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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1 PHYSIOLOGICAL DIFFERENCES BETWEEN SUGAR BEET VARIETIES SUSCEPTIBLE,

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3 *SCHACHTII* (SCHMIDT) UNDER UNINFESTED CONDITIONS

4 **Abstract**

5 The beet cyst nematode (BCN) is a problem to sugar beet growers around the world and can

6 cause severe yield losses. Recently, varieties of sugar beet have been developed which are

7 either tolerant to damage caused by BCN, or alternatively are resistant to BCN. Little is

8 understood about these varieties and how they may have different physiological

9 characteristics when compared with varieties of sugar beet that are susceptible to BCN. This

10 study assessed a range of nine varieties, which were tolerant, susceptible or resistant to BCN,

11 in pot and hydroponic tank investigations to measure differences in their canopy, early rooting

12 and yield traits in the absence of BCN. Two field experiments, using four varieties which were

13 susceptible, resistant or tolerant to BCN, then followed to test the hypothesis that increasing

14 the plant population density (PPD) allows a BCN resistant variety to achieve a greater yield.

15 In the pot and hydroponic experiments, it became clear that the varieties had different growth

16 habits. The resistant variety yielded the least sugar and had the smallest canopy per plant. In

17 the field experiments, which were not infested with BCN, in both years the resistant variety

18 also showed a delayed canopy expansion compared to the other varieties. The rate of

19 expansion could be increased by increasing the PPD. In 2016 this increased PPD resulted in

20 higher yields of the resistant variety. However, due to better canopy development in the

21 following year, a yield penalty was found in 2017 at higher PPDs. Understanding how different

22 varieties need different PPDs may make resistant varieties a more economical option to

23 cultivate in the future. However, the levels of impurities, particularly sodium impurities, in the

24 resistant plants may still make them a less favourable choice to grow.

25 The light tolerant varieties showed a distinct increased rooting and canopy expansion rate

26 compared to the other variety types, while the tolerant varieties showed similar rooting and

27 canopy traits to the susceptible varieties but had different yield responses to increased seed

28 rate.

31 **1. INTRODUCTION**

32 Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is widely grown across the world as a source of

33 sucrose. Like all crops, sugar beet suffers threats to achieving maximum yield due to a range

34 of pests and diseases and careful management of these threats is required to limit yield loss.

35 One pest which poses a threat to sugar beet crops all over the world is the beet cyst nematode

36 (BCN), *Heterodera schachtii* (Schmidt). Commonly found in sugar beet crops grown on sandy,

37 loamy or organic soils, the nematode can cause severe yield losses, especially in water limited

38 conditions (Cooke, 1987). In Europe alone, BCN was estimated to cause annual losses of

39 over €90 million (Müller, 1999). However, with modern varieties this figure may be much lower.

40 BCN can go unnoticed at low population densities below the plant's tolerance threshold to

41 damage. Yield will still be lost even if no symptoms are displayed such as stunted plant

42 development canopy wilting and yellowing of leaves (Dewar and Cooke, 2006) and therefore

43 infestations are probably more widespread than expected by sugar beet growers and levels

44 of yield loss are difficult to quantify. Control of BCN has traditionally been limited to the

45 enforcement of long rotations, of over five years between host species (Koch and Gray, 1997),

46 either through government intervention or contract clauses with sugar processors (Cooke,

47 1987; Ministry of Agriculture, Fisheries and Food, 1977). Nematicides have also been an

48 option for control, but have now been withdrawn from sale due to concerns about their harmful

49 effects (Dewar and Cooke, 2006; Hauer et al., 2016). Other options, such as biofumigation

50 and resistant brassica cover cropping may also provide control for BCN. However, these

51 techniques can produce variable results (Hauer et al., 2016; Held et al., 2000; Hemayati et al.,

52 2017; Lazzeri et al., 1993).

53 Advances in sugar beet breeding have led to the development of varieties of sugar beet which

54 are tolerant, light tolerant or resistant to infestation by BCN. Tolerant varieties, which can

55 compensate for losses to infestations of BCN and allow economically viable yields on infested

56 land, were developed by introgressing genes such as *HsBvm-1* from *Beta vulgaris* ssp.

57   *maritima*, a close relative of sugar beet (Stevanato et al., 2015). Introduced to the UK in 2009,

58   the market share of these tolerant varieties has grown annually from 0.59% in 2009 up to

59   6.69% in 2017 (M Culloden Pers. Comm. – Head of Agriculture, British Sugar). Whilst these

60   varieties have both gained in popularity and yield potential over this period, there is much that

61   is not understood about their physiology and appropriate uses in the field. It is hypothesised

62   that they may have higher levels of photosynthetic assimilation to counteract losses to the

63   BCN or greater levels of early root growth to grow away from infested patches of soil. Varieties

64   marketed as 'light tolerant' were previously available in the UK. Whilst they have since been

65   superseded by higher yielding fully tolerant varieties, they were marketed as having a greater

66   yield potential than tolerant varieties, but would only be beneficial to use in fields with low BCN

67   populations (Kerr and Stevens, 2014). Whilst popular at the moment, tolerant varieties may

68   be of limited use in the long term as they still cause the build-up of BCN populations in the soil

69   (Hauer et al., 2016; Krüssel and Warnecke, 2014). Resistant varieties (which can actively

70   reduce BCN populations) have been available to growers in continental Europe since the mid-

71   1990s (Müller, 1999; Zhang et al., 2008) and were developed by introgressing the HS1[pro1]

72   gene from *Patellifolia procumbens* into sugar beet (Panella and Lewellen, 2007). The

73   resistance mechanism enables the sugar beet to recognise the invading nematode during the

74   development of its feeding cell (syncytium). The hypersensitive response results in the death

75   of cells surrounding the syncytium and the nematode is deprived of nutrients which prevents

76   successful BCN reproduction. As the nematode is prevented from viably reaching mature

77   stages, when greater and more damaging feeding occurs (Müller et al., 1981), the yield of the

78   crop is also protected. The final populations in the soil are lowered when a resistant variety is

79   grown and therefore these varieties may be a good option for growers with BCN infestations

80   who need to plant other host species, such as oilseed rape or vegetable brassicas, in their

81   crop rotations and want to reduce their BCN population levels.

82   BCN infestation is usually very patchy in fields and rarely is it found in all parts of a field

83   (Cooke, 1987). Therefore, growing a tolerant or resistant variety may have a negative impact

84   on overall field yield due to their potentially lower yield performance in the absence of BCN

4

85   and associated higher seed costs of such varieties (British Sugar, 2017). This study aimed to

86   better understand the performance of a range of sugar beet varieties in terms of early rooting

87   habits, canopy expansion and size, photosynthetic activity and their subsequent yield and

88   quality. The experiments were conducted in the absence of any BCN infestation to understand

89   if any physiological differences between the varieties could be identified in uninfested

90   conditions and compare yield without the associated losses from BCN.

91

92   **2. MATERIALS AND METHODS**

93   2.1 Pot Experiment

94   An experiment was established in an unheated glasshouse on 11 May 2015. Nine varieties of

95   sugar beet, varieties 1 to 9 detailed in Table 1, were grown in five blocks, organised as a

96   randomised block design, with two replicates of each variety in each block (n=90).

97   Seeds of each variety were sown into five litre pots filled with a 20:80 mixture of sterilised

98   Kettering loam (24% clay content) (Boughton, Kettering, UK) and coarse sand mixed to create

99   a loamy sand soil texture. Three seeds of each variety were planted in each pot and thinned

100   to one plant at 8 days after sowing (DAS). Plants were given 1.2g of nitrogen fertilizer each

101   using ammonium nitrate (NH_4NO_3) and a 0N-36P-36K fertilizer with additional trace elements

102   (Hortifeeds, Lincoln, UK) was used to meet all of the plants nutritional requirements. All

103   fertilizer was applied to the pot prior to sowing. Plants were supplied with regular irrigation to

104   prevent water stress throughout the experiment.

105   Leaf and canopy expansion were measured during the canopy expansion phase of the plants.

106   Canopy expansion was measured using a digital camera (Canon Eos 1100D fitted with 18-

107   55mm Lens, Canon Inc. Japan) mounted on a copy stand from which canopy cover could be

108   derived by the thresholding of green pixels using ImageJ (Rasband, 2016).

109   A Li-Cor LI6400XT (Li-Cor Inc. Lincoln, NE, USA) was used to measure photosynthetic

110   assimilation (A_{max}), under the following conditions: a saturating photosynthetically active

111   radiation (PAR) level of 1200 µmol m^2/s, CO_2 set to 400µmol/mol, flow rate of 500 µmol/s and

112   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

141   Inc. Japan) and copy-stand. The photographs were then analysed using RootReader2D

142   version 2.3 (Clark et al., 2013) to measure primary and lateral root lengths.

143

144   2.3 Field Experiments

145   Field experiments were sown at The University of Nottingham's Sutton Bonington Campus on

146   7 April 2016 and on 10 April 2017. Both fields were of a freely draining slightly acid loamy soil

147   type (Hallett et al., 2017). In both years the same four varieties of sugar beet were sown: one

148   susceptible to BCN, one light tolerant, one tolerant and a new BCN resistant variety (varieties

149   2, 3, 7 and 10 respectively from Table 1), as the resistant variety previously used was not

150   available. These varieties were selected as they were commercially available in the year of

151   sowing except the resistant variety used was a coded variety under development and not

152   commercially available in any market. Each variety was sown at rates of either 119,000,

153   153,000 or 211,000 seeds per hectare in a Latin square design (n=48) with four replicates of

154   each treatment.

155   Plots containing six rows of sugar beet (7.5 x 3 metres in total, 50 cm row spacing) were sown

156   with a Wintersteiger Monoseed K seed drill (Wintersteiger AG, Reid im Innkreis, Austria). Plots

157   were fertilized with 120 kg ha^{-1} of nitrogen using ammonium nitrate (NH_4NO_3) in both years.

158   Doses of N were split 1/3, applied prior to emergence, and 2/3 applied before the two true

159   leaves stage. In 2016, at 49 DAS, the plots sown at 153,000 seeds ha^{-1} were thinned manually

160   to reduce the population due to a malfunction with the drill. This ensured three distinct

161   population densities were established.

162   Canopy cover was monitored using a digital camera fitted with a wide-angle lens (Canon

163   EOS1100D and 10-18mm lens). The lens' zoom was fixed at 10 mm and the camera mounted

164   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

165   height, rows two, three and four (which were to be harvested) filled the image. Each

166   photograph captured 2.7m of row length (8.1m in total). Images were taken from either end of

167   the plots with the combined area covering 72% of the harvested plot area. The use of a laptop

168   allowed for remote imaging of each plot and storage of the photographs. At the four-leaf stage

169 the photographs were used to count established plants in the plots. Photographs were taken

170 on a weekly basis during the canopy expansion phase of growth. The green area of each

171 image was estimated using the threshold calculated by ImageJ (Rasband, 2016) to measure

172 canopy cover.

173 Canopy development was modelled using a three-parameter log-logistic model fitted in R (R

174 Core Team, 2016; Ritz et al., 2015) using the calculated values for each plot from the image

175 analysis. The model then estimated maximum cover, slope and the inflection point of each

176 plot's canopy. Inflection point (IP) denotes the time when the canopy reached 50% maximum

177 canopy cover and is therefore a measure of the speed at which the canopy expands. A larger

178 and more vigorous canopy has a lower IP than a smaller and slower canopy (Fig. 1).

179 The plots were harvested on 4 October in both years. Three rows were harvested using a

180 Garford Victor harvester (Garford Farm Machinery Ltd, Peterborough, UK) to determine yield

181 and impurity levels at the BBRO tare house. An additional ten beet from row 5 were harvested

182 by hand to measure total biomass of each variety. The ten beet were weighed, subsampled

183 to five, then these leaves and storage roots were washed to remove soil and lateral roots, and

184 then chopped before being dried at 70°C until constant weight.

185

186 2.4 Data analysis

187 Genstat 17th Edition was used for appropriate ANOVAs for each experimental design,

188 regression and comparison of regression analysis (VSN international, Hemel Hempstead,

189 UK). Graphs were prepared using GraphPad Prism v.7 (GraphPad Software Inc. La Jolla, CA,

190 USA). Tukey's multiple comparison test was used to compare the results of the ANOVAs

191 reported in the tables and graphs.

192

193 **3. RESULTS**

194 3.1 Pot experiment

195 Significant differences in canopy expansion were observed between the varieties when grown

196 in the glasshouse (Table 2). At 28 DAS the resistant variety (9) had the smallest canopy cover

197 and was significantly smaller than all varieties except 6 and 7 (P<0.001). At 35 DAS the

198 resistant variety still had the smallest cover and was significantly lower than varieties 2,5 and

199 8 (P=0.007). At 42 DAS there were no significant differences between the varieties, but the

200 resistant variety remained the smallest.

201 When total leaf area was measured at harvest (Fig.2a) varieties 2, 3, 4 and 6 had greater leaf

202 areas than the remaining varieties and the resistant variety (9) still had the smallest leaf cover

203 of all (P<0.001). In terms of photosynthetic assimilation of CO_2, the resistant variety had similar

204 A_{max} values to the susceptible and most tolerant varieties, however the light tolerant varieties

205 (3 and 4) and tolerant variety number 8 had the lowest mean A_{max} values (Fig. 2b).

206 When the plants were harvested there were also significant differences in sugar yield (Fig.

207 2c). Variety 9 yielded the least, but was only significantly lower than variety 5 (P=0.022). A

208 significant difference in root:shoot ratio was also found from the biomass data. Variety 6 had

209 the lowest ratio (P<0.001) (Fig. 2d).

210 The resistant variety had the second lowest percentage of sugar in the root but was only

211 significantly lower than variety 5 (P= 0.006, Fig 3). All varieties had between 16.79 and 17.79%

212 sucrose content.The resistant variety produced significantly greater levels of sodium impurities

213 than all of the other varieties (P<0.001). Apart from variety 6, all varieties produced similar

214 amino nitrogen impurities to the resistant variety (P=0.001). Significant differences were found

215 in relation to the levels of potassium impurities measured too (P<0.001) Variety 8 and 3

216 produced the lowest (26.47 and 26.33 mg per 100g of beet) and variety 2 the greatest

217 (30.14mg per 100g of beet).

218

219 3.2 Hydroponic tank experiment

220 Image analysis of the three-week old seedlings showed significant differences in root growth

221 between varieties (Fig. 4). The two light tolerant varieties (varieties 3 and 4) had longer roots

222 than the other varieties that were tested (P<0.001). Consistent rankings of the total root length

223 and primary root length show the differences in the varieties are driven mainly by the

224 differences in the length of the primary root. This is further supported by no significant

225 differences being found in the ratio of primary to lateral roots of any of the varieties.

226

227 3.3 Field experiments

228 Both years experienced similar levels of total rainfall and thermal time over the course of the

229 experiments (Fig. 5). In 2016 June rainfall was exceptionally high (three times the long term

230 mean) which resulted in delayed and reduced total expansion of the canopies. April, May and

231 June were also warmer in 2017 (Fig. 5) and therefore significantly different responses were

232 observed in terms of both canopy development and subsequent yield.

233

234 3.3.1 Canopy Development & Yield Responses

235 In 2016, the maximum canopy size, estimated by the log-logistic model, was found to differ

236 significantly between the seed rates and varieties (Table 3a) but there was no interaction

237 between seed rate and variety. The lowest seed rate produced an estimated final mean

238 canopy cover of 78.6%, the intermediate seed rate produced a mean canopy cover of 89.5%

239 and the highest seed rate produced the largest mean canopy cover of 93.8% (P<0.001). The

240 varieties produced distinctly different maximum canopy covers, with the resistant variety

241 producing the lowest level of 83.8% followed by the susceptible variety at 87.6%, then the

242 tolerant variety at 88.5% and finally the greatest mean cover was achieved by the light tolerant

243 variety at 89.3% (P=0.031).

244 In 2017, with much warmer conditions during canopy expansion, the canopy model predicted

245 that all treatments would reach or exceed 99% canopy closure (Table 3b). There was a

246 significant response to seed rate only, with the lowest seed rate predicted to produce the

247 largest canopy. However, the averages for all seed rates shows all would meet or exceed

248 100% canopy cover.

249 In 2016, (Fig. 6a) a positive yield response to increasing PPD was found. Three distinct

250 responses are shown; the tolerant and resistant varieties showed the same response to

251 increasing the population, although their intercepts differed, reflecting the different yield

252 potential of these varieties. The susceptible variety had the shallowest slope, indicating that it

253 would benefit least from increasing PPD and the light tolerant variety had the steepest slope

254 as it responded greatest to increasing PPD (P=0.046).

255 In 2017, (Fig. 6b) the opposite response to increased PPD was found. The yield responses of

256 all varieties show a negative relationship to increased PPDs. The varieties all responded

257 similarly (equal slopes) although there were different yield potentials indicated by their

258 significantly different intercepts. The tolerant and resistant varieties had greater yield potential

259 across all populations in 2017 (P<0.001) than the light tolerant and susceptible varieties.

260 Using the inflection point (IP) as a measure of the vigour of the canopy, a significant negative

261 relationship was found in 2016 between IP and sugar yield (P=0.013) in response to the

262 increased PPD (Fig 6c). Similarly to the response to PPD, three distinctly different responses

263 in the relationship between IP & subsequent yield were found. The susceptible variety had the

264 shallowest response to the change in IP (by increasing PPD) and yield, whilst the resistant

265 and tolerant variety show a more negative response and the light tolerant variety has the most

266 negative response. The tolerant, light tolerant and susceptible varieties had more vigorous

267 canopy expansion at all seed rates than the resistant variety.

268 In 2017, the opposite response to PPD and variety was found (Fig 6d). Variety response can

269 be seen to be the same as 2016, with delayed canopy development exhibited by the resistant

270 variety. However, this resulted in a yield benefit rather than penalty. Parallel responses again

271 can be seen and all varieties, excluding the tolerant variety, had similar intercepts (P<0.001).

272 3.3.2 Biomass partitioning

273 In 2016, the higher seed rates produced less storage root biomass in relation to the canopy

274 biomass across all varieties (P=0.028, data not shown). In 2017, root biomass production was

275 not found to differ across the treatments. However, there are trends, although not significant,

276 relating to the amount of canopy biomass produced (data not shown). The higher seed rates

277 produced higher levels of canopy biomass (P=0.052) and the resistant variety consistently

278 produced less canopy biomass at all PPDs than the other varieties (P=0.076).

279

280 3.3.3 Root sucrose concentration and impurities

281 Sucrose levels in the roots show similar patterns in both years. In 2016 the light tolerant variety

282 showed a significantly lower level of sucrose than the other varieties (P<0.001). In 2017, the

283 susceptible and tolerant varieties had significantly higher sucrose concentrations than the light

284 tolerant and resistant varieties (P<0.001) (Fig 7a). In both years the resistant variety had

285 higher sodium impurity levels than the susceptible variety. The light tolerant variety had the

286 highest levels in 2016 (P=0.001) and the resistant variety the highest in 2017 (P≤0.01) (Fig.

287 7b). Amino nitrogen and potassium impurity levels were not significantly different in either year

288 between the varieties (data not shown).

289

290

291 **4. DISCUSSION**

292 The results from these experiments have gained an insight into how BCN tolerant, resistant

293 and susceptible varieties grow and develop their roots and canopies. As all experiments were

294 conducted under BCN free conditions further work is required to understand if these varieties

295 respond differently under infestation.

296

297 4.1 Canopy:

298 Interception of light is directly related to yield of sugar beet (Jaggard and Qi, 2006). In the pot

299 experiment we found that the resistant variety had similar photosynthetic rates to other

300 varieties and therefore it seems likely that its reduced yield was due to the smaller canopy,

301 and thereby reduced light interception, and the overall smaller size of the plants produced

302 (since the root:shoot ratio was not different to most of the varieties). Less vigorous canopy

303 development by the BCN resistant variety was also identified in both years of field

304 experiments, indicated by their delay in reaching the IP.

305 Our results indicate that introducing tolerance traits to sugar beet has not led to significant

306 reduction in canopy vigour, whereas the same cannot be said for the resistant varieties tested.

307 The lower vigour and overall canopy size per plant seen in the pot experiment was also

308 observed in the field. This reduced vigour might be a result of the breeding process to

309 introgress the HS1^{pro-1} gene from *P. procumbens*, to introduce resistance to BCN, and

310 associated linkage drag of undesired genes (Flint-Garcia, 2013). Alternatively, the breeding

311 lines used in the 1990s when these varieties were developed (Müller, 1999) may have had

312 less vigorous canopies than today's elite cultivars.

313 Our investigations have revealed differences in the way that modern BCN resistant varieties

314 develop their canopies compared to susceptible and tolerant cultivars. Understanding the

315 lower level of canopy vigour and growth may assist these varieties in being approved for

316 cultivation in the UK. The results of these field experiments will prove useful in this case, as

317 they consistently show that BCN resistant varieties have a delayed time to reach their IP and

318 that increasing the seed rate can accelerate the rate of canopy development. Sowing at a

319 higher seed rate would incur extra seed costs so would require consideration as to whether

320 the additional return would be worth the extra investment in seed.

321 In 2016, when canopy expansion was limited due to the excessive wet weather, the trend was

322 for greater yield under higher PPDs, likely due to the larger canopy development which

323 occurred before the wet weather caused canopy expansion to stop. The opposite was the

324 case when weather conditions led to rapid canopy closure in 2017. More data is required to

325 confirm that the yield penalty when growing a BCN resistant variety compared to a tolerant or

326 susceptible variety can be overcome with a greater PPD. The opposing yield responses in the

327 two years of field experiments require further experiments with more BCN resistant varieties,

328 seed rates and replicated over more sites and seasons to test the generality of this response.

329 The canopy biomass response seen in 2017 is likely a result of shade avoidance (Ballaré and

330 Pierik, 2017). At the higher PPDs plants were shaded by each other and had to compete with

331 each other for light. Excessive canopy growth resulted and extra resources were invested into

332 the canopy and not used for root growth and yield. In this case, the resistant variety, with its

333 less vigorous canopy, seems to have benefitted and yielded better than expected. As the

334 canopy was smaller there was less competition for light and each plant could dedicate more

335 resources to developing roots, and therefore more yield, rather than having to grow

336   excessively large canopies in an attempt to outcompete neighbouring plants. In years less

337   favourable to canopy expansion than 2017, sowing the resistant variety at a higher rate may

338   be justified in order to ensure better yields and overcome the less vigorous canopy trait.

339   4.2 Rooting:

340   In oilseed rape (*Brassica napus*), positive relationships have been reported between primary

341   root length in hydroponic pouches and field emergence and yield, as well as between lateral

342   root density in hydroponics and the in-leaf concentration of calcium and zinc in field (Thomas

343   et al., 2016). Furthermore, Bussell et al. (2016) found a relationship between the number of

344   lateral roots produced in hydroponic pouches and nitrogen uptake of sugar beet grown in pots.

345   The lack of significant variation in rooting between tolerant, susceptible and resistant varieties

346   in our hydroponic experiment highlights the possibility that if rooting traits differ between

347   susceptible and tolerant varieties they may require infestation with *H. schachtii* to become

348   apparent. It was hypothesised that tolerant varieties would root deeper or more rapidly than a

349   susceptible variety as a method to grow into areas of soil which are not so heavily infested

350   with *H. schachtii*. This was not evident in the hydroponic system and an alternative method of

351   screening for early rooting differences may be needed, as introducing the nematode would

352   require the plants to be grown in soil rather than hydroponics. However, the light tolerant

353   varieties did show enhanced root growth in the pouches compared to the other varieties

354   tested. It is possible that the enhanced root growth observed in the light tolerant varieties may

355   be the mechanism which allows these varieties to outyield susceptible sugar beet varieties at

356   low BCN population. Growing the variety in infested conditions would be needed to test this

357   hypothesis.

358

359   4.3 Impurities:

360   The impurity levels found in the pot experiment and two years of field experiments show that

361   the resistant varieties always had significantly greater levels of sodium than the susceptible

362   control varieties. Sugar beet is known to be able to use sodium as a replacement osmoticum

363   in cases of insufficient potassium availability (Subbarao et al., 2003) and the elevated levels

14

364 may be due to the varieties being more closely related to wild *P. procumbens* and therefore

365 have different osmotic requirements than the elite susceptible varieties usually grown in the

366 UK. However, the enhanced sodium levels seen in the resistant varieties might also be due to

367 the resistance mechanism to BCN. The higher concentration of sodium may make conditions

368 less favourable to the nematode when they establish their feeding cell (syncytium) (Kyndt et

369 al., 2013) in the roots of the sugar beet and assist with the destruction of the syncytium via the

370 hypersensitive response (Heijbroek et al., 1983; Huang, 1998).

371 The associated sugar losses due to raised impurity levels must still be considered (Dutton and

372 Huijbregts, 2006). BCN resistant varieties would be less favourable to sugar processors due

373 to this. Although, if it could be demonstrated that resistance and impurity levels are not

374 associated, breeding could be expected to develop BCN resistant varieties with reduced

375 impurity levels in the future.

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380 **5. CONCLUSIONS**

381 Tolerant varieties appear to have equally vigorous canopy expansion patterns compared to

382 susceptible varieties. This finding is supported by yield results from sugar beet variety trials

383 (BBRO, 2017). However, no obvious physiological differences have been measured during

384 this investigation to highlight BCN tolerance mechanisms, and they may only reveal

385 themselves in BCN infested conditions. The importance of quantifying canopy development

386 has been shown and the techniques used during this research may be used in other aspects

387 of sugar beet variety breeding.

388 Most importantly, this study has revealed more about the agronomy of BCN resistant varieties.

389 It has demonstrated that they have a less vigorous canopy which can be overcome and

390 manipulated by adjusting the plant population density. More experiments on more sites and

391 over a range of seasons would reveal if an increased seed rate could be justified and calculate

15

392   exactly how much higher a seed rate BCN resistant variety needs to be sown at to

393   compensate for the delayed canopy expansion. This is vital to assist such varieties becoming

394   listed for use in new markets, such as the UK, and allow growers to benefit from their active

395   control mechanisms for BCN and maintain and enhance yields on BCN infested fields regularly

396   cultivated with sugar beet.

397

398   **6. ACKNOWLEDGEMENTS**

408

409   **7. REFERENCES:**

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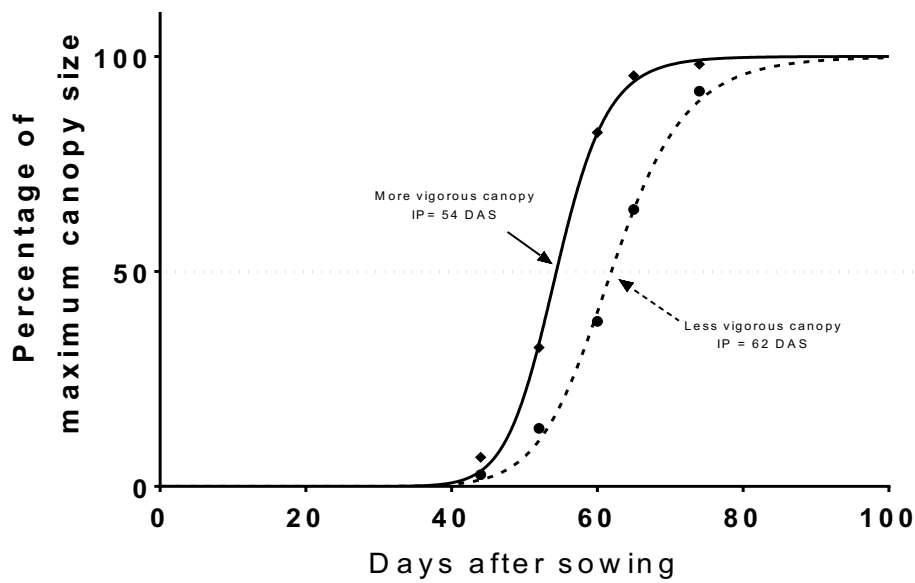
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Figure 1 - Two contrasting canopy development curves as measured in the field trial at Sutton Bonington in 2017. Data from images taken in the field were used to calculate the canopy cover. These data were then inputted into a model to calculate the 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).
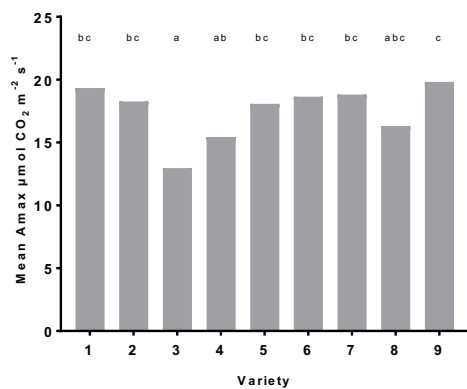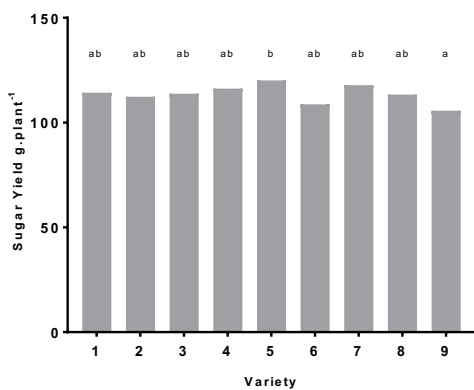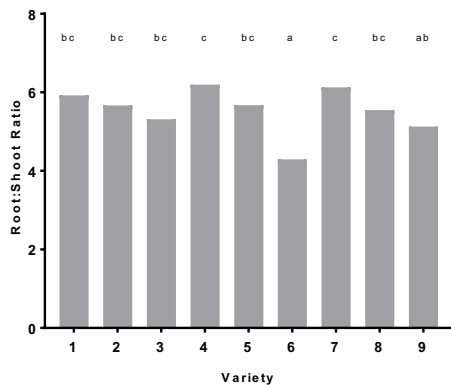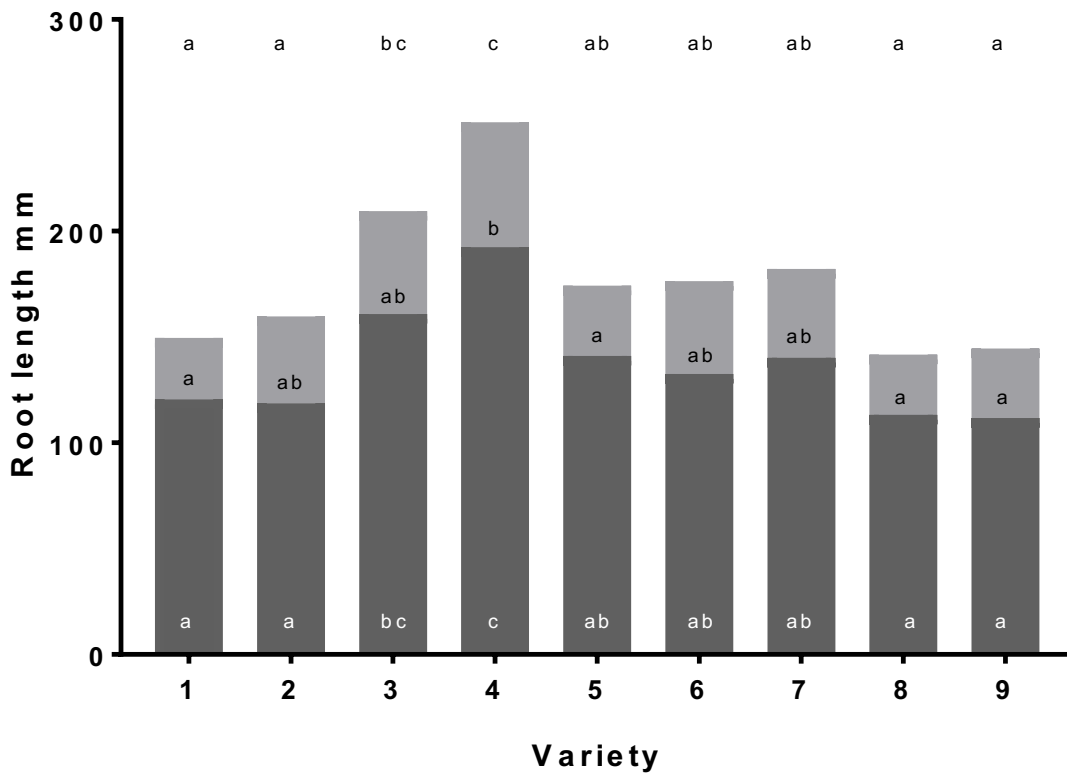
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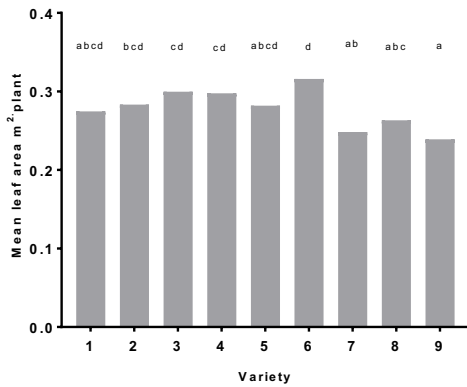
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Figure 2- Results from the pot experiment conducted in 2015 to compare differences between a range of sugar beet varieties which vary in their susceptibility to *H. schachtii*. A shows the mean leaf area per plant (m^2) at harvest (P<0.001), B displays the Mean CO_2 assimilation (μmol $CO_2 m^{-2} s^{-1}$) measured between 57 and 122 days after sowing (*P*<0.001) from the different varieties. C shows the mean mass of sugar (g of sucrose per plant) produced by each plant at harvest (*P*= 0.022) D shows the mean ratio of root to shoot biomass produced by each variety (P<0.001). All plants were grown in the absence of *H. schachtii.* Different lower case letters represent significantly different results at P=0.05.

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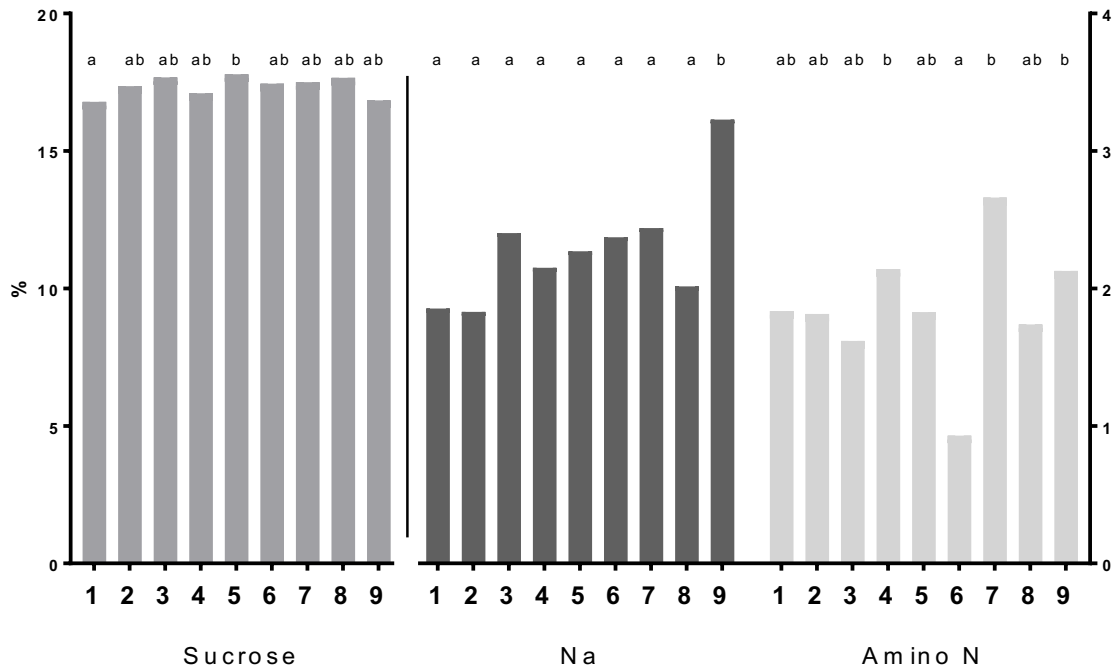
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Figure 3- The nine varieties of sugar beet, which differ in their susceptibility to *H. schachtii*, were grown in a pot experiment at Sutton Bonington. After the roots were harvested a brei paste sample was made. The brei sample was measured for sucrose 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

522

300

Root length mm

200

100

0

a · a · bc · c · ab · ab · ab · a · a

b

ab

a · ab · a · a · a

ab

a · bc · c · ab · ab · ab · a · a

1   2   3   4   5   6   7   8   9

Variety

523
524

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
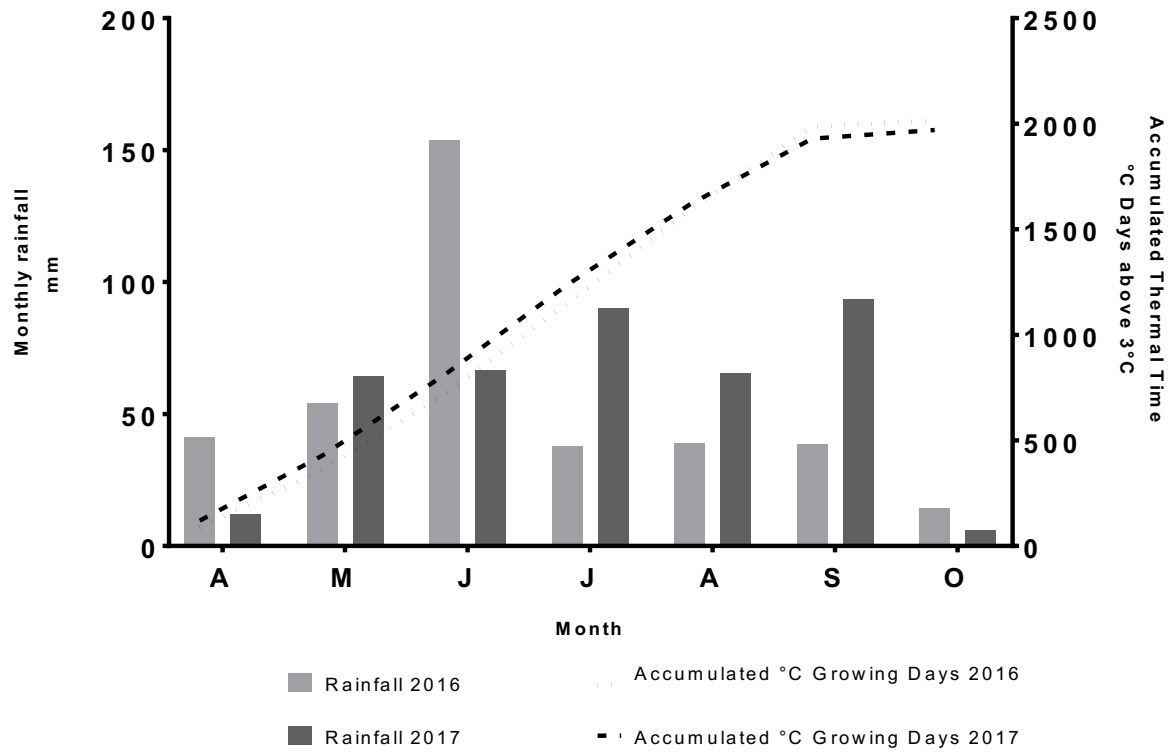
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Figure 5 – Temperature and rainfall were measured throughout the experiment using the meteorological station at Sutton Bonington during the field experiments investigating the response of four sugar beet varieties, which varied in their susceptibility 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
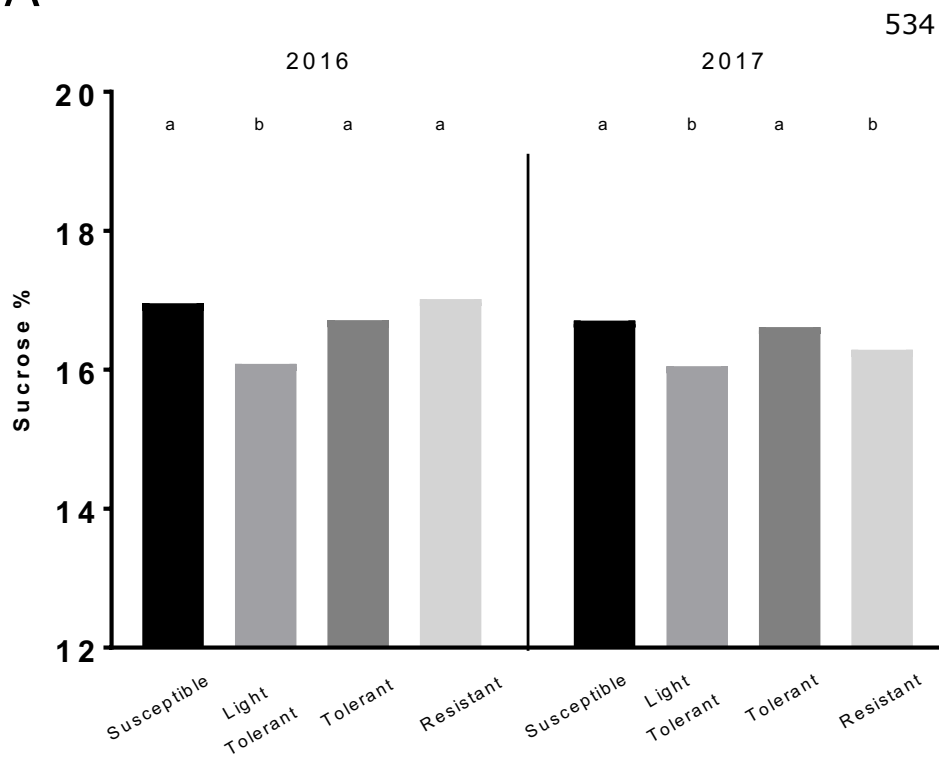
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Figure 6 PRINT IN COLOUR
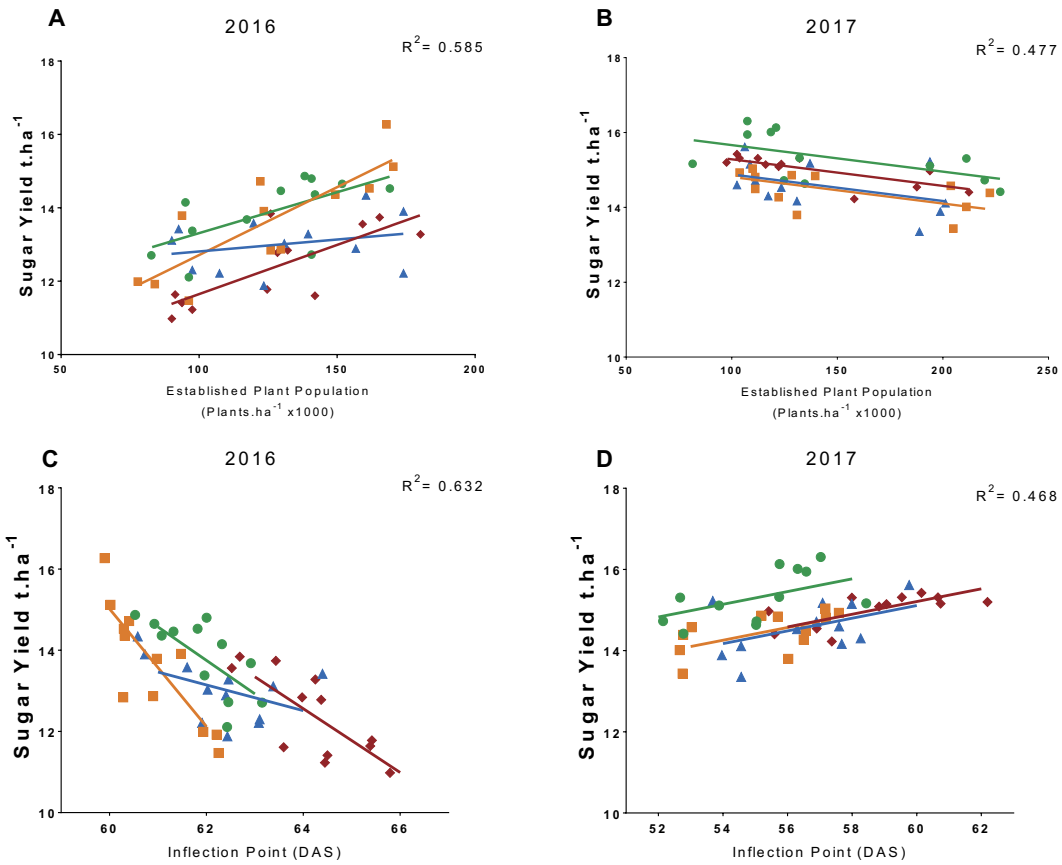
Figure 6 – Data showing the regression relationships between the established plant populations (A - 2016 & B - 2017) upon sugar yield and the response of the increasing inflection point (IP) of each variety (C -2016 & D - 2017) from the field experiments at Sutton Bonington. Resistant ▬ Susceptible ▬ Light tolerant ▬ Tolerant ▬.

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A

B
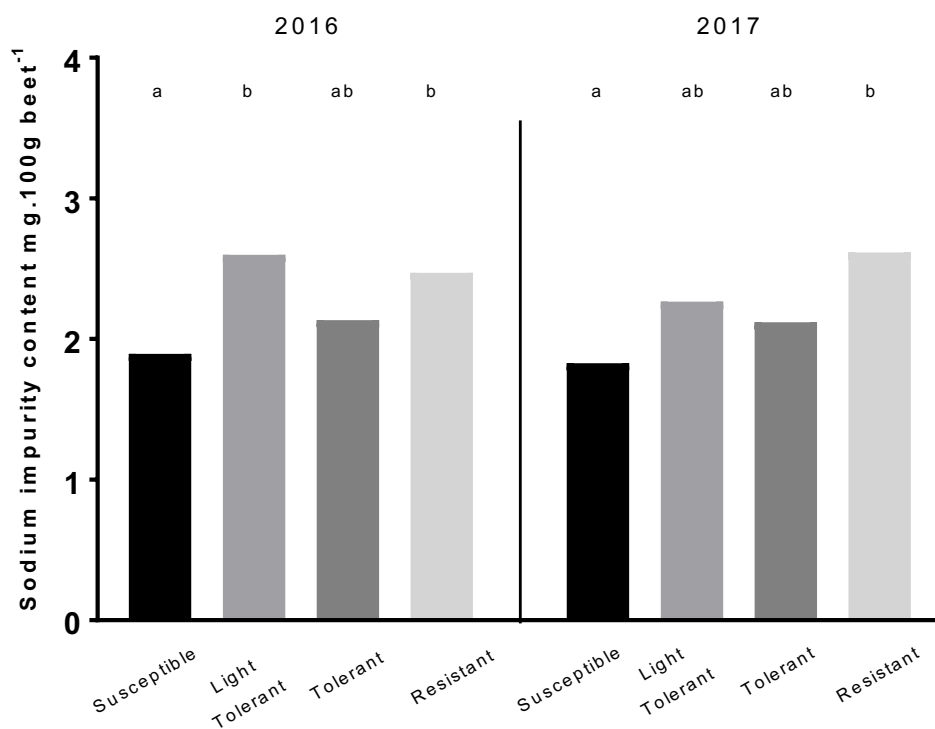
Figure 7 – Mean levels of Sucrose in the storage root (A) (P<0.001 for both years) and Sodium impurities (B) (P=0.006 in 2016, P=0.014 in 2017) in the sugar beet measured in the samples at harvest from the field trials at Sutton Bonington Significant differences were only detected between the varieties 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

535   Table 1
536

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)

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538

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

539   Table 2
540

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

29

541 Table 3 – Mean maximum canopy values estimated using a three-parameter log-logistic
542 model. In 2016 significant differences were found between the varieties and seed rates,
543 but no interaction between the two. In 2017 there were only significant differences found
544 between the seed rate. Different lower case letters adjacent to the means signify a
545 significant difference at P=0.05.
546
547   A- 2016
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		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	
	153000	89.9	90.2	90.3	87.7	89.6 b	<0.001
	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				

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553   B- 2017
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		Variety					
		Susceptible	Light Tolerant	Tolerant	Resistant	Mean	P
Seed Rate (Seeds ha-1)	119000	104.4	102.6	101.5	104.3	103.2 b	
	153000	100.7	100.3	100.5	101.4	100.7 a	0.001
	211000	101.1	100.1	99.7	99.4	100.1 a	

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