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

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ABSTRACT

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

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Pot experiment

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

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

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

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

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

2.2. Hydroponic tank experiment

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

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