017 were undertaken as part of a jointly funded PhD programme with the University of Nottingham and BBRO with additional support from The Morley Agricultural Foundation. The work in 2019 formed a part of an Innovate UK Knowledge Transfer Partnership Project (KTP No. 11116) jointly funded by HM Government and BBRO. We also wish to thank Cranfield University for loaning their irrigation equipment in 2019. The authors also wish to thank all of the support staff at the University of Nottingham and BBRO for their assistance in managing the experiments.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Nicol JM, Turner SJ, Coyne DL, Den NL, Hockland S and Maafi ZT, *Current Nematode Threats to World Agriculture, Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer, Dordrecht (2011).
- CABI, *Heterodera schachtii* (beet cyst eelworm), *Invasive Species Compendium* (2018) <https://www.cabi.org/isc/datasheet/27036> [accessed 17 February 2018].
- Dewar AM and Cooke DA, Pests, in *Sugar Beet*, ed. by Draycott AP. Blackwell Publishing Limited, Oxford, pp. 316–350 (2006).
- Müller J, The economic importance of *Heterodera schachtii* in Europe. *Helminthologia* **36**:205–213 Biol Bundesanstalt Land & Forstwirtschaft, Inst Nematol & Wirbeltierkunde, D-48161 Munster, Germany. Muller, J (reprint author), Biol Bundesanstalt Land & Forstwirtschaft, Inst Nematol & Wirbeltierkunde, Toppheideweg 88, D-48161 Munster, Germany (1999).
- Blok VC, Tylka GL, Smiley RW, de Jong WS and Daub M, in *Resistance Breeding*, ed. by Moens M, Perry RN and Jones JT. Cyst Nematodes, CAB International, Wallingford, Oxfordshire, UK, pp. 174–214 (2018).
- Cooke DA, Beet cyst nematode (*Heterodera Schachtii* Schmidt) and its control on sugar beet. *Agricul Zool Rev* **2**:135–183 (1987).
- MAFF, The Beet Cyst Nematode Order 1977, ed. by Ministry of Agriculture Fisheries and Food, 988, HM Government, London, UK, (1977).
- Hauer M, Koch HJ, Krüssel S, Mittler S and Märlander 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).
- Wright Alistair JD, Back Matthew A, Stevens Mark, Sparkes Debbie L, Evaluating resistant brassica trap crops to manage *Heterodera schachtii* (Schmidt) infestations in eastern England. *Pest Management Science* **75**:438–443 (2019). <https://doi.org/10.1002/ps.5134>.
- Koch DW and Gray FA, Nematode-resistant oil radish for *Heterodera schachtii* control in sugar beet-barley rotations. *J Sugar Beet Re* **34**:31–43 (1997).
- Schlatter C, Clariva™ seed treatment nematicide, a breakthrough for sugar beet production. *75th IIRB Congress* **101**:IIRB (2015).
- Panella L and Lewellen RT, Broadening the genetic base of sugar beet: introgression from wild relatives. *Euphytica* **154**:383–400 (2007).
- Cai DG, Kleine M, Kifle S, Harloff HJ, Sandal NN, Marcker KA et al., Positional cloning of a gene for nematode resistance in sugar beet. *Science* **275**:832–834 CHRISTIAN ALBRECHTS UNIV KIEL, INST CROP SCI & PLANT BREEDING, D-24118 KIEL, GERMANY. AARHUS UNIV, DEPT MOL & STRUCT BIOL, DK-8000 AARHUS, DENMARK. DLO, CTR PLANT BREEDING & REPROD RES, NL-6700 AA WAGENINGEN, NETHERLANDS. CHRISTIAN ALBRECHTS UNIV KIEL, INST PHYTOPLA (1997).
- Blake A, Drought s hidden nematode threat. *Crop Produc Magaz* **13**:56–57 (2011).
- Märlander B, Hoffmann C, Koch HJ, Ladewig E, Merkes R, Petersen J et al., Environmental situation and yield performance of the sugar beet crop in Germany: heading for sustainable development. *J Agron Crop Sci* **189**:201–226 (2003).
- Wright AJD, Bussell JS, Stevens M, Back MA and Sparkes DL, 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 (2018).
- Stevanato P, Trebbi D, Panella L, Richardson K, Broccanello C, Pakish L et al., 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 (2015).
- Lange W and de Bock TSM, Pre-breeding for nematode resistance in Beet. *J Sugar Beet Res* **31**:13–26 (1994).
- Wright AJD, PhD update- Beet cyst nematode. *Br Sugar Beet Rev* **85**:50–53 (2017).
- Bowen S and Townsend T, Beet Yield Challenge 2019. *Br Sugar Beet Review* **88**:50–51 (2020).
- Wright A, Stevens M, Back M and Sparkes D, Interactions between *Heterodera schachtii* and Sugar Beet. *Aspects Appl Biol* **134**:81–88 (2017).
- Burks E, Morris N and Stevens M, Beating cyst nematode. *Br Sugar Beet Rev* **79**:26–29 (2011).
- 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).
- Uddling J, Gelang-Alfredsson J, Piikki K and Pleijel H, Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth Res* **91**:37–46 (2007).
- ICUMSA, *ICUMSA Methods Book*. Bertens, Berlin (2007).
- Reid E, A rolling method for opening cysts of potato root eelworm. *Plant Pathology* **4**:28–29 (1955).
- Jay S, Gorretta N, Morel J, Maupas F, Bendoula R, Rabatel G et al., Estimating leaf chlorophyll content in sugar beet canopies using millimeter- to centimeter-scale reflectance imagery. *Remote Sens Environ* **198**:173–186 (2017).
- Reuther M, Lang C and Grundler F, Nematode-tolerant sugar beet varieties - resistant or susceptible to the Beet cyst nematode *Heterodera schachtii*? *Sugar Industry* **142** (5):277–284 (2017).
- Westphal A, Vertical distribution of *Heterodera schachtii* under susceptible, resistant, or tolerant sugar Beet cultivars. *Plant Dis* **97**:101–106 (2013).
- Jaggard KW and Qi A, *Agronomy, Sugar Beet*. Blackwell Publishing Ltd, Oxford. pp. 134–168 (2006).
- Grunewald Wim, Cannoot Bernard, Friml Jiří, Gheysen Godelieve, Parasitic Nematodes Modulate PIN-Mediated Auxin Transport to Facilitate Infection. *PLoS Pathogens* **5**:e1000266 (2009). <https://doi.org/10.1371/journal.ppat.1000266>.
- BBRO and BSPB, Recommended List of Sugar Beet Varieties 2022, <https://bbro.co.uk/media/50482/22-treated-rl-1.pdf>, 2021.
- Watts W, *Integrated Biomethods for the Management of Globodera Pallida* (Stone.). Harper Adams University, Shropshire, United Kingdom (2013).
- Karssen G, Wesemael W and Moens M, Root-knot nematodes, in *Plant Nematology*, ed. by Perry RN and Moens M. CAB International, Wallingford, Oxon, pp. 73–108 (2013).
- Stevens M, Liu H-Y and Lemaire O, Virus diseases, in *Sugar Beet*, ed. by Draycott AP. Blackwell Publishing, Oxford. pp. 256–285 (2006).
- Asher MJC and Hanson LE, Fungal and bacterial diseases, in *Sugar Beet*, ed. by Draycott AP. Blackwell Publishing, Oxford, pp. 286–315 (2006).
- Perry RN and Moens M, *Glossary, Plant Nematology*. CAB International, Wallingford, Oxon, UK, pp. 523–531 (2013).
- BBRO, BBRO Recommended Sugar Beet Variety List 2015, 2014. <https://bbro.co.uk/media/1148/2015-rl-table-final.pdf>.