Physiological differences between sugar beet varieties susceptible to, tolerant or resistant to the beet schactii (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.

Keywords

Sugar beet, *Heterodera schachtii*, canopy imaging, sugar yield, canopy vigor.

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 (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-1990s (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 to 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 1.2g of nitrogen fertilizer each using ammonium nitrate (NH_4NO_3) and a 0N-36P-36K 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 18-55mm 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 400µmol/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 hours. 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 hours. 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 18-55mm 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.

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 x 3 metres 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 10-18mm 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 2.7m of row length (8.1m 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.

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 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 ($P<0.001$) Variety 8 and 3 produced the lowest (26.47 and 26.33 mg per 100g of beet) and variety 2 the greatest (30.14mg per 100g 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 all seed rates shows 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 show 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). 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 ($P\leq0.01$) (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 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 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 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). 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 a seed rate BCN resistant variety needs to be sown at 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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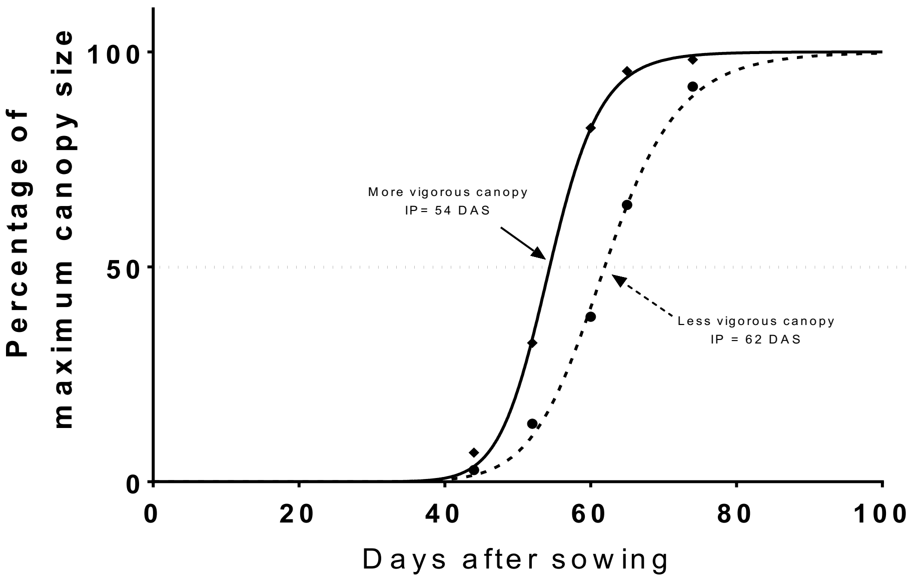

Figure 1 - Two contrasting canopy development curves as measured in the field trial at Sutton Bonington in 2017. Data from images taken in the field were used to calculate the canopy cover. These data were then inputted into a model to calculate the Inflection point (IP) of the different canopies and 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).

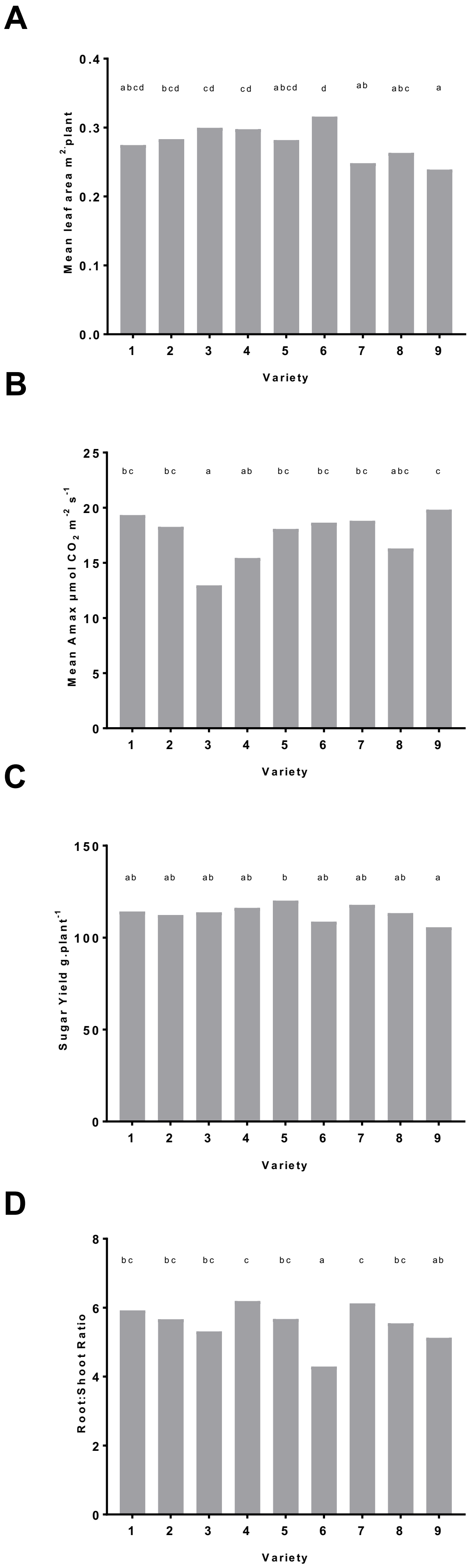

Figure 2- Results from the pot experiment conducted in 2015 to compare differences between a range of sugar beet varieties which vary in their susceptibility to *H. schachtii*. A shows the mean leaf area per plant (m^2) at harvest ($P<0.001$), B displays the Mean CO_2 assimilation ($\mu mol\ CO_2 m^{-2} s^{-1}$) 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 $P=0.05$.

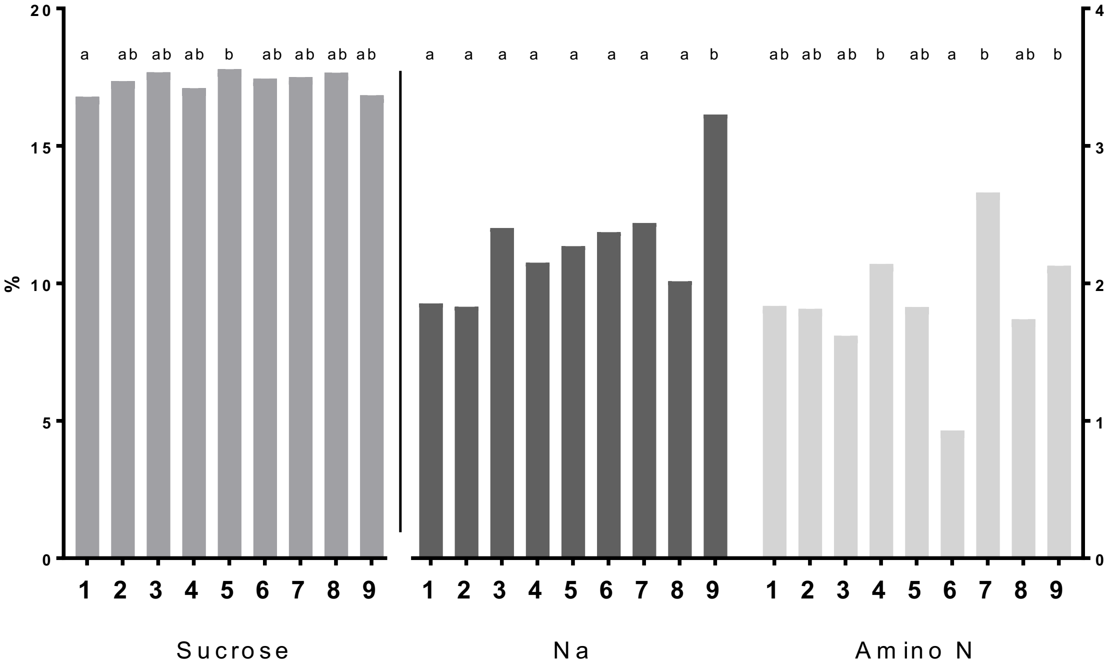

Figure 3- The nine varieties of sugar beet, which differ in their susceptibility to *H. schachtii*, were grown in a pot experiment at Sutton Bonington. After the roots were harvested a brei paste sample was made. The brei sample was measured for sucrose content and impurities of sodium and amino nitrogen (measured in mg of impurity per 100g 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

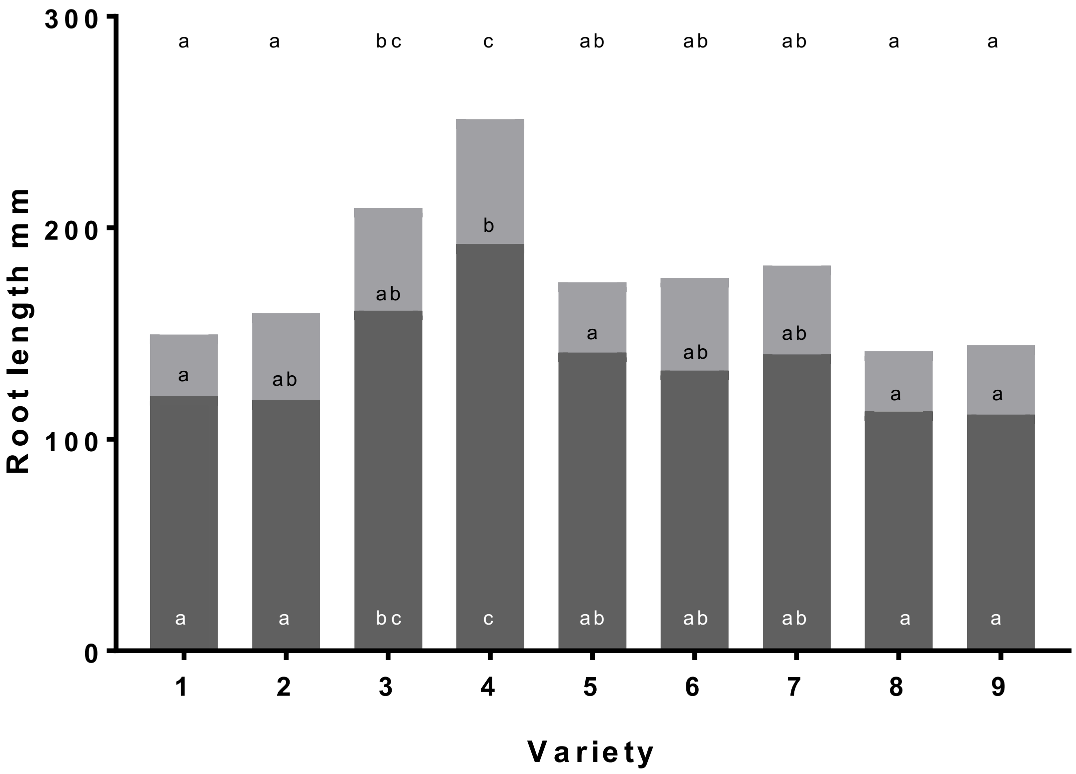

Figure 4- A hydroponic tank experiment was conducted to investigate differences in the early rooting development of nine varieties of sugar beet which vary in their susceptibility to H. schachtii. Primary root lengths ■ and lateral root lengths ■ were measured of each plant using computer image analysis (*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

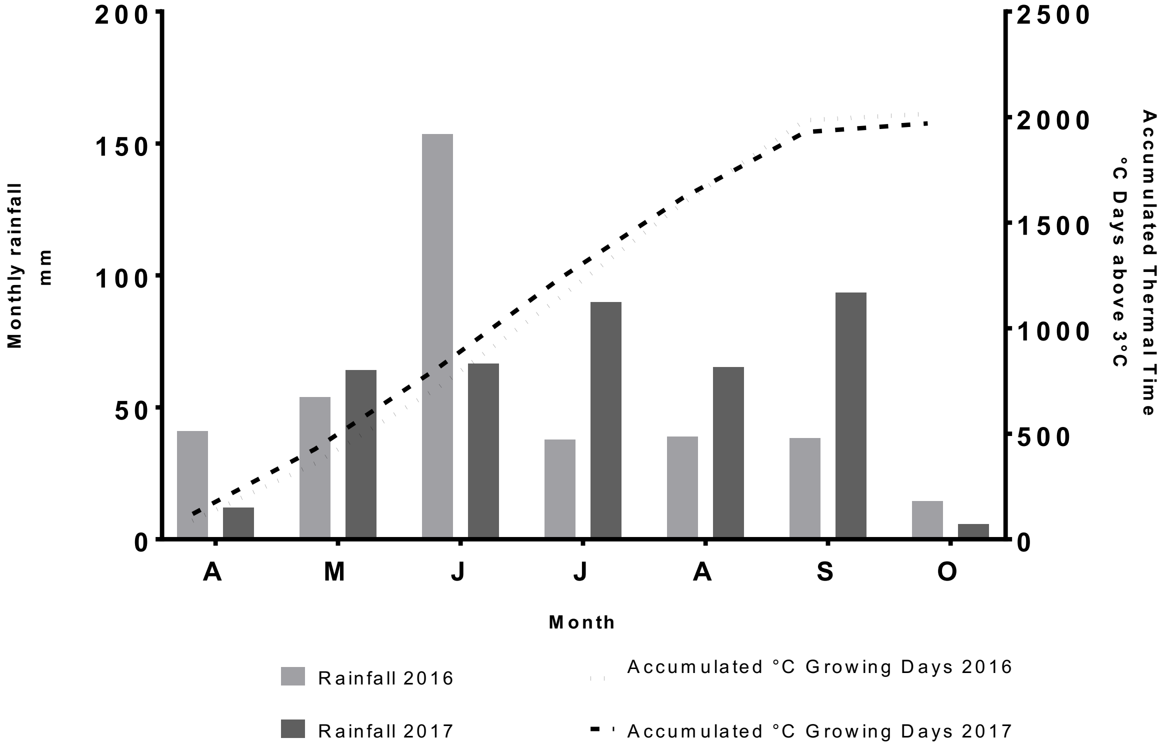

Figure 5 – Temperature and rainfall were measured throughout the experiment using the meteorological station at Sutton Bonington during the field experiments investigating the response of four sugar beet varieties, which varied in their susceptibility to H. schachtii, to increased plant populations.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

Figure 6 PRINT IN COLOUR

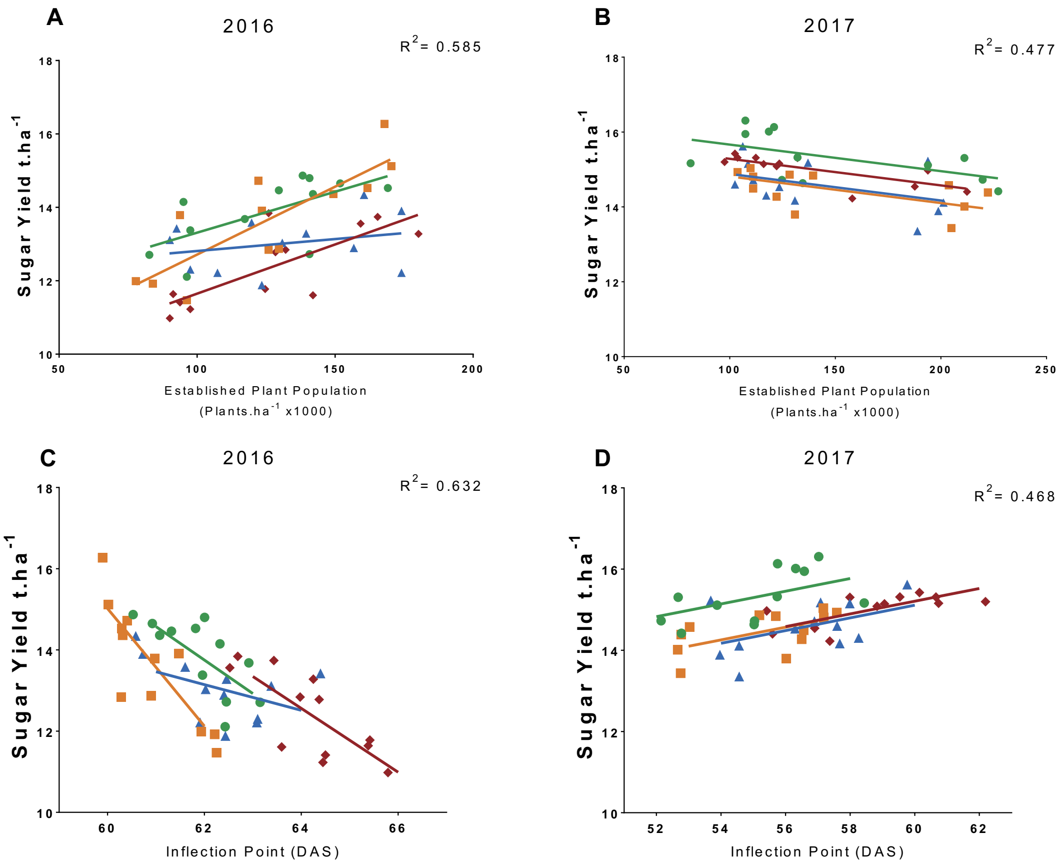

Figure 6 – Data showing the regression relationships between the established plant populations (A - 2016 & B - 2017) upon sugar yield and the response of the increasing inflection point (IP) of each variety (C -2016 & D - 2017) from the field experiments at Sutton Bonington. Resistant 🟥 Susceptible 🟦 Light tolerant 🟧 Tolerant 🟩.

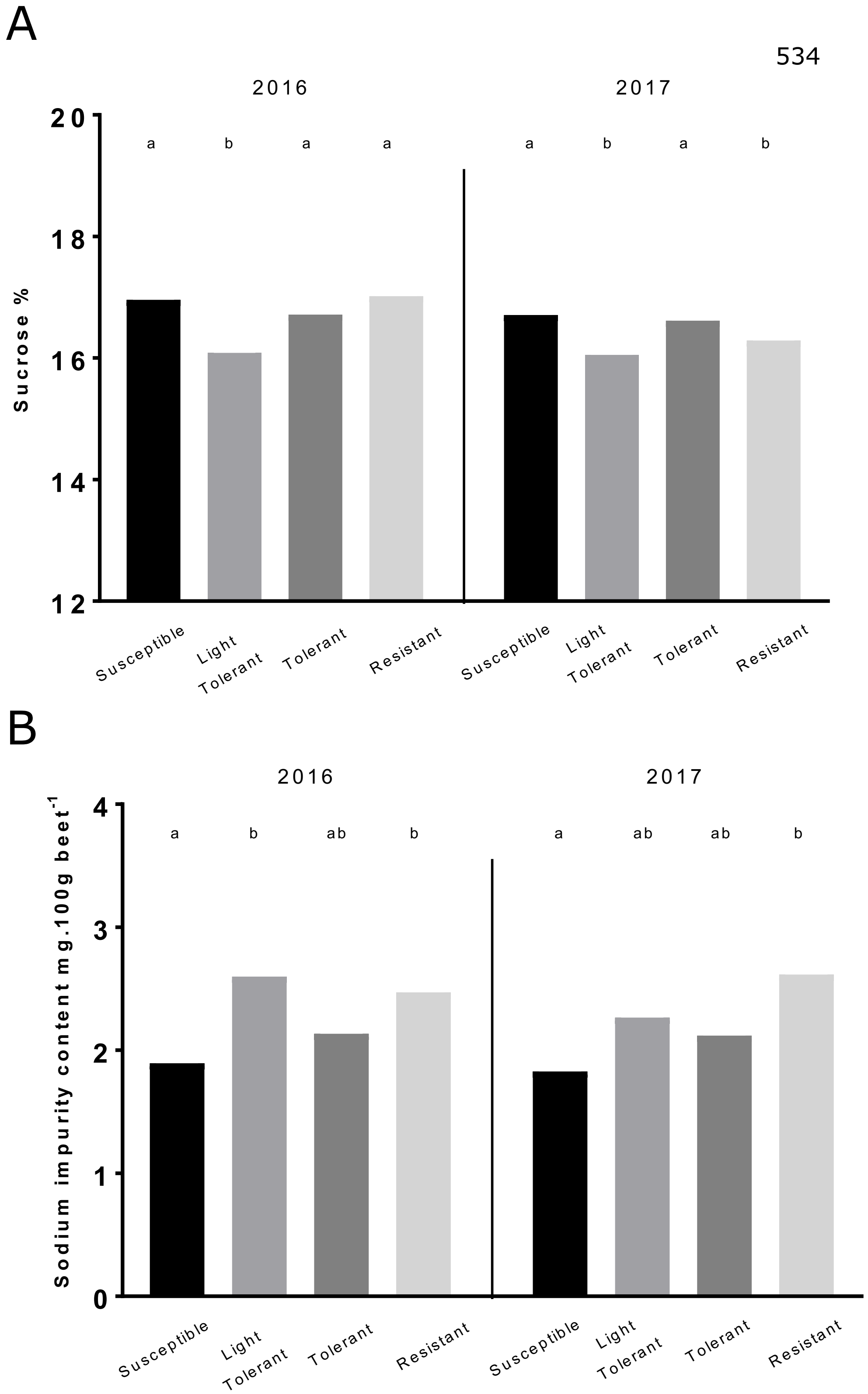

Figure 7 – Mean levels of Sucrose in the storage root (A) ($P<0.001$ for both years) and Sodium impurities (B) ($P=0.006$ in 2016, $P=0.014$ in 2017) in the sugar beet measured in the samples at harvest from the field trials at Sutton Bonington Significant differences were only detected between the varieties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

Table 1

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***	**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	SES Vander Have
2	Susceptible		2011	Strube
3	Light Tolerant	Able to compensate for damage caused by low levels of BCN infestation	2014	Syngenta
4	Light Tolerant		2010	Syngenta
5	Tolerant	Trait is not related to resistance. Tolerant plants are able to sustain growth & yield when parasitized by BCN (Perry and Moens, 2013) Has increased yield performance relative to an infested susceptible plant (BBRO, 2014)	2013	SES Vander Have
6	Tolerant		2013	Betaseed
7	Tolerant		2015	SES Vander Have
8	Tolerant		2012	Strube
9	Resistant +	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	Syngenta
10	Resistant +		n/a	Syngenta

* As claimed by sugar beet breeders upon submission into recommended list (RL) trials.

+ These varieties have never been commercially registered in the UK

Table 2

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

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	0.001
	153000	100.7	100.3	100.5	101.4	100.7 a	
	211000	101.1	100.1	99.7	99.4	100.1 a