



**Harper Adams
University**

**A Thesis Submitted for the Degree of Doctor of Philosophy at Harper Adams
University**

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HARPER ADAMS UNIVERSITY

**The role of agronomy and variety on tissue integrity and damage of sugar
beet (*Beta vulgaris*) during production processes.**

**A thesis submitted in partial fulfilment of the requirements of Harper Adams
University for the degree of Doctor of Philosophy**

By

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Declaration

I, **Paul Zauzau Chunga** declare that this thesis is a result of my own original effort and work. Where assistance was sought, it has been duly acknowledged/cited and referenced. None of this work has been presented/accepted for the award of any other degree or diploma at any University.



Signature

20/11/2023

Date

Abstract

Sugar beet (*Beta vulgaris*) is the second most important sugar crop in the world after sugar cane. The crop supplies about 55 % of the sugar consumed in the UK. Currently, sugar losses in storage are circa 0.1-3 % per volume per day. This substantial loss is attributed to leaching and respiration which are promoted by easily damaged weak tissues. Currently, there is little information on how variety, harvesting conditions and nutrition affect beet damage in the UK. This study hypothesised that variety, growing environment and nutrition influence physiological and cellular factors that contribute towards sugar beet root tissue strength. Specifically, the study aimed to 1) identify sugar beet varieties for tissue damage susceptibility and resilience, 2) identify morphological and tissue basis resilience to root breakage, 3) study the effect of environmental factors on tissue resilience and 4) study the effect of nutrient status during growth on tissue resilience and root impurities. Field experiments were planted at Bracebridge, Lincolnshire and Fotheringhay, Northamptonshire, in 2019, 2020 and 2021 seasons in randomised complete block designs. In 2020, 2021 and 2022, a susceptible variety was planted in the field and polytunnel to assess the effect of moisture and temperature, while in 2021 and 2022, two susceptible varieties were planted in the field to assess the effect of Ca and B. Results show that varieties significantly differ in root tip diameter after damage ($p < 0.05$), width ($p < 0.001$), length ($p < 0.001$), puncture resistance ($p < 0.001$) and compression resistance ($p < 0.001$). Root tip diameter after damage correlated with tissue compression, root width, length, and root weight while surface damage is linked to puncture resistance and moisture content prior to harvesting. Maintaining high water status for seven consecutive weeks prior to harvesting accelerated tissue frailness but also increased root tissue and surface damage while harvesting temperature had no effect. Ca application reduced surface damage while B increased resistance to compression. Impurities were not affected by Ca, B and variety but by season. Our results suggest that variety choice, harvesting time and plant nutrition are key in minimising root tissue damage.

Published work

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Dedication

I dedicate this to my late mum Elube Justina Thambeya Ngwira. A teacher by profession who strongly valued education and taught many people (me included) at Chasenje Primary School in Mzimba district of Malawi-Africa. Popularly known as **“a madam a nyaNgwira”**, you were such a charming mum with a great personality. You taught me the principles of life and I still remember how you hustled as a single parent to raise me and my sister. I wish you had a chance to read this thesis, but God had his plan. May you continue resting in peace a madam a nyaNgwira.

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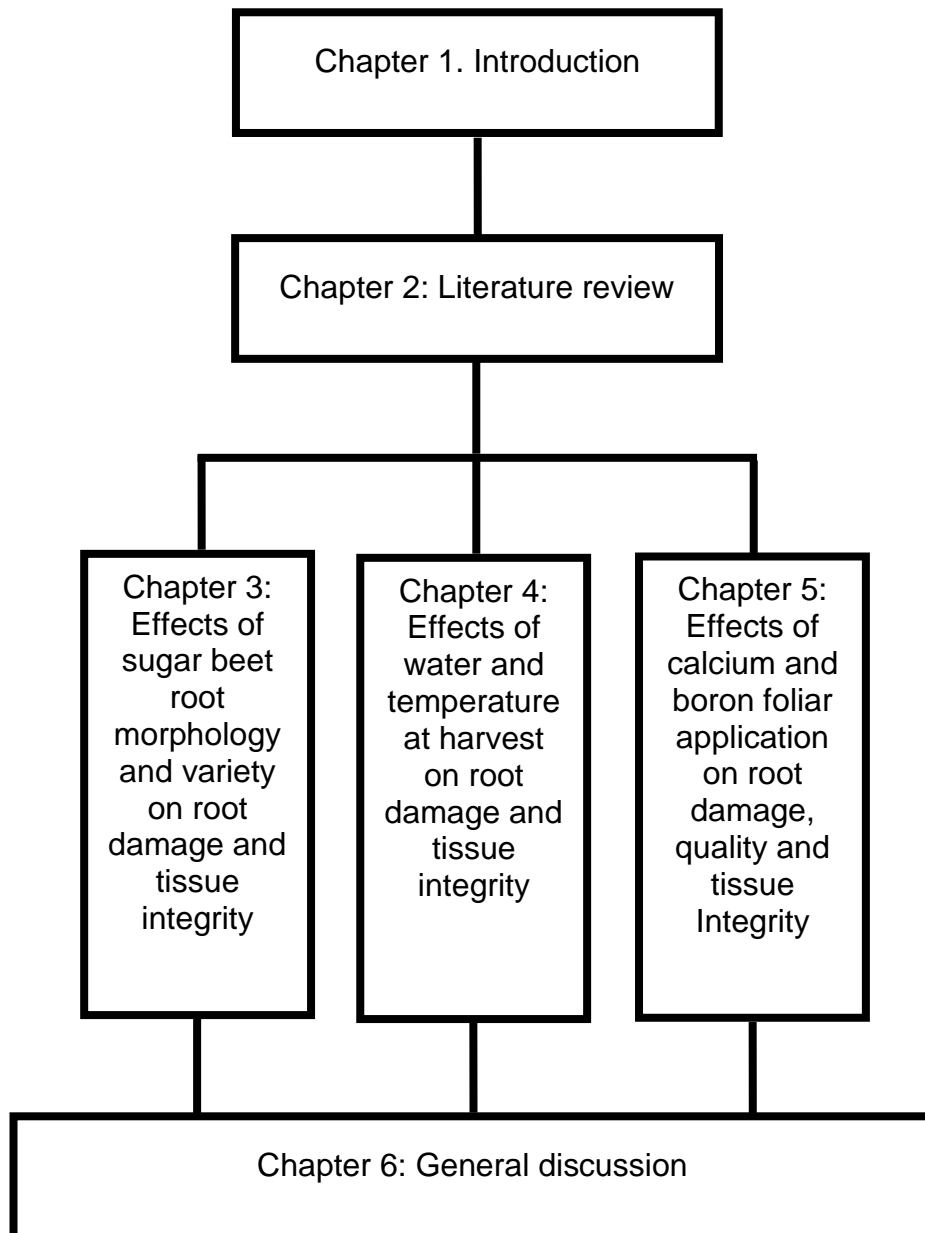
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List of abbreviations

B	Boron
BBRO	British Beet Research Organisation
BSPB	British Society of Plant Breeders
Ca	Calcium
CEC	Cation exchange capacity
CFIA	Canadian Food Inspection Agency
Cu	Copper
DEFRA	Department for Environment, Food and Rural Affairs
FAO	Food Agricultural Organisation
Fe	Iron
GDD	Growing degree days
HAU	Harper Adams University
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
OECD	Organisation for Economic Co-operation and Development
P	Phosphorus
pH	Power of hydrogen
RLD	Root Length Density
RWC	Relative water content
SPAD	Soil Plant Analysis Development
S	Sulphur
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
Zn	Zinc

Thesis map



Chapter 1: Introduction

1.1 World sugar beet production

Sugar beet (*Beta vulgaris* L.) is the world's second most important sugar crop after sugar cane and provides 30 % of world sugar (OECD-FAO, 2018; Ahmad et al., 2017; Iqbal and Saleem, 2015; Nedomova et al., 2017). It is grown in many areas of the world but tends to thrive in the temperate climates found in large parts of Europe, North America, Asia and some parts of northern Africa (**Figure 1-1**) (Draycott, 2006; Fitters et al., 2017). The world's total sugar beet production was estimated at 270 million tonnes in 2021 with the Russian Federation, USA, Germany, France, and Turkey (**Figure 1-2**) as the world's top producers accounting for 52% of total production (FAOSTAT, 2022).

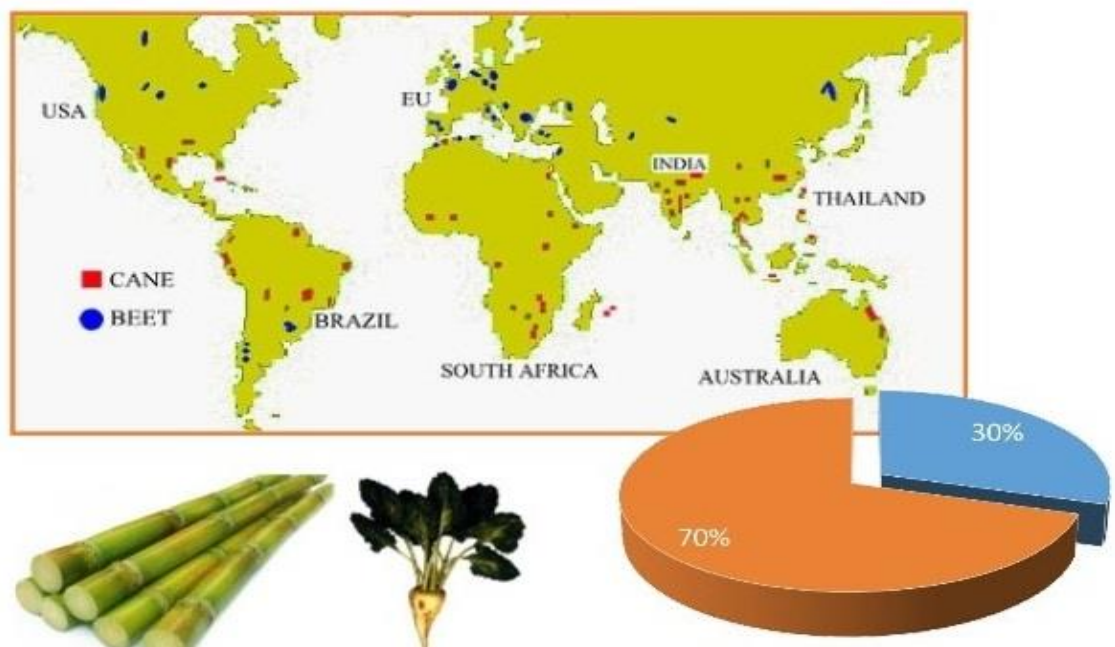


Figure 1-1: World sugar beet and cane producing countries (Askaravut, 2013)

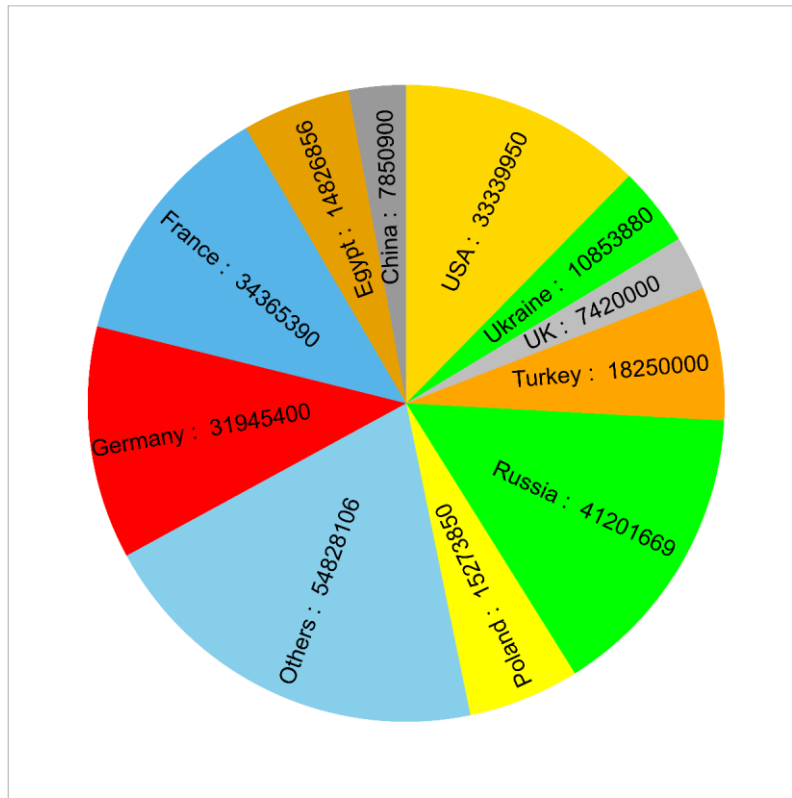


Figure 1-2: World sugar beet production in tonnes during 2021 season (FAOSTAT, 2022).

1.2 United Kingdom's sugar beet industry

Britain's first sugar beet crop was grown and processed in Norfolk over 100 years ago (British Sugar, 2019). It is estimated that the crop provides 55 % of the sugar consumed in the UK and involves about 3,500 growers on approximately 100,000 hectares of land (British Sugar, 2019; Okom et al., 2017). The industry supports approximately 9,500 jobs in the wider economy (British Sugar, 2019) with a farm gate value of £246 million (British Sugar, 2019; DEFRA, 2018; Tzilivakis et al., 2005). Richter et al., (2006) reported that in England and Wales, about 1.7 million hectares of arable land are suitable for sugar beet cultivation but only a small proportion (10%) is usually utilised for sugar beet production. The UK's sugar beet production is concentrated within a distance of 30 miles from the four British Sugar factories (Newark, Wissington, Bury St Edmunds and Cantley) located in the east of England (Fitters et al., 2017). During the 2021 crop growing season, UK was globally ranked 10th with a total production of 7.4 million tonnes (representing 3 % of global production) from 95200 hectares (FAOSTAT, 2022).

1.3 Root damage concerns and sugar losses

Despite its economic significance, reports indicate that damage which is usually perpetuated by post-harvest handling problems leads to substantial losses of sugar, mostly during storage. Jaggard et al., (Jaggard et al., 1997) observed that sugar loss on farmers' fields across England was circa 0.14% per day mostly in clamps with damaged roots. High field root yield losses of about 9.1 t ha⁻¹ and a total of 2-3% sugar losses per clamp were observed in storage (Hanse et al., 2018; Van Swaij et al., 2003). However, recent figures by indicate that average sugar losses from harvested sugar beet roots during storage are reported to be approximately 0.1% of total sugar volume per day. An earlier monetary quantification by Brown et al., (2002) indicated that UK sugar beet growers lose about £12 million per year due to harvesting and storage losses. Sugar losses in storage are heavily linked to increased levels of respiration which are accelerated by root damage on tips and surface (Fugate et al., 2010; Kenter et al., 2006a).

The aim of this research was to

- a) identify agronomic practices that may lead to more robust sugar beet roots
- b) identify physiological traits that may be selected for in breeding programmes to develop more robust sugar beet varieties
- c) to identify best environmental conditions that would help to enhance root robustness during post-harvest operations.

Chapter 2: Literature review

2.1 The sugar beet crop

2.1.2 Origin and Taxonomy

Sugar beet originated in the middle east, near the Tigris and Euphrates rivers where it is believed that wild beets spread west into the Mediterranean and north along the Atlantic Sea coast where it was widely used for various culinary purposes (CFIA, 2020). It was not until the 17th century, however, that beet was first grown on a field scale (**Figure 2-1**), and only then as fodder for cattle and sucrose was obtained in 1747 in German from beet by the chemist Andreas Margraff, and the first sugar beet factory was built in Silesia in 1802 (Berlowska et al., 2018; Hoffmann et al., 2020). The crop is also believed to have been a common element of the Egyptian diet during the building of the pyramids (USDA, 1998). Many of the names for beet in different ancient languages (selg in Arabic and silg in Nabataean) are apparently derived from the Greek word sicula, which means beet from Sicily. The crop was known as silga in 800 BC and in the second century, beet was commonly mentioned in the literature by Roman writers as beta (Winner, 1993).



Figure 2-1: A sugar beet crop at vegetative stage (Bayer, 2018)

The crop belongs to domain: Eukaryota, kingdom: plantae, phylum: Spermatophyta, subphylum: Angiospermae, class: Dicotyledonae, order: Caryophyllales, family: Chenopodiaceae, genus: Beta. The genus beta is divided into four species; vulgaris, corollinae, patellares and nanae (De Bock, 1986). Cultivated sugar beet originate from wild vulgaris section known as maritime beet (*B. vulgaris subsp. maritima*) through breeding selection (Winner, 1993).

2.1.3 Sugar beet uses.

Sugar beet is mainly utilized as a source of sugar mostly in the temperate region. The sugar beet root contains a high level of sucrose, which varies between 12% and 21% on a fresh weight basis, depending on cultivar and growing conditions (Pan et al., 2015). It provides 30 % of the world's sugar mainly as white sugar (Wojtczak et al., 2014). The rest of the sugar consumed in the world is made from sugar cane which is mainly grown in tropical countries (OECD-FAO, 2018). Apart from acting as a sugar source, the crop is economically important as its products such as pulp, molasses and leaves can also be utilised. For example, leaves are a natural fertiliser for the following crop, the sugar extracted is an ingredient for many industries, such as food, pharmaceutical, and cosmetic, the pulp is utilised for animal feed, molasses are a key ingredient in the production of alcohol and, lastly, carbonation sludge provides nutrients for agricultural soils (**Figure 2-2**).

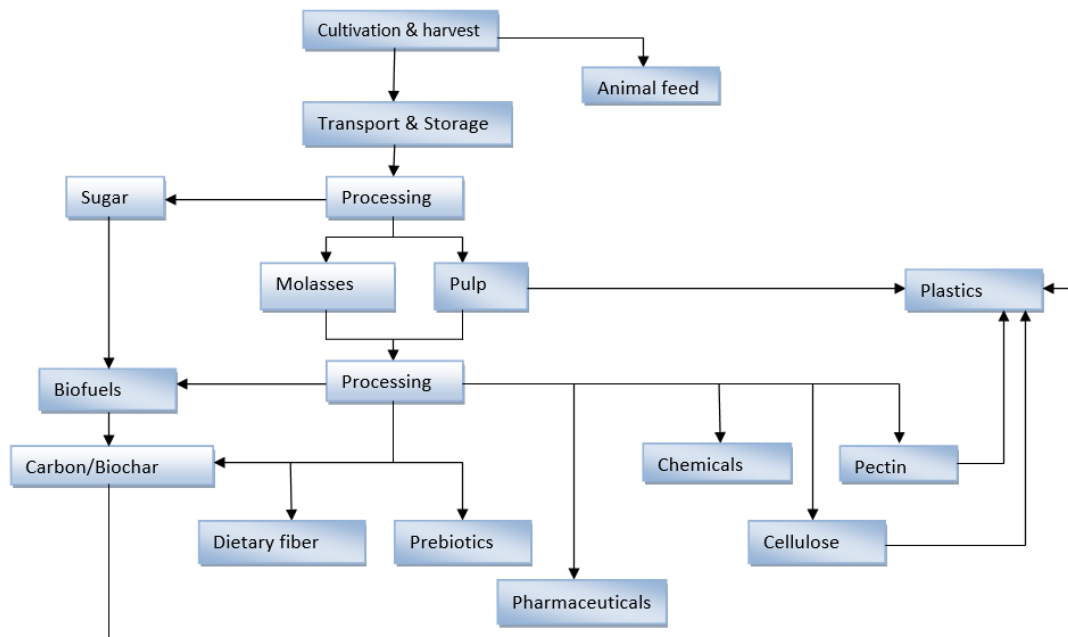


Figure 2-2: Schematic of complete utilisation of sugar beet into value-added products (Finkenstadt, 2014).

2.1.4 Genetics and breeding

The original seed of *Beta ssp.* is multi-germ, where one fruit is composed of up to five single seeds, each containing one germ and monogermity was discovered to be caused by one gene on linkage group 4 (Sadras and Calderini, 2020). The first monogerm beet plants were domesticated as diploids ($2n = 2x = 18$) but later, polyploids especially tetraploids ($2n = 4x = 36$) were produced which were then used to make triploids ($2n = 3x = 27$) (Draycott, 2006; Lange et al., 1999). In general, the introduction of monogerm varieties in the mid-1960s eliminated the labour requirement to single seedlings in the field and allowed a much higher efficiency in cultivation (Sadras and Calderini, 2020) but tetraploids were discovered to have significantly lower root and sugar yield compared to diploids. Hence, triploids were produced by cross-pollinating diploid and tetraploid seed-parents (USDA, 1998). Triploids varieties registered substantial root and sugar yield compared to diploids and tetraploids (Draycott, 2006). An earlier study showed that triploid beets exceeded the diploids in root weight by 12.2%, in yield of sugar per beet by 14.9%, in dry top weight by 17.8%, in area index of the leaves by 34.4% and in area index of the stomata by 42.6% (Peto and Boyes, 1940).

Breeding remains the driving force for yield improvement. The most common methods of selection used by UK sugar beet breeders include mass selection,

progeny selection and line breeding, recurrent selection and inbreeding (Winner, 1993). Currently, there are six companies involved in sugar beet breeding in the UK: Betaseed; KWS UK Ltd; Limagrain UK Ltd; Strube Sugar Beet UK Ltd; SESVanderHave UK Ltd and MariboHilleshog (MH). These companies use thousands of isolation tents to maintain genetic purity of individual parent lines where genotypes from new crosses are developed. They are later grown and tested in field trials and their agronomic performance is compared against existing commercial varieties for a period of not less than 10 years and only the promising ones are maintained and put on the Recommended List. Currently, the 2023 Recommended List has 22 varieties (BBRO, 2022; BBRO and BSPB, 2019).

The goal of breeding programmes is to develop sugar beet varieties with higher root yield, sugar content, better extraction yield, higher seed germination percentages and lower bolting tendency; as well as physical attributes of the root adapted to mechanical harvesting, low soil tare and higher resistance to diseases and pests (BSPB, 2014; Guss, 2006). Breeding programs rely on the genetic diversity existing among wild relatives which act as a gene reservoir. Hybridisation with these wild relatives has approximately contributed to half of the tremendous root and sugar yield increase (Hoffmann and Loel, 2015). Compared to mid-sixties, currently recommended varieties in UK and other European countries have registered relatively higher sugar content, low impurities (Amino N, Na and K), increased tolerance against various pests and diseases, high seed quality and high bolting resistance. Since the introduction of hybrid sugar beet, yield has increased by approximately 1.5 % per year with sugar content increasing from 6 to 16 % and root yield increased from 4.5 to 90 t ha⁻¹ (Sadras and Calderini, 2020).

2.2 Physiology of sugar beet

2.2.1 General morphology

Sugar beet is a biennial plant with an epigeal germination which leads to the development of a rosette of glabrous, dark green, glossy leaves with prominent midribs and strong petioles (Elliott and Weston, 1993). The crop grows up to 120 cm in height and consists of three main parts (**Figure 2-3**) namely crown, neck and cone-shaped root where the crown produces leaves (Guss, 2006; Schulze-Lammers et al., 2015). The root is stout, sometimes conspicuously swollen forming a beet together with the hypocotyl, and sometimes forming a branched taproot.

Stems are decumbent, ascending, or erect and branched. Leaves are varied in size, shape and colour, often dark green or reddish and rather shiny, frequently forming a radicle rosette and flowers are hermaphrodite arranged in small cymes (Guss, 2006).

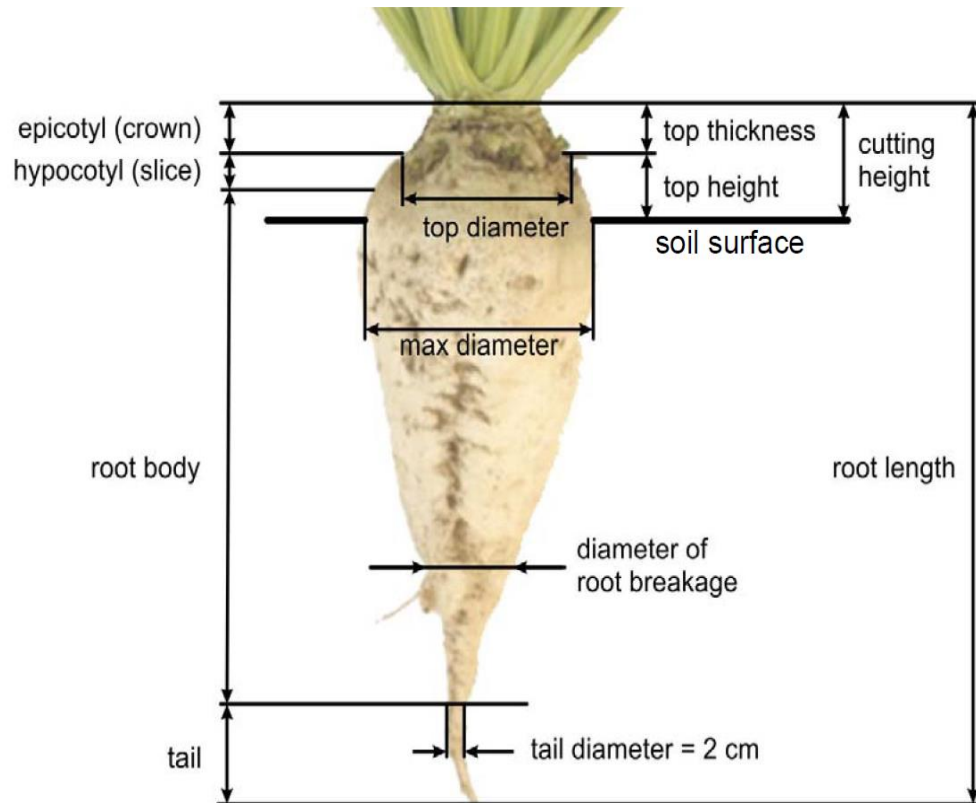


Figure 2-3: Morphology of a sugar beet root (Schulze-Lammers et al., 2015)

2.2.2 Root composition and growth

Sugar beet produces sucrose in the first year with the root acting as a sink (FAO, 2009). It has very high soluble sugar content, high pectin and hemicellulose carbohydrate contents, and relatively low lignin contents which vary regionally and seasonally as a function of many interacting factors including plant biology, location, agronomy, harvest, and post-harvest practices (**Figure 2-4**). On wet basis, the root consists of 75 % water, 2.5 % non-sugars, 17.5 % sugar and 5 % pulp (Bichsel, 1987; Zicari et al., 2019) while on a dry basis the root consists of 70 % sucrose and 30 % others (**Figure 2-5**) (Zicari et al., 2019). The roots are classified according to their size and sugar concentration. The first class is the Z type which are characterised by less parenchymatous zones, small size and high sugar concentration while the second type is the E type which have larger roots, extensive

parenchyma development and are generally characterized by low sugar concentration (Milford, 1973).

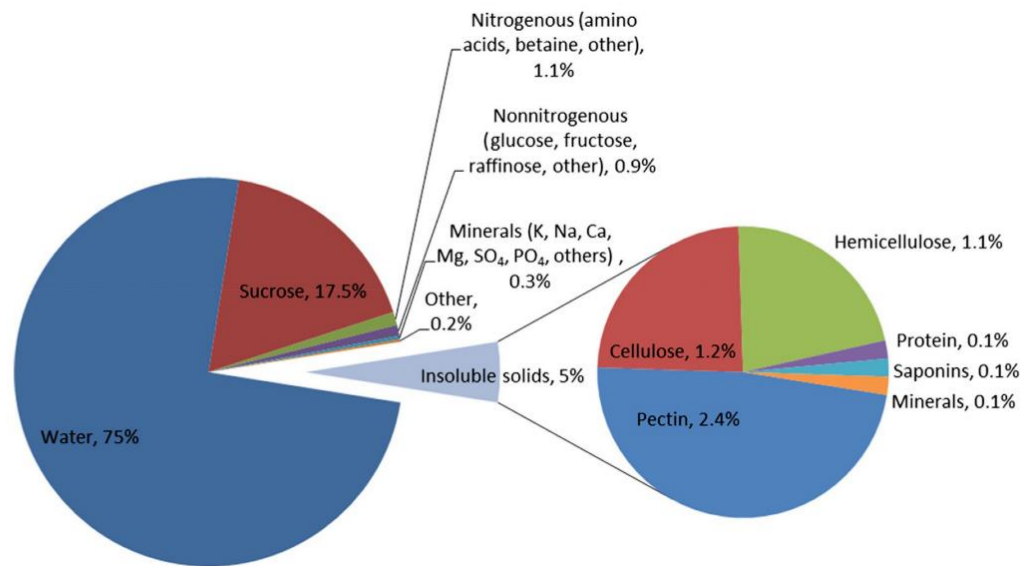


Figure 2-4: Sugar beet root composition on wet basis (Zicari et al., 2019).

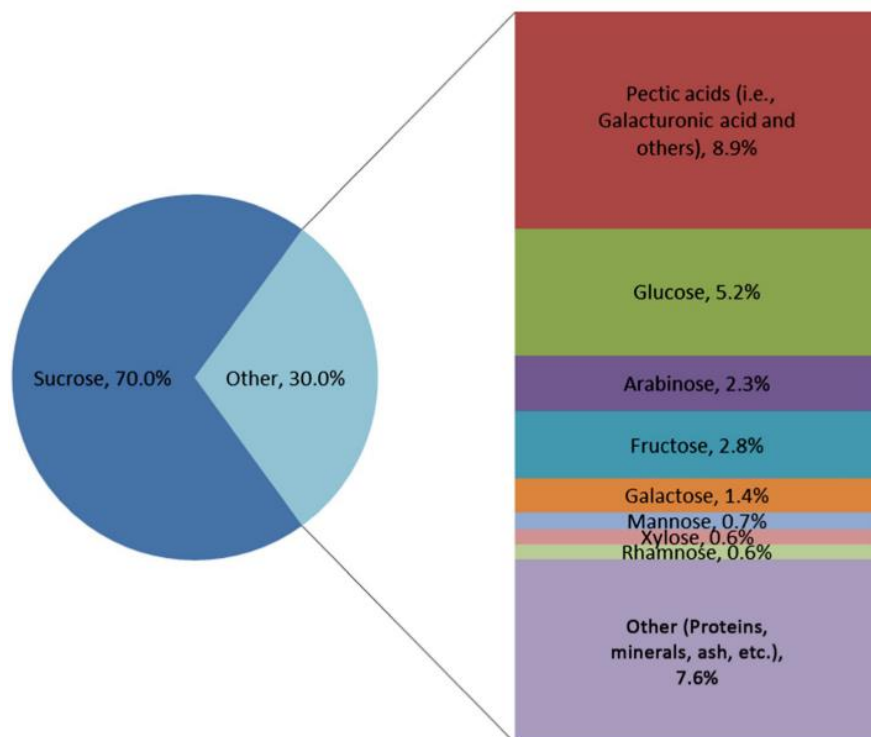


Figure 2-5: Sugar beet root composition on a dry basis (Zicari et al., 2019)

After establishment, sugar beet accumulates more leaf biomass and a small root up to the 8 - 10 leaf stage where roots and leaves grow simultaneously. In the later growth stages the root exceeds the weight of above-ground biomass (Elliott and

Weston, 1993; Milford, 1973). Sugar beet roots, have a capability to grow up to 1.5 m down the soil profile (**Figure 2-6**), if there are no soil restrictions, regardless of water availability (Fitters et al., 2017). Stevanato et al., (2010) evaluated 18 sugar beet varieties and reported that a high yielding variety (L18) was discovered to have the deepest root growth of 2.90 m compared to the lowest yielding variety which had a shorter root of 2.55 m. However, root related traits have been reported to be significantly different in various zones depending on the variety. Fitters et al., (2022), examined the root length density (RLD) trait for various sugar beet varieties and reported that RLD is sometimes dependent on the interaction between variety and depth where a variety known as Aurora had a very low RLD in the 30–60 cm section compared to Haydn and BTS 340 while Hornet and BTS340 showed an increase in RLD at 15–30 cm compared to 0 –15 cm.



Figure 2-6: Sugar beet roots growing deep into the soils under dry conditions

The centre of the beet root is occupied by a solid star-shaped body referred to as the central core which measures only a few millimetres across but occasionally it is much thicker and uniform throughout its entire length though, it may taper abruptly from the neck region downward (Artschwager, 1952). After the central core, there are cambium rings (**Figure 2-7**) that lie between primary xylem and phloem where cell division takes place during root growth (Green et al., 1986). According to Hoffmann et al., (2020), there is a temporal and functional connection between leaf formation and cambial ring development. Each leaf initiates the formation of at least

two cambium rings where the first cambium originates from procambium and interfascicular parenchyma, while a second cambium ring develops in the phloem parenchyma inside the endodermis (Zamski and Azenkot, 1981). There can be 12-15 cambium rings by maturity of which 4-5 develop during the first 8 weeks after germination (Bellin et al., 2007). This information was also reported by Doney et al., (1981) who after evaluating various sugar beet varieties discovered that the tap root differentiates into a complete set of cambial rings in the first 30-35 days of growth and also suggested that differences in root size are a result of genetic differences in cell volume or cell division rate, or both. The first two months of growth are particularly important in determining the final sugar beet yield. The first 6 cambium rings make up a large proportion of the storage root and contribute most to the final yield of the root, while rings 9 and above are highly concentrated in the peripheral and make almost no contribution to the expansion of the storage root (Elliott and Weston, 1993). The inner rings are mature at harvest time, equidistant and relatively broad; those near the periphery are narrow and close together and in a typical mature sugar beet root, the ratio of total radius of mature to immature rings is 10:1 (Artschwager, 1952). Hoffmann (2010), observed that the number of cambium rings is not significantly affected if stress such as drought is introduced six weeks after sowing. She attributed this to the fact that most of the rings will have already formed by this stage.

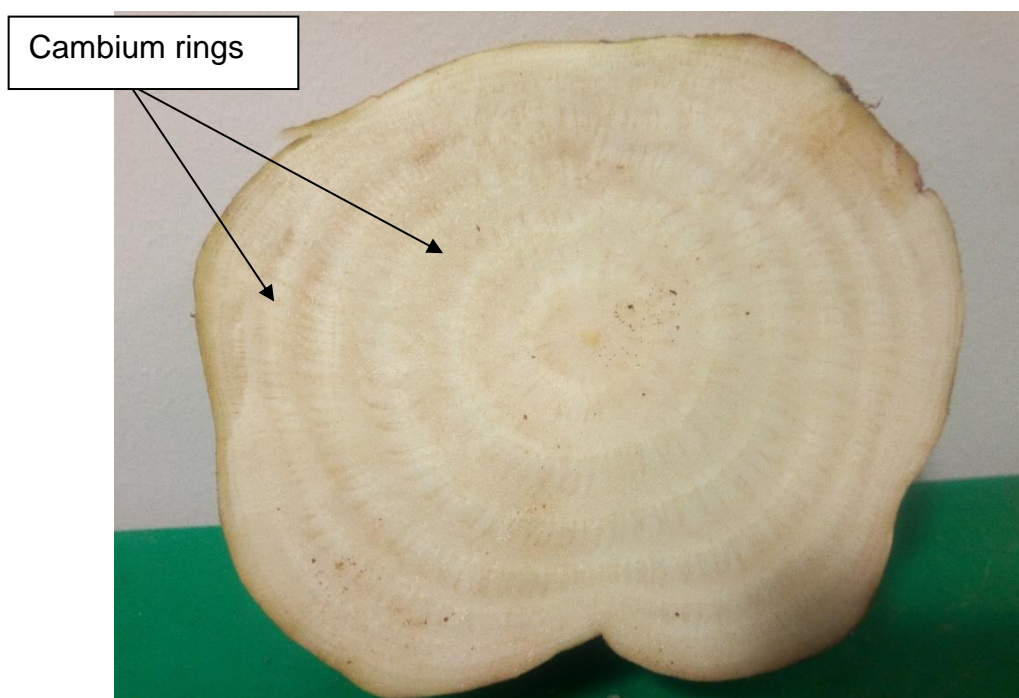


Figure 2-7: A cross-section of a sugar beet root showing cambium rings

2.2.3 Sucrose accumulation

Sucrose is formed through photosynthesis and forms approximately 98 % of the extracted sugars in harvested roots (Trebbe and McGrath, 2003). Photosynthesis is a process by which carbohydrate molecules are synthesised by turning sunlight, water and carbon dioxide into oxygen and energy, in the form of sugar (Nevins, 1995). During photosynthesis, starch is synthesised and stored in the chloroplast matrix and sucrose is synthesized in the leaf cytosol, and it is transported to sink tissues where it accumulates to high concentrations (Jung et al., 2015). It is loaded in the phloem by coupling to a proton transport mechanism driven by a vectorial plasmalemma ATPase (Giaquinta, 1983) where it is transported to the sink (root) and stored in the vacuoles of parenchyma cells (Giaquinta, 1983; Zamski and Azenkot, 1981). Trebbi et al., (2003) reported that sucrose is the main component (>98 %) of the extracted sugars from sugar beet, and only traces of glucose and fructose were detected. They further stated that sucrose content increased dramatically from less than 2 % to more than 10 % (fresh weight) between the 5th and 8th weeks and a further smaller increase was observed during the last two weeks when lines reached more than 12% of sucrose in fresh weight. They explained that differences in sucrose content between the lowest sucrose content variety USH20 and the highest sucrose content germplasm SR96 lines was statistically significant after the 6th week post emergence and no significant differences between entries was observed for sucrose content expressed on a dry weight basis, which increased from 5 to more than 55% during the tenth week.

A difference in sugar concentrations for various varieties was also reported by Doney et al., (1981) who microscopically measured cell size and cell number from stained root cross sections and reported an inverse relationship between sucrose percentage and cell size with high-sucrose varieties having small cells and low-sucrose varieties having large cells. Hoffmann (2010) reported that when the crop is water stressed there is a heavy reduction in sugar concentration in the roots during this stage highlighting that the initial 20 weeks are crucial for sugar accumulation in sugar beet roots. Similarly, Bellin et al., (2007) analysed field-grown sugar beet and reported that during early beet development sucrose concentration had already achieved 40 % of their final values. They further reported that sucrose levels rose from 10 to 17 % over the thermal time of 1300 - 1400 °Cd which is the recommended thermal time for sugar beet to reach physiological maturity

(Neamatollahi et al., 2012). These findings point to the fact that early days are important in sugar beet production as they determine the concentration of sucrose, hence any form of stress whether biotic or abiotic must be avoided.

2.3 Commercial beet production

2.3.1 Variety choice and seed treatment

Sugar beet is vulnerable to various diseases like damping off and black leg that are caused by soil-borne pathogens hence the need to treat the seed with recommended fungicides before planting. Since thiram (Dithiocarbamate) was banned and has not been in use since January 2020, Vibrance SB (sedaxane, fludioxonil and metalaxyl-M) is recommended for seed treatment as it has been proven to control a wide range of soil-borne diseases including rhizoctonia, pythium species and phoma (BBRO, 2022). Beet cyst nematodes (BCN) are present in some areas of sugar beet production and may cause significant yield reduction. In these areas, BCN resistant varieties are recommended (BBRO, 2022). It is a legal requirement that a variety is added to the Recommended List (RL) before it can be sold or used by growers. National list trials are used to determine whether a genotype has value for cultivation and use (VCU), and is distinct, uniform and stable (DUS) (BBRO, 2022). Currently, the 2023 RL has 22 varieties (**Table 2-1**).

Table 2-1: Sugar beet Recommended List for 2023 (BBRO, 2022)

Rz1 rhizomania varieties	Daphna	BTS1140	Kortessa KWS	BTS1915	Harryetta KWS	Katjana KWS	Annatina KWS	BTS3610	Wren	Morgan	Stewart	Tawny	Evalotta KWS	Adder	BTS3020	BTS5770	Button	Lacewing	Philina KWS	Maruscha KWS	BTS Smart9485	Smart Rixta KWS
Root yield	102	101	99	107	104	102	101	101	104	101	100	102	100	104	99	98	101	97	101	91	98	92
Sugar content %	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	18	17	17.3	17	17	17	17
Rust (1-9)	6	6	8	7	5	4	5	6	6	4	4	6	2	6	8	8	4	4	3	6	5	5
Powdery mildew (1-9)	5	5	6	4	5	5	5	5	5	5	5	6	5	5	5	5	5	4	5	3	6	5
Cercospora (1-9)	7	6	7	7	6	7	6	7	7	7	6	7	5	6	7	7	7	7	7	8	6	6
Aggressive rhizomania																			Y			
BCN	Y				Y	Y											Y	Y				
Year first listed	2017	2018	2018	2020	2022	2021	2022	2022	2021	2022	2022	2022	2020	2022	2021	2021	2022	2020	2018	2021	2022	2021
Breeder	KWS	BTS	KWS	BTS	KWS	KWS	KWS	BTS	SV	STR	STR	SV	KWS	SV	BTS	BTS	STR	SV	KWS	KWS	BTS	KWS
UK Agent	KWS	LG	KWS	LG	KWS	KWS	KWS	LG	SV	STR	STR	SV	KWS	SV	LG	LG	STR	SV	KWS	KWS	LG	KWS

Note:

- 1) BTS = Betaseed, KWS = KWS UK Ltd, LG = Limagrain UK Ltd, STR = Strube UK Ltd, SV = SESVanderHave UK Ltd
- 2) For disease score, 1=low resistance 9 = high resistance

2.3.2 Soil pH

The crop is recommended for soils with pH 6.5 - 8.0 (BBRO, 2022). Low pH values limit availability of some nutrients like P that are essential for sugar beet plant growth. A 36-year long-term field experiment revealed that sugar beet reacts to low soil pH levels more sensitively than cereal crops like wheat where non-limed soil with a pH of 5.0 significantly reduced sugar beet's nutrient uptake by 46% which translated into a 26% yield loss compared to a limed soil with a pH value of 6.5 (Von Tucher et al., 2018). While determining the point of liming under glasshouse experiments, Meyer and Wood (1976) discovered that sugar beet responded markedly to lime where soil pH values were less than 5.3 and this was ascribed primarily to the elimination of toxic elements such as Al, with secondary benefits from improved P and N availability. Matsi et al., (2005) reported that soil pH seemed to be one of the main factors negatively affecting the availability of soil Fe and Mn and significant decreases in sugar beet yield were observed because of an increase in soil pH.

2.3.3 Land preparation

Sugar beet can perform well in a range of soils except heavy clays, thin chalk soils and very stony soils on which drilling and harvesting can be very difficult (Finch et al., 2014). The purpose of land preparation is to provide necessary soil conditions that will dispose previous crop residue, improve drainage and to ultimately provide a medium to fine and uniform seed-bed which will enhance successful establishment of seedlings. Land preparation carried out under non-optimal conditions regarding cultivation timings can affect seedling establishment and in the long run reduce yield by 30 % or more (BBRO, 2022). Poor establishment falls into several categories and are associated with the seed, drilling, pests, diseases and soil problems which kill seedlings including wind erosion, waterlogging, desiccation, slumping of the surface and capping (Gummerson, 1989). Heavy, light and medium soils should be ploughed before the end of October, shortly before drilling in late March or early April, and mid-October respectively to avoid weathering, slumping, erosion and compaction (BBRO, 2022). Poor seed-bed preparation affects seed germination which results in reduced plant establishment and poor crop stands. It is recommended that the seed-bed should be prepared to a depth of 5-7 cm and should have a high proportion of small particles in the planting zone, so that you get good seed-to-soil contact (BBRO, 2022). Conducting an experiment in Brooms Barn

experimental station in Suffolk-UK, Gummerson (1989) who prepared seedbeds of various conditions by varying soil type, cultivation depth, moisture and texture, concluded that differences in emergence between seed-beds were large only when conditions were dry, but in all years, it was advantageous to level the seed bed in autumn or winter. It was suggested that seed beds with a dense soil below the seed and fine aggregates above gave the most suitable conditions for rapid and successful emergence.

2.3.4 Drilling

Key factors that affect final crop yield when drilling sugar beet are date, moisture and soil temperature. (**Figure 2-8**) In the UK, sugar beet is usually drilled by the end of March and any further drilling after mid-April results in yield losses (BBRO, 2022). Seed is commonly drilled at 2 - 3 cm but drilling depth can be increased in drier conditions but not deeper than 5 cm.



Figure 2-8: Drilling sugar beet (Bayer, 2011)

Moisture is an important factor during drilling as it has an impact on sugar beet germination and growth. Stout et al., (1956), maintained moisture percentages of 12, 16 and 21 % in a sandy loam soil (63 % sand, 23 % silt and 14 % clay) and demonstrated that emergence was optimum when soil moisture was between 16 and 21 %. Petkeviciene, (2009) compared 24 varieties from 2000 to 2004 in Lithuania and in support of Stout et al., (1956) concluded that drilling sugar beet when soil moisture content in the seedbed was 16.3 % increased plant density by 3.3 % compared to when the sugar beet was planted at a moisture content of 12%. Hunter (1951) planted sugar beet under five temperatures (5, 8, 10, 15 and 20 °C) and calculated germination percentages after 5, 10, 15 and 20 days and observed that sugar beet did not germinate when drilled at a soil temperature at or below 5 °C and that at 8 °C and 10 °C, sugar beet took 10 and 15 days respectively to reach 50 % germination while 73 and 82 % already germinated after 20 days when the seed were put at 15 and 20 °C. Similar results were reported by Gummerson (1986) who

found out that sugar beet seeds did not germinate at 5 °C after 48 days of adequate moisture and aeration but at 18 °C, 100 % of the seeds germinated within the same period.

2.3.5 Plant population

Most sub-optimal populations in sugar beet fields are due to abiotic (drought, waterlogging and soil capping) and biotic factors (insect damage, virus and fungi). Growers are encouraged to maintain a population of 100 000 plants ha⁻¹, to achieve maximum sugar yield, which is achieved when one seed is planted on rows that are spaced at 45 or 50 cm with an intra-row spacing between 16 to 18 cm (BBRO, 2022). Smit (1993) investigated the effects of plant densities (20000 to 90000 ha⁻¹) on sugar yield using six experiments for two years in north, southwest and central Netherlands. He observed that sugar yield mainly depended on the interaction between site and plant density. This was based on his findings that plant density influenced sugar yield more in the trials in the north and south than in the central region on reclaimed marine soil.

Sogut and Arioglu (2004) established sugar beet populations at 116 000, 94 000, 81 000, 71 000 and 58 000 ha⁻¹ in May and March with an aim of assessing the effect of planting date and population on sugar beet yield in Turkey. They concluded that across sowing dates, the highest sugar beet population (116 000 ha⁻¹) produced 10 tonnes ha⁻¹ more root yield than lower plant populations resulting in a 30 % greater sucrose yield. Cakmakci and Oral (2002) supported these results by concluding that low sugar beet plant population results in poor quality plants and increased non-sugar content. They observed maximum differences between large (103 600 ha⁻¹) and small plant densities (55500 ha⁻¹) of 37.9, 5.6 and 15.8 % for leaf, sugar and root yield, respectively. However, Draycott and Webb (1971) who evaluated sugar beet performance under varying plant populations and N levels at Broom's Barn in the UK and demonstrated that increasing plant population beyond 32 000 plants per acre (80 000 ha⁻¹) did not increase sugar yield. Lauer (1995) evaluated the effect of N and plant density in a split plots design by using N rates of 0, 112, 168, 224, 280, and 336 kg ha⁻¹ with plant populations of 37 100, 61 800, 86 500, and 111 200 ha⁻¹ and reported that plant density had no effect on root yield ha⁻¹. However, sucrose content increased with 5g Kg⁻¹ as plant density increased from 42 000 to 112 000 ha⁻¹.

These findings suggest that when planting sugar beet, recommended populations must be considered as populations higher than 100000 plants ha⁻¹ result in small roots, while populations lower than 80000 plants ha⁻¹ result in big roots but of low quality. High populations result in competition for N, space, sunlight, and other factors that might limit vegetative growth hence small roots. While low populations result in luxury feeding that produces overgrown roots with low sugar content.

2.3.6 Harvesting

In most parts of Europe and America, sugar beet harvesting normally starts in September and ends in November with an exception of some areas where the crop is harvested in the spring (Clarke and Clearly, 2003). The aim of the harvesting process is to make sure a farmer is lifting sugar beet that is clean, unbroken, and free of non-sugar beet related material like leaves and stones. In the UK, sugar beet is harvested during autumn and winter using harvesters (**Figure 2-9**) which can harvest four to six rows simultaneously and up to 1000 tonnes per day (British Sugar, 2019; Clarke and Clearly, 2003). The harvesters remove leaves which are left on the field to help improving soil structure by forming part of the organic matter (BBRO, 2022).



Figure 2-9: Sugar beet harvesting using machinery in one of the fields in UK (Bayer, 2011).

2.3.7 Mechanical processes in the harvester

Main root damages on sugar beet, such as surface and root tip breakage damage are reported to originate from operations very early in the harvesting chain, in

particular during lifting and cleaning in the harvester (Hoffmann et al., 2018b). Harvester type influences harvest quality where some harvesters register lower topping diameter, higher portion of leaf residues, lower root tip and surface damage than others (Hoffmann, 2018). During the harvesting process, basic operations by the harvester include removal of the leaves, crowning of the top portion, lifting, cleaning and loading (Winner, 1993). The process starts with the defoliator which removes the green leaves and slices a slab from the top of the sugar beet root. This removed slab is the growing point of the sugar beet and contains high levels of impurities, which impede the factories' ability to extract the sugar from the remainder of the harvested root. The roots are then lifted by a pinch wheel and carried through cleaning aggregates (axial rollers/cleaning turbines) the transfer web and the discharge elevator, where the beet is separated from the adhering soil and transported into the holding tank.

2.3.8 Root and surface breakages in a harvester

Mechanical processes in a harvester are the main source of damage as the beet are heavily impacted by the machine due to frontal collision with machine parts and with each other. Root breakage develops during lifting, cleaning within the harvester, loading and unloading in the hopper phase where devices apply a combination of vertical and horizontal forces to the root. During lifting, the forces are resisted by a soil reaction causing root tip breakage whenever the set-up stress exceeds the strength of the root material (Verulen, 2001). During hopper loading, hopper storage and hopper unloading, the root tip and surface damage occur due to impact, when beet are not delivered smoothly from one transport device to the other, or when drop heights are excessive. Impact of excessive drop heights on sugar beet were well demonstrated by Akesson and Stout (1978) who concluded that root tip diameter after damage, cracks and respiration rate increased when drop height was increased from 1.5 to 6 feet (0.5 to 1.8 m). Surface damage during cleaning in the axial rollers or turbines occur due to spilling of beet and beet fragments.

2.3.9 Cleaning systems

Harvesters differ in terms of topping and lifting devices, transport and cleaning aggregates. The degree of root damage and injuries for various harvesting systems depend on cleaning aggregates and the intensity of cleaning (Huijbregts et al., 2013; Schulze-Lammers et al., 2015). Hoffmann et al., (2018b) studied two commercial beet harvesters in Germany. The two harvesters were a Maxtron beet harvester (harvester 1) which was equipped with main webs and 10 axial cleaning rollers and

Oppel wheels (**Figure 2-11**) and the Holmer T4 40 (harvester 2) which used 3 cleaning turbines and walking shares (**Figure 2-12**). They found out that harvester type influenced root tip breakage with turbine cleaning systems having a higher root damage compared to axial rollers (**Figure 2-10**). However, surface damage was only influenced by the cleaning intensity suggesting that settings and aggressiveness of harvesters is an important factor when minimising damage during harvesting. Similar results were reported by Bentini et al., (2002) who observed that harvester forward speed of 6 km h⁻¹ caused the fewest taproot breaks and bruises compared to 8 and 10 km h⁻¹. They described the relationship between taproot breakage and impact velocity change and showed that this was statistically significant for lifting shares and roller bed, while the relationship was not significant for transfer web and turbines.

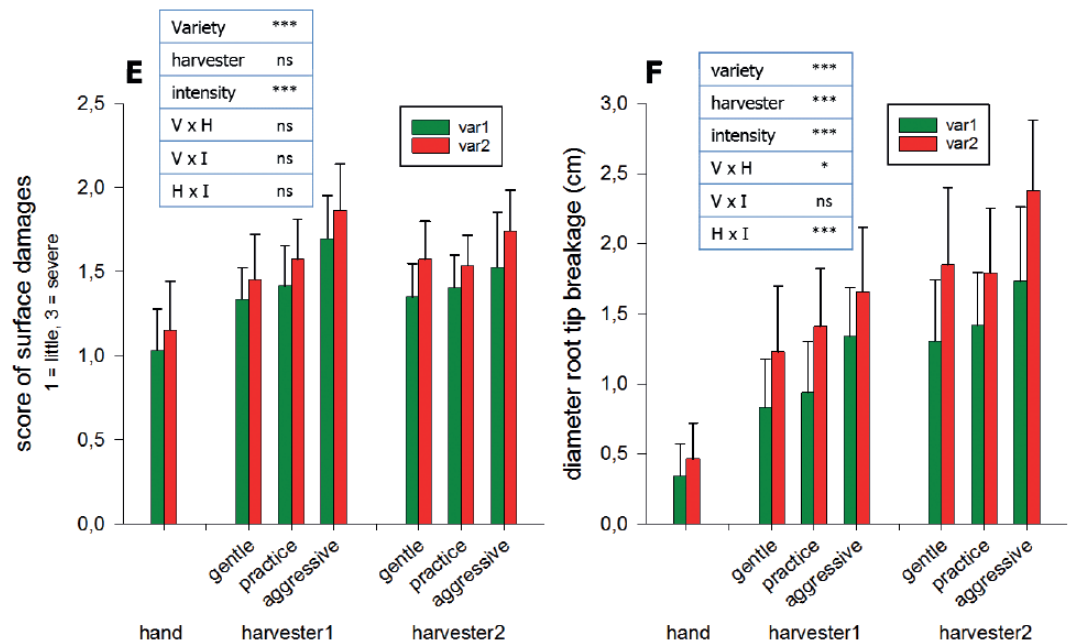


Figure 2-10: Effects of harvester type and speed on surface damage and root tip damage (Hoffmann et al., 2018b).



Figure 2-11: A main web with a roller table on a Maxtron 620 harvester (GRIMME, 2020)

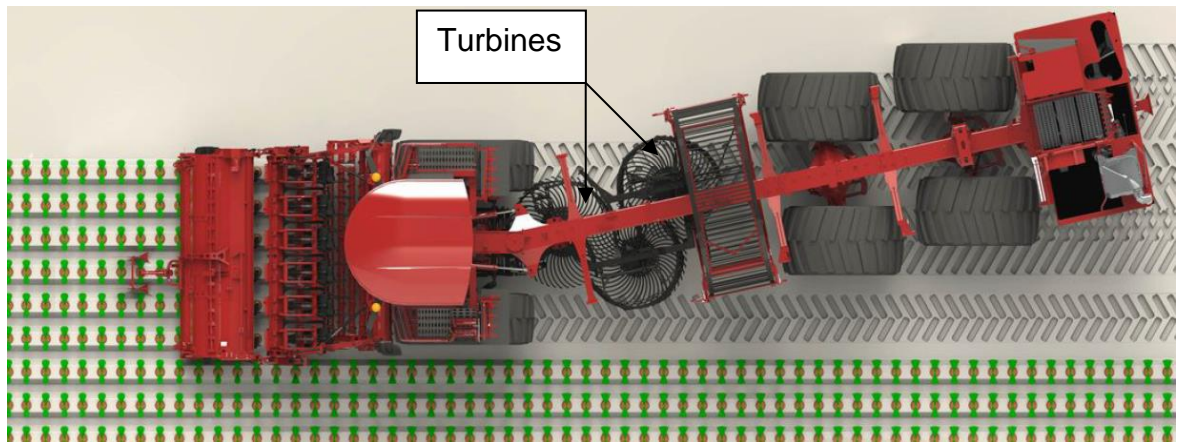


Figure 2-12: A diagrammatic view of a Holmer T4 40 with a turbine cleaning system (Holmer, 2020)

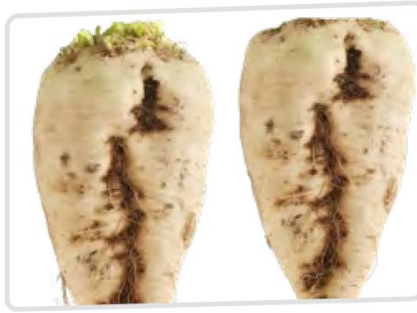
2.3.10 Harvester settings

BBRO (2022) recommends that harvester operators should be fully trained and familiar with equipment as it will help them to adjust the machine accordingly to minimise excess damage or breakage. Settings for harvesters are also crucial as they help to reduce damage through excess topping, crowning (**Figure 2-13**), cracking and root tip damage.

UNDER-CROWNED



OPTIMAL CROWNING



OVER-CROWNED



Figure 2-13: Different crowning conditions for sugar beet harvested using a beet harvester (BBRO, 2022)

Table 2-2: Recommended harvester settings for various conditions in UK (BBRO, 2016)

Type of loss/damage	Dry soils condition	Wet soils condition	Crop conditions
Whole root losses	Set lifting mechanism deeper Fit discs in place of shares Decrease forward speed Add Opel wheel star wheels Check condition of shares - if worn, replace or repair if possible	Increase or decrease forward speed Set lifting mechanisms deeper Replace discs with shares or close discs	
Root tails broken off at lifting	Reduce forward speed	Adjust depth of lifting mechanism - raise/lower	
Root damage - chipping, breakage and cracking in the cleaning mechanism	Set lifting mechanism deeper Fit turbine gate plates Reduce turbine speed Fit ringed turbines and/or more helper tines Consider increasing or decreasing forward speed Remove agitator rollers from chain cleaning systems	Reduce turbine speed Increase forward speed Check lifting accuracy	
Excessive soil adhering to harvested roots		Increase turbine speed Remove gate plates Fit pig tines instead of railed gates Fit lifting shares in place of discs Raise lifting mechanism Fit agitator rollers and chains Increase turbine gate gaps Increase angle of roller bed and lower grub chain	

Small beet			Fit gate plates Reduce pitch of cleaning/transport chains or fit plastic pipe over chain links to reduce pitch Close turbine finger wheel gaps Close Oppel wheel gaps
Gappy beet			Open discs and move further from skids Sharpen topper knives Reduce scalper arm pressure
High weed infestation			Increase gap between turbine and gates Increase angle of roller bed Replace flails on topper Sharpen knives

2.3.11 Clamping

Sugar beet can be harvested and stored temporarily in a clamp (**Figure 2-14**) which is a compact storage heap, mound or pile used for temporary storage of root crops such as sugar beet. The average duration that sugar beet stays in storage before actual processing in Europe is 80 days (Liebe and Varrelmann, 2016). Schnepel and Hoffmann (2016a) also state that the average duration for sugar beet in storage is often not less than two months across Europe. BBRO (2022) reported that sugar losses in the clamps is due to injuries, infections and poor ventilation which increase respiration rates, with sugar losses in clamps circa 0.1 - 0.04 % of total sugar per day per volume. Over the past decades, clamping of sugar beet has gone through several changes mostly due to increase in production and tonnage. BBRO (2016) recommends that a properly built clamp should not be more than 2.5 m high and 10 m long (**Figure 2-15**). There are several types of clamps and various building protocols (**Table 2-3**) must be followed when one is constructing. The following are BBRO (2022) recommendations a grower should consider when constructing a clamp:

- 1) Build the clamp in an open area to aid ventilation and cooling
- 2) Build the clamp on a firm, well-drained site which will be suitable for loading and unloading
- 3) Never push beet up the face of the clamp. This will break beet, compact the clamp and in turn restrict air movement, allow heat to build-up and increase the rate of sugar loss
- 4) Ensure minimal damage from harvesting



Figure 2-14: A clamp used for storing sugar beet over time

Table 2-3: Description of early, late and A-shaped clamps

Early season clamp	Late season clamp	A - shaped clamp for cleaner loaders
Early in the season beet should be in a clamp for no more than a few days. These clamps should not be covered or have retaining walls.	Late season long-term clamps should be no more than 2.5m high with a level surface so there are no frost pockets.	Where a self-propelled cleaner- loader is used, clamps should be built in an 'A' shape of the correct width to allow the machine to operate effectively. The beet must be placed on a flat un-rutted surface.
Short-term clamps are designed to give maximum surface area and therefore cooling to reduce sugar loss through respiration.	Clamps should be built using straw retaining walls. Bales should be placed on pallets with the open-end facing outward to aid ventilation.	Clamps are normally built on the headland, but consideration needs to be given for machinery to access the clamp easily.
Clamps should be made up of individual loads and be no more than 2m high	Only use clamp sheets if the ground temperature is forecast to be below -3°C.	A-shaped clamps are best built with a harvester or side-delivery trailer rather than a conventional tipping trailer to avoid rutting in the clamp base
	Clamp sheets are made of polyfelt which not only offer protection but also allow the beet to breathe.	

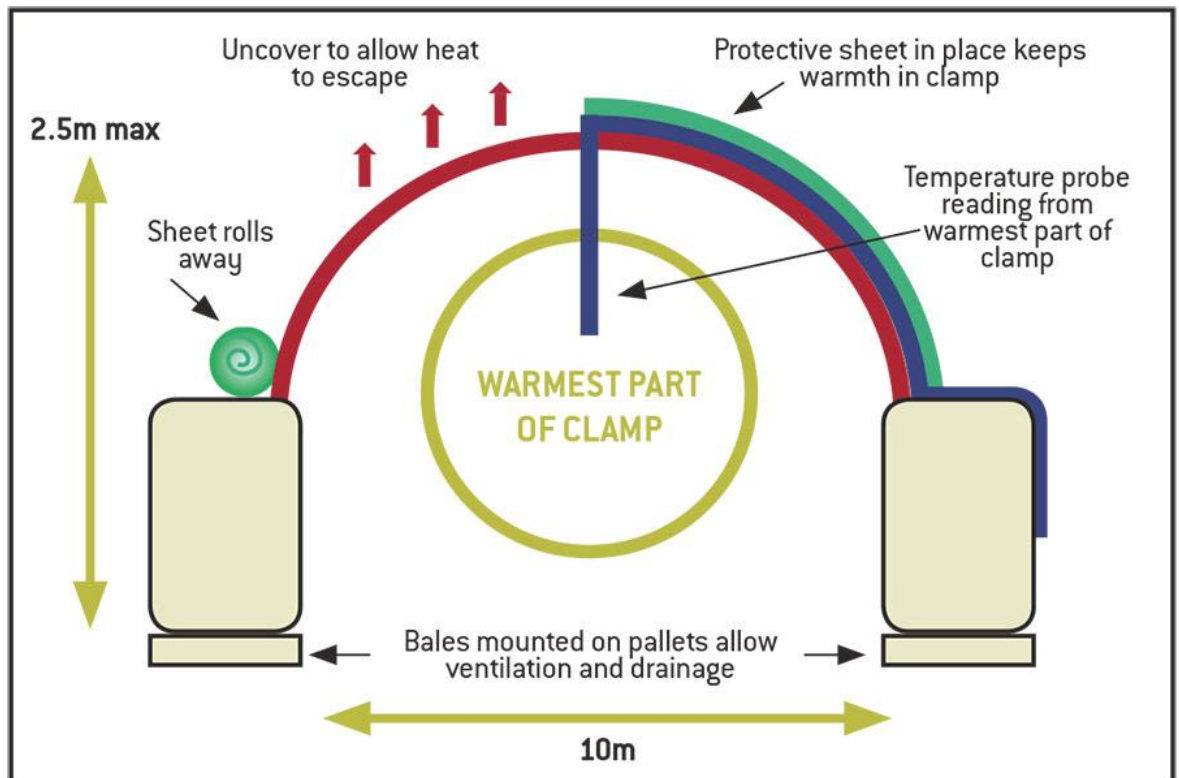


Figure 2-15: Cross-section of a traditional clamp (BBRO, 2016).

2.3.12 Cleaner loaders

Sugar beet roots are unloaded either direct onto the truck for transport to the factory, or the harvester unloads the roots into a chaser bin which unloads in clamps on the field where they are then further cleaned and loaded into trucks at a later time by cleaner-loaders (Bentini et al., 2005). The aim of further cleaning using cleaner loaders is to reduce dirt tare and loose soil being delivered to the processing factory. The cleaning is done either through conventional or self-propelled cleaner loaders which have recently helped to reduce soil tare by an average of 10 % (Marlander et al., 2003; Verulen, 2001). In the UK there are around 200 conventional cleaner loaders and around 20 self-propelled cleaner loaders which are mainly operated by the larger haulage companies to help maximise the turnaround time of the lorries (Fishpool, 2016). The roots are loaded into the cleaner loader by a loading machine (**Figure 2-16**). The loading of the beet into the cleaner loader and the truck also acts as a source of damage for the sugar beet roots. In the cleaner loader beet are lifted by a lugged, endless elevator draper and an endless, upwardly inclined belt having flexible, finger-like projections. The draper and the belt are driven through their respective circuits with the belt being driven at a speed faster than that of the draper. The speed differential between the belt and the draper causes the finger-like

projections to flexibly engage with the beet producing a brushing effect on the beets to clean them while they are being elevated for loading. As the beet impact on the draper, surface and root tip damage can occur. Poorly managed self-propelled and conventional cleaner loaders can lead to root tip and surface damage that amount to total yield losses of 1.2 - 2.4 % respectively (Fishpool, 2016).



Figure 2-16: A truck being loaded with sugar beet by a cleaner loader (red) and a loading machine (yellow) (Holmer 2020)

2.4 Fertiliser requirements and nutrition

Plant nutrition plays an important role in cell structure and development. Availability of essential nutrients involved in physiological process like photosynthesis and respiration is heavily linked to plant tissue strength (Singh et al., 2010). Despite nutrition being directly linked to strengthening of plant cells, most studies have focused on its contribution to economic yield and other yield components like biomass, root length and width of sugar beet. No studies have been done to assess the role of essential nutrients like Ca, B, P, and N on cellular properties that influence textural properties like resistance to puncture and compression in sugar beet. The influence of Ca on mechanical properties in horticultural crops like apples has been studied. Cybulska et al., (2012) conducted an experiment where apples were subjected to Ca lactate solutions of 0 (water), 1, 2, 4 and 6 % for 24 hours and discovered that treatments with increased Ca^{2+} concentration were associated with a significant increase in firmness and puncture resistance of the apples. However, the draw back with this experiment is that it was conducted in the laboratory and not in the field. It is not known how field induced Ca would affect firmness or puncture

resistance for root crops like sugar beet. Studies on a direct effect of B on the membrane potential of sunflower (*Helianthus annuus* [L.], cv Mammoth Grey Stripe) root tip cells show that treatment in 50 μM B caused a significantly greater accumulation of K^+ after 48 hours and a deficiency in B affects the root quality (Schon et al., 1990). However, it is not known how B would affect textural properties of root crops like sugar beet.

In sugar beet production, nutrients are obtained from the soil and where they are inadequate, they are supplemented through fertilisers which in sugar beet are recommended to be applied while making an allowance for organic manure. Specifically, in the UK, it is recommended that N fertilisers are applied early enough to drive canopy development and P, K, Mg and Na fertilisers should be applied pre-ploughing to minimise damage to soil structure (BBRO, 2022). It is recommended that P, K, Mg and Na should be applied using soil analysis results or determined by the previous cropping (**Table 2-4**).

Table 2-4: Major nutrient recommendations (kg ha^{-1}) for sugar beet production in UK (BBRO, 2022)

Nutrient	Soil index			
	0	1	2	3
Nitrogen	120	120	100	80
Phosphate	110	80	50	0
Potash	160	130	100	0
Magnesium	150	75	0	0
Sodium	200	200	100	0

2.4.1 Nitrogen

Plants contain more than 1% of nitrogen (N) and the nutrient is required in large quantities at different stages from sowing to harvest in order to synthesise amino-acids, proteins, nucleic acids, and many other cell constituents (Khattab et al., 2019). The N demand for sugar beet crop is estimated to be in the range of 200–250 kg ha^{-1} , half of which is provided by soil residual and mineralisable $\text{NO}_3\text{-N}$ (Marlander et al., 2003). Excessive use of N fertiliser sometimes results in higher root yield, but it consequently lowers sugar content and increases standard

molasses loss (Marlander, 1990; Prvulovic et al., 2010). Studying the relationship between applied N and uptake on unmanured mineral soils and organic mineral soils of the UK and Belgium, Pocock et al., (1990) observed that N uptake in sugar beet ranged from 65-190 kg ha⁻¹ and 295-383 kg ha⁻¹ on unmanured and manured land respectively. They concluded that fertiliser application on unmanured soils increased N uptake while on manured soil, application of fertilisers did not result into any increase in N uptake. Marlander (1990) reported that while conducting fertiliser trials on alluvial soils in Germany, sugar beet was seen to have optimum values after which sugar beet yield does not increase when further N was applied. Root yield, sugar yield and white sugar yield all increased with increasing N-supply and reached maximum values at 159, 136 and 129 kg ha⁻¹ N, respectively. Similar results were observed by Draycott and Webb (1971) and Hozayn (2014) who discovered that after increasing N levels on calcareous sandy loam soils, an application of between 0.6 and 1.2 hundred weights (27 and 54 Kg) of N per acre gave maximum sugar yield and a further increase in N did not increase sugar yield.

Malnou et al., (2006) conducted field experiments on four sites in the UK to determine the smallest amount of N required to produce an 85% canopy cover on mineral soils and concluded that in the absence of organic manure, 100 kg ha⁻¹ of N is required. However, studies in Greece by Maslaris et al., (2010) who used 0, 60, 120, 180, and 240 kg ha⁻¹ N concluded that root yield showed greater response to N addition (more than the recommended 150 kg ha⁻¹) for soil pH greater than 8.0 (19.1%) and a smaller response (3.9–6.0%) to N was recorded for pH less than the optimal (7.0) for sugar beet. Malnou et al., (2008) carried out three field experiments at Broom's Barn investigating the effects of applying N fertilisers late in the summer. They reported that an application of an extra 60 kg ha⁻¹ of total available N in late summer only increased chlorophyll content and foliage dry weight at harvest but did not influence sugar yield. This can be explained by the fact that sucrose is mainly accumulated during the early stages of development, hence extra application of N only facilitated further vegetative growth and not sugar formation. Lauer (1995) conducted experiments to determine whether with an increase in the campaign time, N rates and plant density requires to be adjusted. He observed that increasing N levels from 0-112 kg ha⁻¹ N increased root yield more (11 Mg ha⁻¹) compared to when N rates were increased from 0-168 kg ha⁻¹ N (5.2 Mg ha⁻¹) and that a further addition of N after 168 kg ha⁻¹ N decreased sucrose content in sugar beet roots.

2.4.2 Phosphorus

Phosphorus (P) is one of the essential nutrients required to complete a life-cycle for sugar beet. It is the second most important nutrient for sugar beet production and plays an important role in energy transfer within the plant and maintains structural integrity of cell membrane (Ahmad et al., 2017). Response to P deficiencies is understood to vary from one species to the other. Studies on sugar beet response to P show that the crop is more sensitive to P compared to cereal crops like maize and wheat (Von Tucher et al., 2018; Zicker et al., 2018). However, after comparing sugar beet and wheat on luvisols (A kind of soil with eluvial horizons from which clay has been leached after snowmelt or heavy rains and illuvial horizons in which clay has been deposited) and oxisols (a soil of an order comprising stable, highly weathered, tropical mineral soils with highly oxidized subsurface horizons), Bhadoria et al., (2002) reported that the efficiency of utilising P when absorbed by a sugar beet and a wheat plant is equal meaning that neither crop has the ability to utilize the Ca or Fe bound P. Atkinson (1973) conducted experiments using 21 dicotyledonous and twenty-four monocotyledonous species and concluded that P deficient species are more sensitive to the physical environment and are heavily affected in terms of root: shoot ratio and hydration of plant cells.

Sailsbery and Hills (1968) reported that in five fields in USA, with soil P levels from 4.5 to 8.4 ppm, there was a marked response in sugar beet top growth early in the season. However, non-fertilized plants accumulated sufficient P for maximum growth later in growth, indicating that the period of P deficiency occurred very early in the growth period of the crops. They also reported that sugar beet yield increased significantly by 2.6 t ha⁻¹, but application of P did not affect sucrose concentration in the beet. Westerman et al., (1977) also studied P application and reported that P application greater than 10 ppm did not affect root yield or sucrose concentration. P deficiencies in early growth stages or during establishment in various plant species have been widely reported. This response has been observed in sugar beet by Terry and Ulrich (1973) who reported that a removal of P from the nutrient supply at the ten-leaf stage (28 days after germination) decreased net rates of photosynthesis by two thirds after thirty days. Sims and Smith (2001) conducted an experiment in which they applied four rates (0, 15, 30 and 45 kg ha⁻¹) of P on a sandy loam soil to determine the effects on early season sugar beet root and shoot growth. They

reported that P fertilisation significantly increased both shoot and root dry matter accumulation and 15 kg ha⁻¹ produced most of the observed response. Root dry matter accumulation to P rates was apparent within 30 days after planting and this response was maintained at the end of the growing season.

2.4.3 Potassium

Potassium (K) is essential for the photosynthetic process, transport of sugars from the leaves to the roots and reduction of oxidative damage and helps in maintaining plant osmotic potential, cell turgor and regulation of the opening and closure of stomata (Oosterhuis et al., 2013). Sugar beet requires 2.9-6.8 kg of K per tonne of storage root (Przemysław et al., 2018) and take up 350 - 500 kg ha⁻¹ K, two-thirds of which is accumulated in the shoot and one third in the storage root at harvest (Draycott, 2006). The crop requires a concentration of 120 to 180 mg of exchangeable K per gram soil to achieve maximum sugar yield (BBRO and BSPB, 2019; Milford et al., 2000). Conducting a 20-year long-term experiment in Germany (Lower Saxony) where 0, 29, 58, 87, 174, and 524 kg ha⁻¹ K were applied to the soil, Romer et al., (2004) concluded that extractable sugar content reached a maximum at a yearly application of 174 kg ha⁻¹ K, the time and source of application had no effect on extractable sugar yield and a K concentration of 110 mg g⁻¹ K is sufficient for maximum extractable sugar yield on alluvial soils. However, this high K application can only be economical in alluvial soils which have a high capacity for fixing K.

Wakeel et al., (2010) conducted an experiment on alluvial soils to check the possibility of substituting K with Na in such soils and they concluded that Na can substitute K in sugar beet nutrition to a high degree. Przemyslaw et al., (2018) tested the four K: Mg: Na cation ratios (1:0:0; 1:0.11:0.09; 1:0.16:0.54 and 1:0.33:2.19) and observed that the effect of these ratios was dependent on site and that K rate reduction from 125 to 24 kg ha⁻¹ combined with the simultaneous increase in the rate of Mg and Na did not result in lower sugar beet yield. K has also been reported to increase yield mostly in conditions where water is sufficient (Grzebisz et al., 2013). Mubarak et al., (2016) conducted a pot experiment to determine how sugar beet responds to K under sufficient (in soils with 60 % water holding capacity) and deficient (in soils with 40 % water holding capacity) water conditions and they reported that applying K in soils with sufficient water holding capacity significantly

increased plant growth, beet yield and sugar content. Milford et al., (2000) applied K ranging from 0 - 600 kg ha⁻¹ on soils of varying types and indexes in 1992 and 1997 and reported that K offtakes were higher on a soil with a high K index than that of a low index and sugar beet yielding 60-70 t ha⁻¹ removed about 70 kg ha⁻¹ of K on low K index sandy loams and 120 kg ha⁻¹ on clay soils of K index 3 and above.

2.4.4 Calcium

Calcium (Ca) is responsible for binding of cell wall's pectin, hence increasing plants rigidity (Burstrom, 1968; Hepler, 1994). A sugar beet crop of 70 t ha⁻¹ contains approximately 100 Kg of Ca (BBRO and BSPB, 2019). Ulrich and Mostafa (1976) reported that when sugar beet is grown in a nutrient solution lacking Ca the root and top fail to develop while when transferred into a Ca deficient solution at 8 leaf stage, the roots become swollen and stubby at the tip. Ca deficiency results in small leaf blades with a black tip at the apex of the petiole. Foliar application of varying Ca and silicon rates at 4-6 leaf stage by Artyszak et al., (2016) increased sugar beet root yield by 22 % but did not have any effect on sucrose content. However, Foliar application of Ca and silicon had no significant effect on such sugar beet root quality parameters features as content of sucrose, alpha- amino-N, K and Na (Artyszak et al., 2016).

Terry and Huston (1975) induced Ca deficiency in sugar beet 28 days after planting for 19, 20 and 21 days and observed that carbon dioxide intake per unit leaf area increased by 15 % but with a reduced leaf area suggesting that for purposes of photosynthesis, small amounts of Ca are needed. In potatoes, Singh and Sharma (1972) also reported a reduced leaf area and less sugar and starch in plants with less Ca. Conducting experiments in Herbaceous peony (*Paeonia Lactiflora* Pall.) in Jiangsu Province in China, Li et al., (2012) showed that breaking force of the top segment of peonies stems was positively correlated with the ratio of water insoluble pectin to water soluble pectin ($R = 0.673$) as well as lignin contents ($R = 0.926$) after Ca applications. However, this study examined the breaking forces in stems of peony plants and not roots and the structure of sugar beet roots and poeny stems are different, hence a need to do a separate study on sugar beet to see if results will be the same. Samarakoon et al., (2017) concluded that for poinsettia (*Euphorbia pulcherrima*) cuttings at the time of harvest from the stock plant, penetration resistance increased by 10 % with the application of 800 mg L⁻¹ Ca compared with the control (0 mg L⁻¹), whereas peak force was greater by 9 %. While for zonal

geranium (*Pelargonium xhortorum*), work of penetration increased 15 % with the application of 800 mg L⁻¹ Ca compared with the control.

2.4.5 Boron

Boron (B) is essential in promoting cell wall formation, carbohydrate metabolism and sugar translocation with sugar beet absorbing B in the form of B(OH)₃ or H₃BO₃ from the soil by roots (Mekdad and Shaaban, 2020). In England, B deficiency frequently occurs in beet and swede on soils derived from Triassic and Devonian sandstones as well as post-glacial sands (Shorrocks, 1997) and samples tested from crops like oilseed rape have been reported to have a 70 % deficiency in B (Jenkins, 2020). Bonilla et al., (1980) conducted an experiment and concluded that B deficiency reduces cell activity, division, differentiation, maturing, respiration and growth. An application of 7 kg ha⁻¹ Zinc and 2.4 kg ha⁻¹ B significantly increased root growth, SPAD value, sucrose %, extractable %, yields, and purity % of sugar beet (Mekdad and Shaaban, 2020). However, the improvement observed cannot be exclusively attributed to B as the foliar fertiliser contained other nutrients including P and N. Bonilla et al., (1980) reported in their studies that conditions of B deficiency and toxicity result in a substantial decrease of the sugar levels in the sap and in the root. This finding was echoed by Pommerrenig et al., (2019) who concluded that B deficiency in common plantain (*Plantago major L.*) affected quantitative distribution patterns of various phytohormones, sugars and macro and micronutrients in a tissue-specific manner where vascular sucrose level dropped, and sucrose loading into the phloem was reduced. This study was done at transport tissues level (vascular and phloem tissues) and did not extend to B's impact on tissue or organ response to damage when exposed to external forces.

It is also reported that B deficiency starts with a white netted chapping of the upper leaf blade surfaces with wilting in new leaves and the growing point turning black (Poindexter, 2012) and a substantial decrease in sugar levels in the sap (28 %) and the root (30 %) (Bonilla et al., 1980). Oertli and Roth (1969) grew plants in solutions containing B ranging from 0 - 40 ppm and reported that levels of B in nutrient solutions suitable for plant growth are highest for sugar beet, lowest for soybean (*Glycine max*), and intermediate for cotton (*Gossipium hirsutum*). They continued to report that B toxicity symptoms appeared at a much slower rate in cotton, while sugar beet showed only slight interveinal yellowing, a pale marginal zone a few millimetres wide, and some marginal necrotic spots in old leaves of the two highest

treatments. They stated that deficiency symptoms appeared in the reverse order with sugar beet suffering first and most severely with a cessation of growth, brown spots in basal regions and on veins of young leaves, necrosis of newly emerging leaves and black roots. However, this experiment focused on an inter-species comparison where conclusions were only drawn on deficiency symptoms and their effect on root yield. It would be interesting to expand the concept to tissue cells resilience and robustness to damage and assess B's contribution to mechanical strength which has not been reported in literature.

Conducting studies on carrots using five commercial cultivars namely; Kuroda (orange), Dragon Purple, Kuttiger White, Yellow, and Nutri-Red, Singh et al., (2010) used 5.0 μM of H_2BO_3 and 3.0 mM CaCl_2 in a feeding solution as sources of B and Ca, respectively where one treatment had both and two others had either of the two. They concluded that elevated levels of B in carrot root tissue reduced the uptake of Ca and other mineral nutrients and enhanced plant cell wall structural integrity, resistance to fracture, and the weight and size (both diameter and length) of carrots. Although they further reported that higher amounts of Ca were accumulated in the plant materials, the additional supply of Ca did not have a significant effect on the mechanical properties of mature plant tissues or on the uptake of B by the plant. They therefore suggested that B cross-linking of pectin (rhamnogalacturonan II) has a greater influence on mature tissue mechanical properties than Ca cross-linking of pectin (homogalacturonan) when supplied during plant growth.

In summary, most studies on Ca and B as cited in this study focused on their contribution as micronutrients on yield and yield related parameters. Very few have researched on their binding role in the cell wall and membranes. Specifically, most Ca studies in sugar beet production have only focused on its contribution to economic yield and quality related parameters like amino-acids and K. Despite some investigations on its role in mechanical strength, their focus was on the leaves because that's where its deficiency is firstly and easily noticed. There are remarkable trends of Ca's influence of strength of the leaves and yields but nothing has been reported on whether it helps to strengthen root tissues. Understanding Ca's role on root strength is important in root and tuber crops like sugar beet or potatoes because it can be agronomically optimised and help growers to minimise root damage. In this study, Ca's contribution in sugar beet root tissues was explored

through establishment of experiments in sites that show deficiency. B has also been highly linked to cell wall formation and vegetative growth of plants but there is little information on how deficiencies or sufficiency would affect mechanical properties in sugar beet roots where damage is an issue of great concern. While B deficiency has not been observed widely, research in the recent past has revealed this to be a widespread problem in crops like cotton, rapeseed, wheat, peanut, sorghum and rice and its deficiency can be corrected by soil application or by foliar feeding (Rashid and Ryan, 2004). Therefore, this study scientifically focused on establishing the impact of the two micronutrients on root tissue damage by performing mechanical tests on root tissues.

2.5 Post-harvest losses in sugar beet

Post-harvest losses are generally classified into two categories. This include losses through beet or parts of beet that are left in the field during harvest and the sugar that is lost through physiological processes initiated through damage or breakages during pre and post-harvest field operations (**Figure 2-17**). Van Swaaij et al., (2003) stated that the main source of loss are; 1) the root tips and other fragments that stay behind after harvest and cleaning which account for 2-3 % of sugar loss, 2) sugar loss during storage by respiration or due to invasion by bacteria and fungi in injured beet accounting for up to 2.5 % of the total amount of sugar and 3) leaching of sugar from injured beet during processing which accounts for 0.8 % of sugar loss. Minimising sugar loss is therefore benchmarked on reducing pre and post-harvest damage to tissues by minimising entry points for pathogens and associated respiration during the storage period (Hoffmann and Schnepel, 2016; Kenter et al., 2006a). Most researchers mainly attribute sugar losses due to pre and post-harvest injuries, storage temperature and length of the storage period (Hoffmann and Schnepel, 2016; Kenter et al., 2006a).

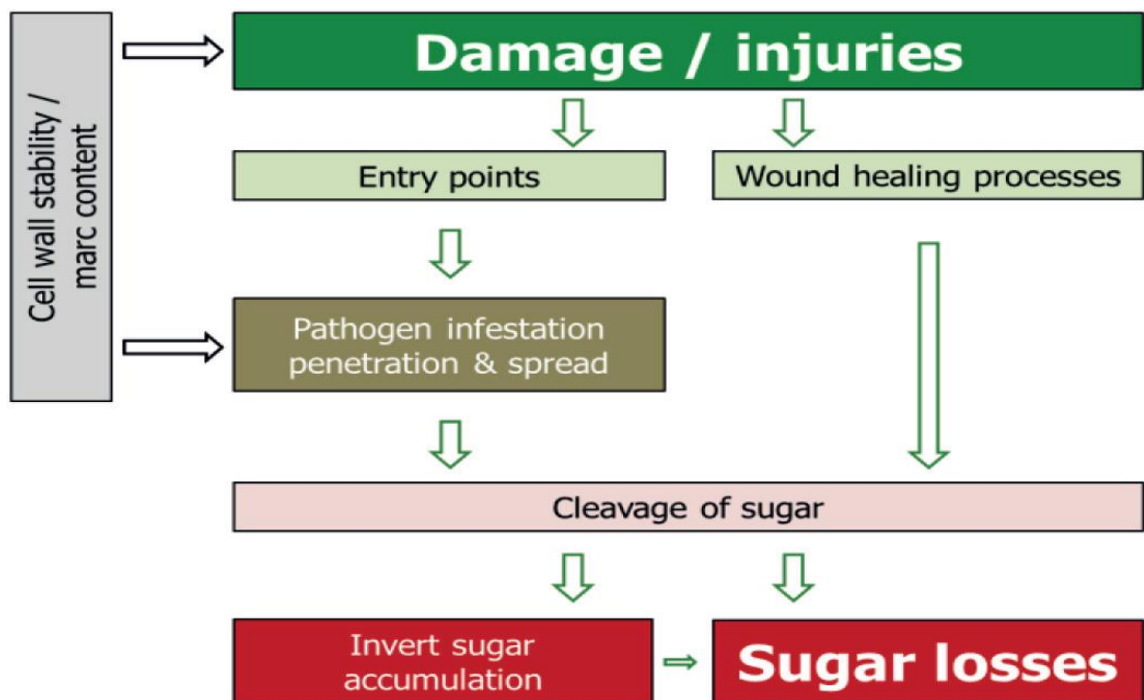


Figure 2-17: Causes for storage losses of sugar beet (Schnepel and Hoffmann, 2016b)

2.5.1 Respiration

Respiration is the process by which carbon compounds are oxidized to provide the metabolic energy and substrates needed for growth and maintenance of all living cells (Klotz et al., 2008). In sugar beet that have been harvested, the process involves conversion of sucrose into energy to maintain the root's physiological integrity and accounts for 50-80 % of the sucrose loss that occurs during storage (Fugate et al., 2010; Kenter et al., 2006a).

Respiration is accelerated by many factors including temperature, injury, oxygen and ethylene content of the sugar beet roots (Parks and Peterson, 1978). An increase in temperature increases cellular reactions, increasing the rate of respiration. Effects of temperature on sugar beet root respiration rates in storage were well explained by Wyse (1973) who observed a threefold increase in respiration when temperatures were increased from 40 to 75 °F (4 to 24 °C). However, Lafta and Fugate (2009) reported that even at the same temperature, variations in relative humidity also alter root tissue properties and increase respiration. They reported that changing relative humidity to 40 from 85% increased respiration rate for the sugar beet variety VDH66156 by 108 % and by 82 % for the variety Beta 4797R. Fugate et al., (2016) also reported that wound induced

respiration is higher at higher compared to low temperatures. This was discovered after storing sugar beet roots at 6 °C and 12 °C for 28 days. However, they also reported that for long-term storage (more than 28 days), low (6 °C) temperatures may also register a high respiration rate of about 52 % compared to high temperature (12 °C) mostly in damaged roots.

Tissue damage also increases respiration rates post-harvest to the extent that the greater the degree of injury by topping and handling, the higher the respiration rate. The increase in respiration due to injury is attributed to wound healing and infection by pathogens that increase ethylene levels (Fugate et al., 2010). Mechanical injuries were well documented by Wyse (1973) who observed that respiration was higher in injured roots than those that were manually lifted (**Figure 2-18**). Kenter et al., (2006a) demonstrated the effects of external damage by injuring various sugar beet varieties using a rotating drum with sharp edged bars and later stored them in climate containers at 5, 12 and 20 °C and took samples after 2, 6, 14, 21 and 27 days. They concluded that sucrose content started decreasing in the damaged beets after 14 days independent of temperature. Whereas the undamaged sugar beet, did not exceed 0.1 % sucrose loss of the original mass per day during 21 days at 20 °C but the damaged sugar beet lost approximately 0.5 % per day.

Akeson et al., (1978) damaged sugar beet using hard rubber, steel and spring rubber from a height of 0.5, 1 and 2 m. Sucrose content for the damaged beet was compared to hand harvested (undamaged) beet after storing at 4 °C, 95 to 100 % RH for 119 and 121 days respectively. There was an increase in sucrose loss mostly when spring rubber was used to impact the sugar beet roots. They also reported that despite the sucrose loss not being statistically significant, there was a close relationship between respiration ($R = 0.95$), sucrose loss ($R = 0.95$) and invert sugars ($R = 0.99$).

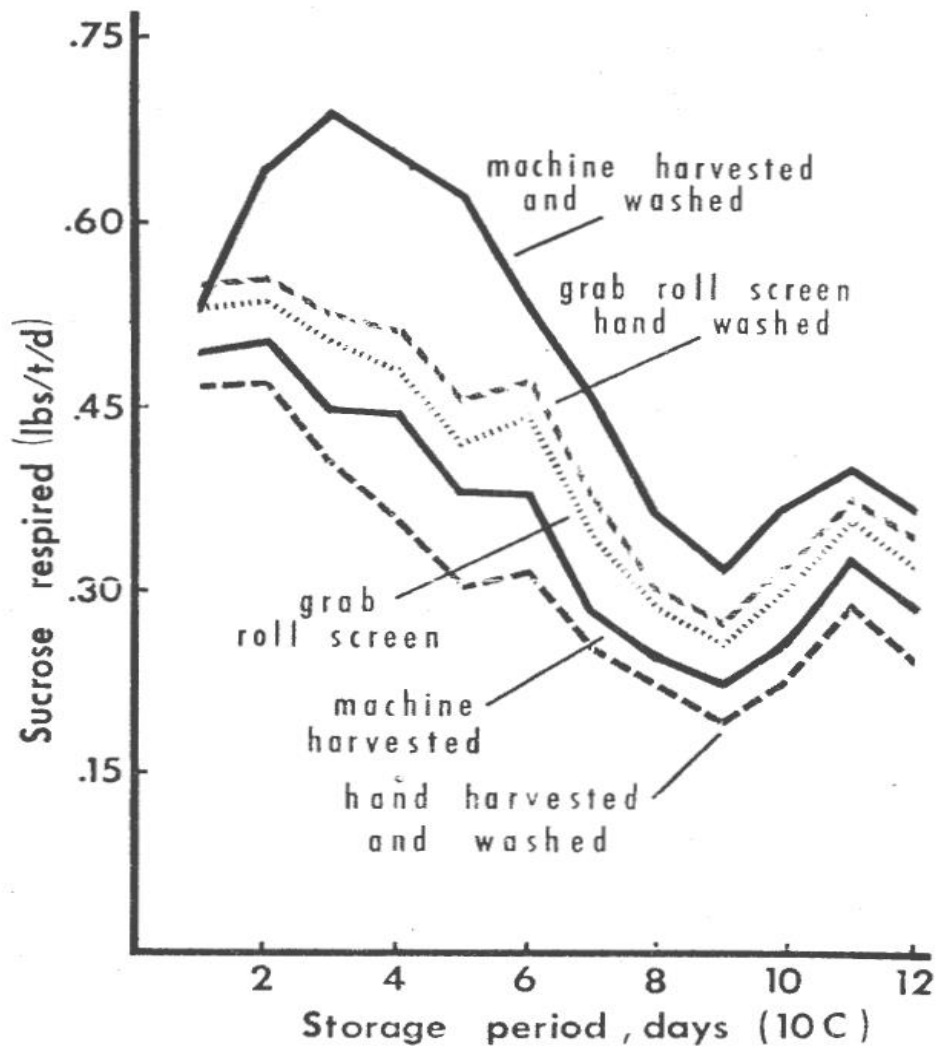


Figure 2-18: Effects of handling method on respiration rate of sugar beet roots (Wyse, 1973).

When sugar beet is stored in a clamp, with limited ventilation a decrease in oxygen concentration decreases respiration rate until a critical point is reached where anaerobic respiration begins. At an oxygen concentration below this point, anaerobic respiration becomes a greater proportion of the total, and the respiration rate increases again. Ethylene content and its effect on respiration were well documented by Fugate et al., (2010), who concluded that post-harvest sugar beet roots increase ethylene production in response to wounding resulting in an increased respiration rate. However, they suggested that ethylene production and ethylene effects on root respiration rate are likely to be small under commercial storage conditions and of limited economic significance.

2.5.2 Tissue damage

Data obtained through tissue tests has been widely used by scientists, consumers and growers to design equipment for sorting, grading, packing, conveying and storage systems. Nedomova et al., (2017) evaluated tissue properties for an early maturing sugar beet variety Gellert after storing for 1, 8, 22, 43, and 71 days at 4 °C and 85 % RH and concluded that failure strength of the sugar beet root tissues increases with storage time.

Kleuker and Hoffmann (2019) evaluated the effects of washing (washed and unwashed), storage time (3, 6, 24, 48, 120 and 168 hours after washing) and sample position (axial and radial) on resistance to tissue puncture and compression for two sugar beet varieties (Z-type and E-type) and they discovered that washing and storage did not significantly affect puncture resistance while sample position had a significant effect as radial measurements produced higher values (5.64 MPa) compared to axial values (5.28 MPa). Firmness was only affected by storage time, and significant differences were observed after 48 hours where it decreased by 0.62 MPa for the Z-type, and 0.37 MPa for the E-type. Sample position affected compression in the sense that inner part tissues of the root produced low resistance values compared to those taken from the root peripheral. The paper explains clear differences observed between the treatments on samples from the glass house. However, in field trials, sugar beet was compared to beetroot and folder beet where genotypic differences were observed. There is no explanation as to how the differences observed among sugar beet varieties in the glasshouse repeat themselves when the crop is grown in the field. This is a gap that therefore needs to be investigated. The paper also describes differences in sample distribution for compression test, but it does not indicate actual puncture resistance changes following the axial axis.

Kolodziej et al., (2019) concluded that to compress sugar beet tissue samples after 120 hours requires 40 % less energy than when the same sample is compressed immediately after harvest. This was concluded after carrying out a study on one sugar beet variety and the tissues were taken crosswise and length wise. Just like Nedomova (2017), this experiment did not consider all the positions of the sugar beet root. This is important as Kleuker and Hoffmann (2019) and Gemtos (1999) established that compressibility of sugar beet root tissues is subject to position

where tissues from the inner part require less energy than those from the outward position. Nause et al., 2020 conducted field trials using 6 varieties at 2 locations harvested in August and November and discovered that varieties with a high puncture resistance had a thinner inner tissue. They further stated that varieties with varying tissue strength also differed in fibre content, however the number of cambium rings had no effect on tissue strength. However, the study mainly focused on varieties only without considering how varying conditions in terms of water, temperature and soil nutrients can affect the tissue strength for sugar beet.

2.5.3 Pathogens

Storage of sugar beet in clamps is prone to sugar losses emanating from infections by pathogens that deteriorate the roots through rotting because clamps provide a favourable environment for growth of pathogens like bacteria and fungi. Cole and Bugbee (1976) reported that changes in sucrolytic activities are greatest (1) when roots were especially wet and dirt covered when piled, creating conditions favourable for the development of storage rots, (2) in roots at the pile surface, where frost damage and microbial infection were apparent, and (3) in roots at the pile base, where conditions that promote fungal and bacterial growth can occur due to inadequate ventilation. Sugar beet rots are mainly associated with fungal species namely; *Botrytis cinerea*, *Fusarium spp.*, *Penicillium spp.* and *Phoma betae* (Klotz and Finger, 2004; Liebe et al., 2016). The pathogens possess invertases that hydrolyse sucrose into invert sugar hence affecting sugar yield (Klotz and Finger, 2004) and sugar losses due to root rot have been reported to be around 1.2 % (Bugbee, 1982). Christ et al., (2011) concluded that *Fusarium redolens* was predominant in freshly harvested beets, while *F. culmorum*, *F. cerealis*, *F. graminearum* and *F. mycoflora* are subject to long-term clamp storage. Mumford and Wyse (1976) artificially inoculated damaged sugar beet roots with *Penicillium* and *Botrytis* fungi and observed that within one month, respiration rate for sugar beet doubled when 20 % of the sugar beet surface was infected by fungi, they also observed a threefold increase for non-reducing sugars when 15 % of the sugar beet surface was damaged. Schnepel and Hoffmann (2016) stored 24 varieties at two locations for 8 and 12 weeks at 8 °C and they observed that sugar losses correlated with invert sugar levels and was mainly dependent on pathogen infestation.

2.6 Factors affecting tissue damage

2.6.1 Mechanical damage

Mechanical damage of sugar beet mostly is a result of harvest machinery, soil type and seasonal factors. Harvesters differ in their harvesting system in terms of topping and lifting devices, but also the transport and cleaning aggregates. Schulze-Lammers (2015) states that harvesting speed, plant population, root diameter and agronomic information such as soil type, moisture and variety must be taken into account since they can increase mechanical damage. Hoffmann (2018b) conducted an experiment to assess damage on two sugar beet varieties when harvested with two six-row harvesters (axial roller versus turbine for cleaning) using three cleaning intensities. They concluded that diameter of root tip breakage and surface damage increased with cleaning intensity.

Soil type also has an influence on sugar beet in the sense that in heavy soils, clay particles adhere to the roots which can be a source of pathogens and requires harsher cleaning (Verulen, 2001). In the 2016 - 17 campaign a total of 4.2 t ha⁻¹ of dirt tare were extracted at the four British factories (Gabarron-Galeote et al., 2019). Effects of soil type on growth and performance of sugar beet were also well documented by Kenter et al., (2006b) who stated that on sandy soils, the growth of sugar beet in summer may be limited by rainfall, while loamy soils can retain water for the crop to use during drought. Qi et al., (2005) conducted studies at Broom's Barn and concluded that new leaves produced by sugar beet plants late in summer are larger on loam soils where water is more readily available than sandy soils leading to a more effective crop canopy and consequently a larger total dry matter and sugar yield. However, research has focused on how the soil type affects root yield and not mechanical properties of sugar beet.

Seasonal effects on mechanical damage were well documented by Van Swaaij et al., (2003) who observed least surface damage in the samples harvested in October for the first and second year while samples harvested in September registered the least damage during the third year. Root tip breakage was statistically worse for samples harvested in November for the second year and September during the third year. These results suggest that time of harvesting is dependent on many weather factors such as rainfall, soil type and seems to have varying effects on root tip breakage as well as surface damage.

2.6.2 Water status

Detrimental effects on plant tissues can be caused by disruption of plant water status through decreased availability of water in the environment during drought or cellular dehydration (Verslues et al., 2006). Water status has been heavily linked to tissue damage or cell strength because it determines turgidity. Largely parenchymatous crops are believed to be brittle and at high turgidity, protoplasts exert a pressure on cell walls reducing the amount of force required to induce fracture (McGarry, 1995). Studies in other horticultural crops like carrots have shown that the cell wall plays an important part in cell damage and that tissue strength is inversely related to turgidity (McGarry, 1993). Sugar beet in the UK is usually harvested in autumn and winter, when soil moisture is commonly close to field capacity (Gabarron-Galeote et al., 2019). Due to differences in soil type and rainfall, harvesting conditions vary across fields and seasons prompting growers to harvest at different soil moisture. However, there is little information on how varying soil moisture influence root tissue water status and strength which are widely linked to post-harvest injuries.

Schafer et al., (2020) compared varieties of sugar beet (Finola and Daphna), fodder beet (Ribambelle) and beetroot (Alto) and concluded that only compressive strength of sugar beet tissues increases with decreasing water content of the roots, but cell wall stability is dependent of the interactive effects of water status and overall cell wall composition. However, in this study, it is not mentioned how the two sugar beet varieties varied in terms of compression when water status was decreasing. Conducting studies on radish and carrots, Herppich et al., (2005) discovered that stiffness for the two crops showed a significant correlation with water status. They further concluded that for carrots, water status' interaction with temperature did not influence tissue strength. However, this study tested this interaction under relatively higher temperatures than the ones prevailing under optimum growing conditions. It is upon this basis that testing root crops resilience to tissue damage under growing conditions is paramount in post-harvest handling as it helps to syphon information that might help to design a harvesting strategy that can help to reduce injuries/damage. Smittle et al., (1974) examined the relationship between bruising induced by a falling bolt and turgidity over a wider range of water status (2.7, 5.6 and 11.2 % water loss) and they found that shatter damage was much worse in

more turgid tissue and was positively correlated with turgor while internal damage was worse in more flaccid tissue and was negatively correlated with turgor. The increase in internal damage for flaccid tissues could be due to The study measured both shatter and internal damage which are influenced by water status of tissues but there is no information whether harvesting sugar beet at field capacity would induce enough turgor pressure to replicate similar results.

Conducting studies on grapes, Nedomova et al., (2016) observed that puncture and compression resistance decrease when the crop is harvested late. However, recent research has suggested that resistance to puncture remains stable throughout the growing season (Nause et al., 2020). This paper did not define environmental conditions that would alter the mechanical properties of the sugar beet varieties used. This is suggested while bearing in mind that growing conditions for sugar beet are different from one farm to the other depending on environmental conditions. Hence studies that will manipulate the water status and assess mechanical properties would be more informing compared to uncontrolled and one site experiments which may lead to wrong conclusions.

2.6.3 Variety

Damage on the root tip and surface of sugar beet roots largely contribute to sugar losses during storage mostly in varieties with weak tissues (Hoffmann and Schnepel, 2016; Kleuker and Hoffmann, 2019). After storing 36 varieties for 8 and 12 weeks across two environments in Germany, Schnepel and Hoffmann (2014) observed significant differences among varieties, environment and their interaction in terms of sugar losses at 20°C where one variety registered the highest sugar loss of 15 % and another variety had the lowest sugar loss of 1 %. These results agree with those of Cole and Bugbee (1976) who reported that in the Red River of North Dakota and Minnesota, sugar beet clamp temperature stabilise to about 5 °C after 150 days and during this time, sugar loss averages around 0.25 g kg⁻¹. Gorzelany and Puchalski (2000) measured forces required to penetrate the skin of four polish sugar beet varieties (Prisma, Matador, Amelia and Milla) where 10 healthy roots of each variety in three sizes (small, medium, large, of 7 - 9; 9 - 12; 12 - 15 cm diameter respectively) were kept either in ambient conditions under a roof with average temperature of 5 °C and relative humidity of 75 % or a room with average temperature of 20 °C and relative humidity of 55 % for 45 days. They discovered

that average values for puncturing the skin of sugar beet varieties rose from 241.2 - 254.1 to 227.4 - 245.8 N for freshly harvested sugar beet after 45 days of storage, respectively. They further stated that average penetration distance required for rupture of the sugar beet increased with storage time. The increase was more pronounced in sugar beet that were stored under high temperature (20 °C). However, the paper does not state whether there were significant differences among varieties and no explanation has been given on how root size affected puncture resistance within the varieties. It would be imperative to indicate how size influence puncture resistance for growers to understand effects of poor crop management which mostly result in overgrown or small roots.

Van Swaaij and Huijbregts (2010) stored 12 varieties from six different countries for two months and observed that variety had an effect on the root tip breakage which highly correlated ($R = 0.66$) with sugar losses. Van Swaaij et al., (2003) investigated the effect of size, variety and harvest date on damage susceptibility in Netherlands using three varieties (Aristo, Cyntia and Madonna). They discovered that variety influenced root tip breakage and Cynthia had the least damage. Surface damage was affected by beet size in the first two years of experimentation where small beets registered greater damage than the large sized beet while in the third year, small beets had the least surface damage compared to the large beets. This clearly shows that much as genotypic differences provide a source for some resistance to root tip damage, other factors are involved. This could be confounded by environmental factors like temperature, soil and moisture that the roots were exposed to before the damage test was carried.

2.7 Assessing root texture

Resistance to compression, puncture and damage are tests used commonly for measuring root properties for agricultural products (Kabas and Ozmerzi, 2008; Sirisomboon and Pornchaloempong, 2011). Compression tests are usually employed to evaluate the resistance to tissue failure by the whole agricultural product while puncture tests are used to determine the penetration resistance at a point (Nedomova et al., 2016b). In the recent past, various appliances have been used to measure textural properties in horticultural crops. Harker et al., (1997) compared a penetrometer and tensile measurement methods on parenchyma tissues of bananas, watermelon, muskmelon, carrot, avocado and apple and

discovered that a tensile strength, along with measurements of puncture and shear resistance, showed a curvilinear relationship with sensory assessments of tissue hardness. However, such methods are only good when measuring puncture and shear resistances of the root skin and not compression resistance which is believed to play an important role on plant tissues resilience to external forces and damage.

Various attempts have been made to perfect textural assessment methods for sugar beet mechanical properties and few variations on lab procedures have been observed. Recently, English et al., (2022) examined textural properties in the field using a hand-held penetrometer (**Figure 2-19**) and observed strong correlation coefficients (0.86 and 0.94) between seasons and they concluded that a handheld penetrometer can be applied as an economic means of quantifying differences in textural properties of sugar beet varieties. Despite being viewed as the most economical way of measuring textural properties for sugar beets, the challenge with this method is that compression resistance values were not reported by the authors hence providing an incomplete information on how the penetrometer might help to assess all parameters related to texture (firmness, puncture, and compression resistance). Most texture assessment methods explained have been commonly applied on old or few sugar beet varieties used within the UK and some parts of Europe. However, some have become obsolete and not fit for the modern science. This means, a researcher must be cautious when choosing a method for evaluating mechanical properties on root crops like sugar beet. It is therefore important to employ modern methods such as texture analysers for generation of data that can be used to technically evaluate the recommended sugar beet varieties.



Figure 2-19: Hand held penetrometer (English et al., 2022)

2.7.1 Puncture Tests

Puncture resistance is one of the mechanical properties assessments that seem to be similar among many researchers. However, there are some minor differences regarding the actual procedure. Kleuker and Hoffmann (2019) calculated puncture resistance and tissue firmness from figures obtained from a TA.HD. plus texture analyser (Stable Micro Systems, Godalming, UK) with a 2 mm cylindrical probe (P/2) using a crosshead speed of 60 mm/minute up to a penetration depth of 5 mm. They found that when the roots are uniform, the method can achieve representative results with a small sample size, but with increasing heterogeneity of the roots a higher number of sampled roots is required.

Nedomova et al., (2017) used a similar method to that of Kleuker and Hoffmann (2019) but using a TIRATEST 27025 (TIRA Maschinenbau GmbH, Germany) with a different crosshead velocity of 20 mm/minute. The crosshead speed used varies across crops for example Canet et al., (2005) used a TA.HD texturometer (Stable Micro Systems, Godalming, UK) with a crosshead velocity of 50 mm/minute in potatoes. Gorzelany and Puchalski (2000) used a steel plunger of 8 mm diameter which was fitted on a micro tensile tester, Zwick model 1425 to measure puncture resistance of four sugar beet varieties (Prisma, Matador, Amelia and Nilla) in Poland.

A similar test was performed on radishes by Lockley et al., (2019) using a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England) by fitting with a P/2 cylindrical probe at a crosshead speed of 2 mms⁻¹ and the test distance was 160 mm. Conducting studies on oranges, Singh and Reddy (2006) fitted the texture analyser (Texture Analyzer (model:TAXT2i, Stable Microsystems, England) HDP/BSK blade set) with a 5 mm cylindrical probe with a load cell of 250 N where puncturing process was operated at a speed of 1 mms⁻¹ at a distance of 6 mm. This clearly shows that various crops require various types of puncturing probes and load cells. For example, in oranges, a load cell of up to 250 N has been used by researchers while sugar beet and other crops like radish, load cells of up to 50-100 N have been used.

2.7.2 Damage tests

Root damage at harvest cannot be completely avoided but it is related with an increase in storage losses, such that several efforts have been made by various researchers to reduce damage Kenter et al., (2006a). In the recent past, old methods of assessing tissue damage on roots crops were improvised though not very efficient. Ibrahim et al., (2001) assessed damage of sugar beet plants by dropping a bolt that weighed 470 g through a 60 cm tube onto the sugar beet samples to generate an equal energy that impacted on sugar beet and he compared non-impacted and impacted beet after 3 hours, 3, 9 and 24 days.

Hoffmann and Schnepel (2016) used modern methods to assess effects of root damage at harvest by applying artificial damage through rotation of sugar beet roots for 60 seconds in a rotating drum (**Figure 2-20**). The diameter of the tip breakage in this experiment was measured in the following diameter classes: 0-2cm, >2-4cm,

>4-6cm, >6-8cm and >8cm as described by (BBRO, 2022; Schulze-Lammers et al., 2015). Calibration factors are used to determine the relative weight of root lost within each breakage diameter class. Commercially, BBRO (2022) developed a protocol (**Table 2-5**) for assessing root damage which suggests that if breakage diameter for every 10% of the sample is between 8-10 cm, then yield loss will be about 3 tonnes ha⁻¹.

Table 2-5: Commercial protocol for assessing sugar beet damage by BBRO (2022)

Root breakage diameter (cm)	For every 10% of roots in each sample	Yield loss t/ha
2-4	10%	0.5
4-6	10%	1
6-8	10%	2
8-10	10%	3

The speed of the rotating turbines varies among studies, Van Swaij et al., (2003) conducted a damage test on three sugar beet varieties by rotating the sugar beet in a drum at a speed of 45 rpm for 15 seconds. While by Kenter et al., (2006a) adopted the same method by increasing the time to 45 seconds. The change of rotating duration to 45 seconds by Kenter et al., (2006a) and 60 seconds by Hoffmann et al., (2016) was not accompanied by any technical or scientific justification. However, Van Swaij et al., (2003) stated that the damage caused by the standardised treatment in the turbine correlated well ($R = +0.94$) with damage caused by machine harvesting in the field.



Figure 2-20: A rotating drum used to artificially cause root tip breakage and surface damage in sugar beet roots (Schnepel and Hoffmann, 2016b).

2.7.3 Compression test

Methods for compression resistance analysis such as near-infrared spectroscopy have provided a poor ability to predict the compressive mechanical properties of both beet slices and intact beets compared to visible texture analyser (Pan et al., 2015). Kleuker et al (2019) used 75 mm compression plates (P/75) until rupture with a crosshead speed of 60 mm/minute until the maximum pressure at rupture was measured as compressive strength. This method is better compared to the old pendulum method which made it impossible to entirely remove vibration effects in the sugar beet root or in the arm during the rebound course of the pendulum. Bentini et al., (2005) evaluated sugar beet compression by using the pendulum method at three heights (100, 200, and 300 mm above the height of the fixing point of the root on the pendulum arm) but improved it by calculating impact peak acceleration, impact velocity change and impact duration in the compression phase. Most researchers have found it difficult to calculate the rebound height for this method making it difficult to estimate the impact (Opara and Pathare, 2014). However,

Opara et al., (2007) improved the method by developing a new system that helps to determine the pendulum height during rebound where a video camera was used and this was perceived to be better than visual assessment of the rebound height.

Kolodziej et al., (2019) evaluated sugar beet compression failure by cutting samples of 9 mm in diameter and 20 mm high from the central part of the root axially and laterally using a punching die and deformed them by 5 mm using a cylindrical hammer that had a 3.5 kg mass at a velocity of 1 m s^{-1} where an Endevco model 2311-100 piezoelectric force sensor of 2 mV N^{-1} sensitivity with a measuring range of 222.5 N was installed to measure the force required to compress the sugar beet samples. Errington et al., (1997) Carried out compression tests on tomatoes using a Stable Micro Systems TA-XT2 texture analyser fitted with a 10 cm diameter flat circular plate and using Xtra Dimension v3.2 data capture and analysis software using a force of 4 N and at a crosshead speed of 10 mm s^{-1} . In an improved version, Oey et al., (2006) inserted a stereomicroscope (SMZ1000, Nikon, Japan) equipped with a CCD camera (type TK-1360B colour $\frac{1}{2}$ inch CCD, JVC) on a miniature tensile (Deben Microtest, Suffolk, U.K.) to measure and monitor compression of the cells in apples.

2.8 Conclusions

Research on sugar beet tissue damage has been conducted in several countries with a focus on storage factors like time, temperature and their effects on sugar beet mechanical properties in storage. Despite some harvesting campaigns getting prolonged in most countries across Europe, little attention has been given to how late harvesting accelerates or improves root damage despite the adverse effects that prevailing conditions may have on plant tissues. At the same time, most researchers have reported results for storage trials without assessing the underlying physiological factors like to compression, shear, and puncture resistance. The effect of sugar beet varieties on sugar losses regarding year and site has been well documented in literature but the physiological factors that underpin genotypic differences in tissue damage have not been extensively highlighted. A further check on the literature shows that most studies on variety tissue strength have not been extensively done on varieties commonly used in the UK.

There is also clear direction regarding some agronomic practices on sugar beet like nutrition, water or moisture regimes, drilling, pH values, harvesting and clamping. However, there is no explanation as to how a deficiency or excess of these factors contribute to sugar beet plant tissue's vulnerability to damage in the field. For example, excess water during harvest may have compelling effects on plant tissues and their response to damage during mechanical harvesting. However, there is an information gap as to what is the best moisture content for a grower to consider when harvesting. Research outside the UK has also been widely conducted on root tip and surface damage on sugar beet varieties. However, there are no studies that have evaluated the recommended varieties used in the UK. Despite the literature's strong indication that plant nutrition is a focal point for plant tissue's rigidity and strength, the effect of plant nutrients that are directly involved in building plant cell walls on tissue damage has not been evaluated. B and Ca were well documented to have a direct effect on building the cell walls and making the cells strong but there are no studies on how these can be maximised to minimise damage related losses. This, therefore, calls for an intensive physiological and environmental study through usage of efficient methods of assessing genetic resilience to damage.

2.9 Research hypotheses

Having carried out an extensive literature review that revealed existing gaps in this research area. The study addressed some gaps by focusing on the following hypotheses.

- Variety affects sugar beet root tissue damage susceptibility and resilience
- Root morphology influences the variety's resilience to damage
- Textural properties affect sugar beet root resilience to tissue damage
- Harvesting time influences textural properties and the root's resilience to tissue damage.
- Water status at harvest affects sugar beet's tissue damage susceptibility
- Root temperature at harvest affects sugar beet's tissue damage susceptibility
- Interaction between temp and water status affects sugar beet's tissue damage susceptibility
- A foliar B application affects sugar beet response to root damage, textural properties and impurities
- A foliar application of Ca affects sugar beet response to root damage, textural properties and impurities

Chapter 3: Effects of sugar beet root morphology and variety on root damage and tissue integrity

3.1 Abstract

Despite varieties being reported to statistically differ in terms of resilience to tissue damage, there is no information that correlates physiological and morphological properties to sugar beet tissue strength among recommended varieties and environments in the UK. Specifically, the aims of this experiment were to 1) identify weak and strong sugar beet varieties for tissue damage susceptibility and resilience, 2) identify morphological factors that affect a variety's resilience to root breakage, 3) identify textural properties that affect resilience to root breakage 4) study the effect of delayed harvesting on textural properties and resilience to tissue damage. Sugar beet roots were sampled from field experiments at Bracebridge, Lincolnshire and Fotheringhay, Northamptonshire during 2019, 2020 and 2021 seasons. The experiments were laid in a randomised complete block design with eight varieties from the 2019 Recommended List and three replications. At Bracebridge harvesting was done twice with the first one at physiological maturity and the second one 30 days later in order to assess the effects of sequential harvesting. Tissue properties were assessed post-harvest, and it was observed that root tip diameter after damage correlated to tissue compression, root width, length, and root weight. Surface damage negatively correlated with puncture resistance and cumulative rainfall 30 days prior to harvest. Puncture resistance decreased from the crown to the tip and compression resistance was lowest in the root's central region than peripheral and the middle. Our results suggest that variety and morphology form part of the key factors in minimising tissue damage. Scheduling of harvesting was also seen to be a factor affecting compression suggesting that root maturity contributes towards tissue texture.

3.2 Introduction

Sugar beet (*Beta vulgaris*) is a biennial plant with an epigeal germination which develops into a rosette of glabrous, dark green, glossy leaves with prominent midribs and strong petioles (Elliott and Weston, 1993). Its stems are decumbent, ascending or erect, and branched which can grow up to 120 cm in height. The root system consists of three main parts namely crown, neck and cone-shaped root where the crown produces leaves (Guss, 2006; Schulze-Lammers et al., 2015). Root tip and surface damage occur during lifting, cleaning within the harvester, and loading and unloading in the hopper phase where devices apply a combination of vertical and horizontal forces to the root. As the beet is strongly anchored into the soil by an extensive network of rootlets, the applied uprooting forces are resisted by the soil causing root breakage whenever the set-up stress exceeds the strength of the root material (Verulen, 2001). During hopper loading, hopper storage and hopper unloading, the root tip and surface damage occur due to impact, when beets are not delivered smoothly from one transport device to the other, or when drop heights are excessive. Despite reports indicating that varieties respond differently to damage and the strong link between damage and sugar losses, there is no information that correlates sugar beet physiological and morphological properties that link to tissues' resilience to damage. There is also little information regarding robustness to damage and textural properties among the currently recommended varieties and environments in the UK. This study compares selected recommended varieties in the UK and studies the underlying physiological and morphological factors that correlate with resilience or susceptibility to damage.

This study hypothesised that variety and agronomic management influence the physiological and morphological properties that contribute towards root tissue strength of sugar beet varieties. The aims of the study were to: 1) identify sugar beet varieties for tissue damage susceptibility and resilience, 2) identify morphological factors that affect a variety's resilience to root breakage, 3) identify textural properties that affect resilience to root breakage 4) study the effect of delayed harvesting on textural properties and resilience to tissue damage.

3.3 Materials and methods

3.3.1 Sites

The study was conducted at Bracebridge (53°09'43.4"N 0°28'51.6"W) and Fotheringhay (52°32'22.4"N 0°24'59.0"W) in East Midlands of England during 2019, 2020 and 2021 seasons. The soils were analysed at NRM laboratories - Cawood scientific by following analytical procedures of MAFF (1986). The soils at Fotheringhay are predominantly sandy loam (**Table 3-1**) and apart from nitrogen which was lower, the nutrient contents and pH were within normal ranges required for sugar beet production. The field used for the first-year experiment at Bracebridge was a loamy sand and the field used for the second-year experiment was a sandy loam. From the day of planting to harvesting, Fotheringhay received a total amount of 546, 350 and 340 mm of rain in 2019, 2020 and 2021 respectively while Bracebridge received 776 mm in 2019, 269 mm in 2020 and 347 mm in 2021. Bracebridge experienced a sporadic distribution of rain from the day of planting to the second harvesting (**Figure 3-2, Figure 3-3, Figure 3-4**) in all years, while Fotheringhay experienced severe drought 30 days after planting in 2020 (**Figure 3-3**). From the day of planting to harvesting, Bracebridge had an average temperature of 11 °C with minimum and maximum temperatures of 1 and 26 °C in 2019. In 2020, the average temperature for Bracebridge was 11 °C with a minimum of -1 °C and a maximum of 24 °C from the day of planting to harvesting. While in 2021, Bracebridge recorded an average temperature of 11 °C with minimum and maximum temperatures of -2 °C and 26 °C, respectively from the day of planting to harvesting. Fotheringhay had an average temperature of 12 °C and minimum and maximum temperatures of -2 °C and 26 °C respectively in 2019 from the day of planting to harvesting. In 2020, Fotheringhay registered an average temperature of 13 °C and minimum and maximum temperatures of -1 and 24 °C. In 2021, Fotheringhay recorded minimum, maximum and average temperature of -2, 26 and 12 °C, respectively.

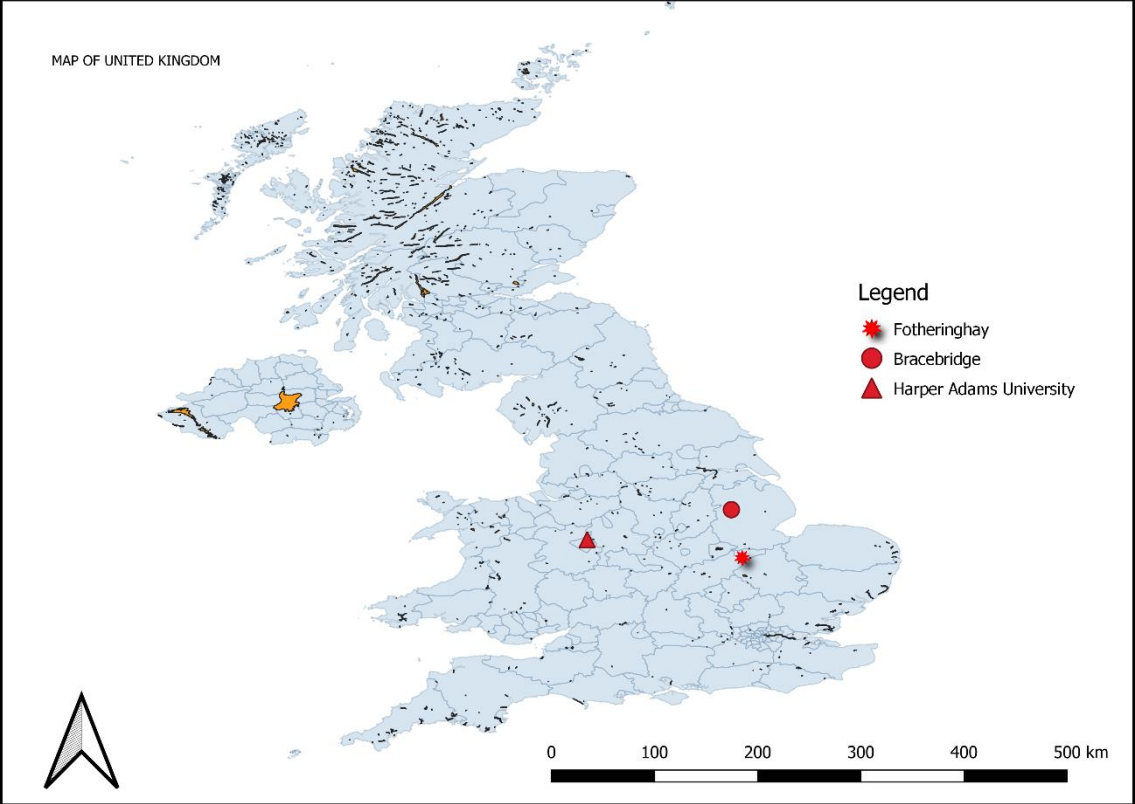


Figure 3-1: Map showing trial sites

Table 3-1: Soil characteristics for Bracebridge and Fotheringhay.

Measurement	Bracebridge			Fotheringhay		
	2019	2020	2021	2019	2020	2021
pH	7.9	8.5	8.1	7.1	7.6	7.7
P mg L ⁻¹ (Available)	24	46	43	19	41	43
K mg L ⁻¹ (Available)	189	127	178	149	181	179
Mg mg L ⁻¹ (Available)	53	47	52	84	37	59
Cu (EDTA Extractable) mg L ⁻¹	3	6	8	4	4	4
B (Hot Water Soluble) mg L ⁻¹	1.3	1.2	1.9	1.8	0.9	0.8
Na (Ammonium Nitrate Extractable) mg L ⁻¹	5	5	7	11	12	6
Zn (EDTA Extractable) mg L ⁻¹	4	5	7	2	2	4
Ca (Ammonium Nitrate Extractable) mg L ⁻¹	1699	1816	2123	2381	1629	2067
Fe (DPTA Extractable) mg L ⁻¹	16	23	20	60	50	56
Organic matter (LOI) %	3	3	4	5	4	4
S (Phosphate Buffer Extractable) mg L ⁻¹	16	13	13	34	8	28
Mn (DPTA Extractable) mg L ⁻¹	7	7	6	9	10	7
Sand (2.00 - 0.063mm) %	81	75	78	59	57	56
Silt (0.063 - 0.002mm) %	10	11	10	26	27	28
Clay (< 0.002mm) %	9	14	12	15	16	16
Textural Classification	Loamy sand	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Estimated CEC meq 100 ⁻¹	12	13	15	17	12	15

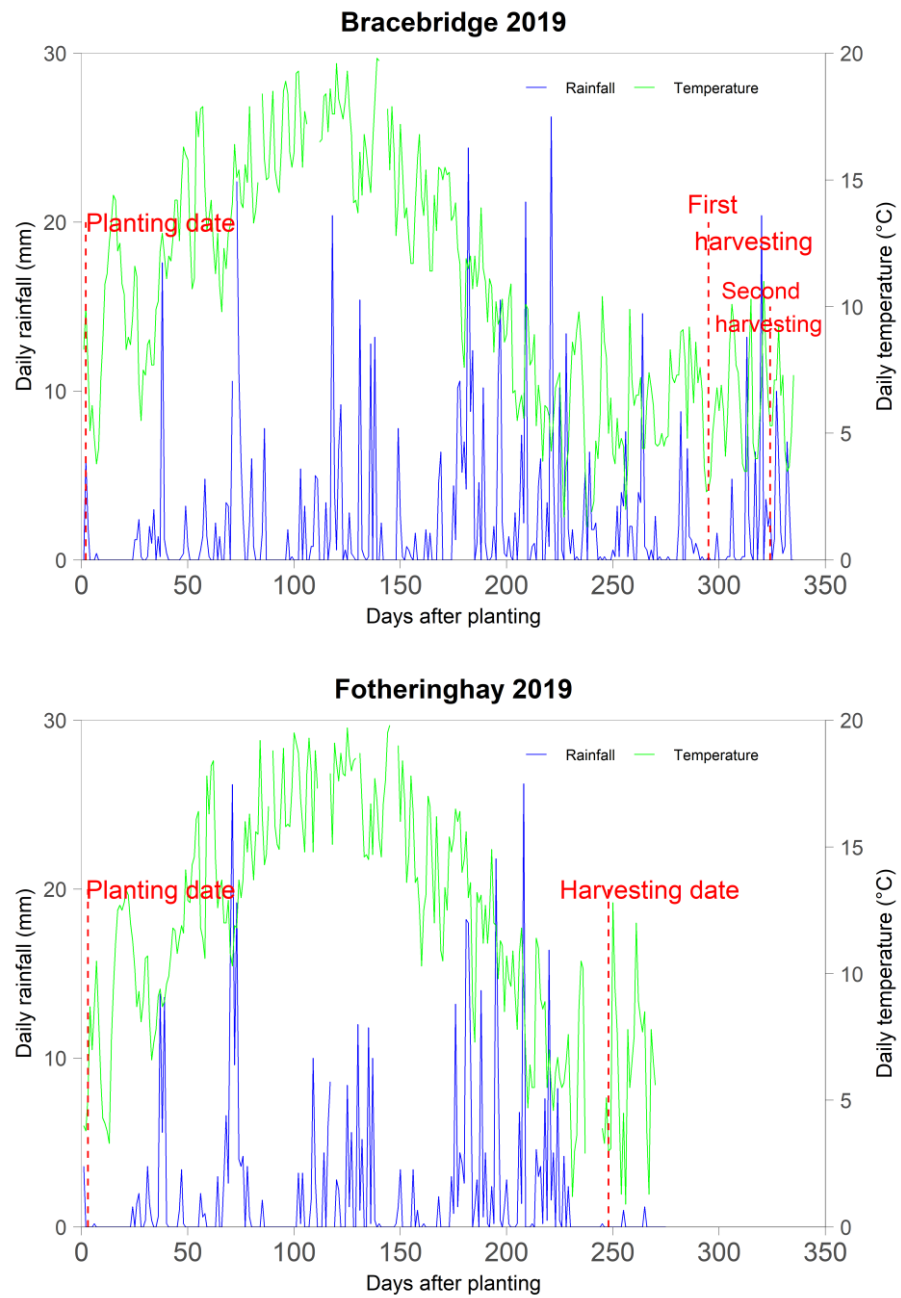


Figure 3-2: Daily rainfall and temperature distribution collected on the experimental site using a BBRO weather station at Bracebridge and Fotheringhay in 2019.

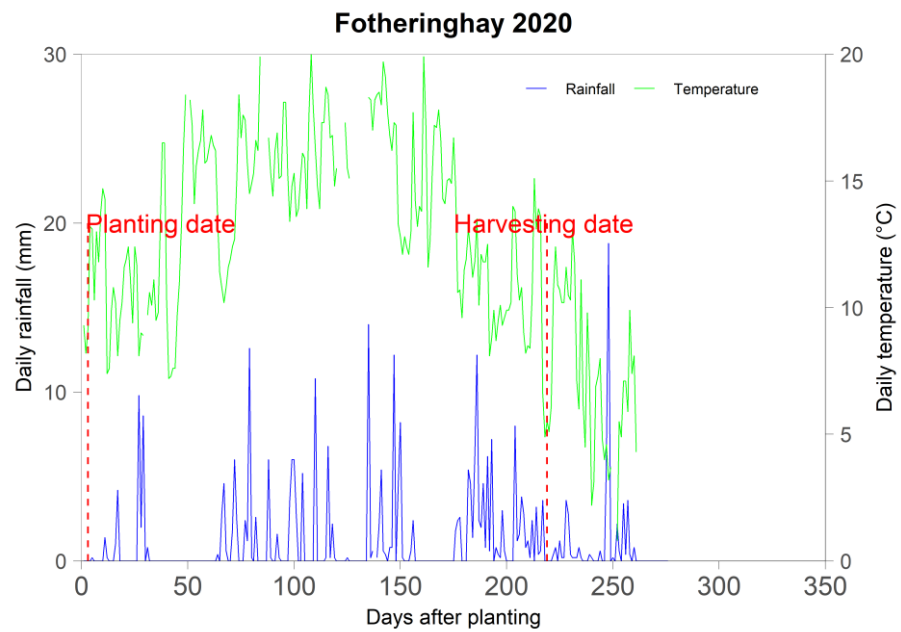
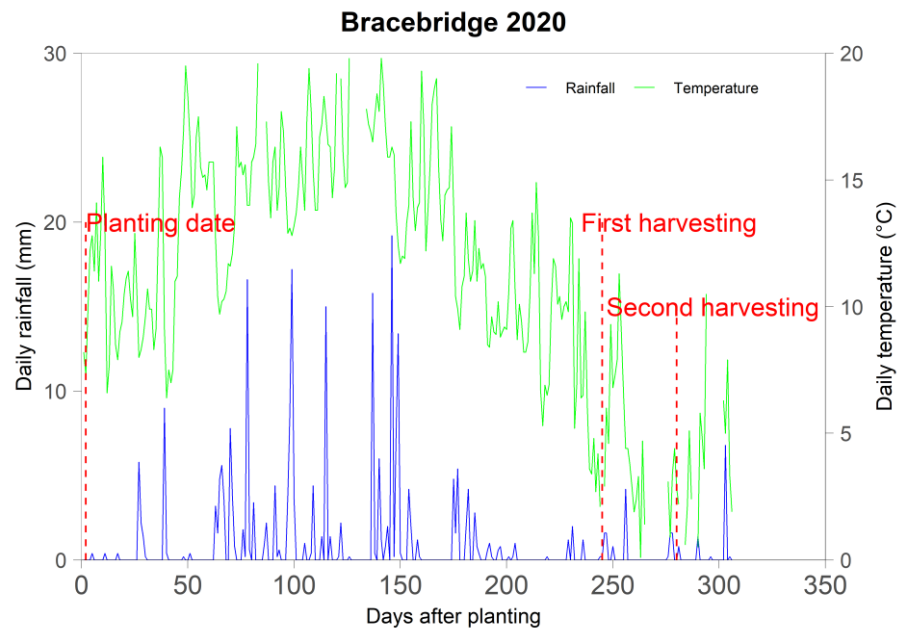


Figure 3-3: Daily rainfall and temperature distribution collected on the experimental site using a BBRO weather station at Bracebridge and Fotheringhay in 2020

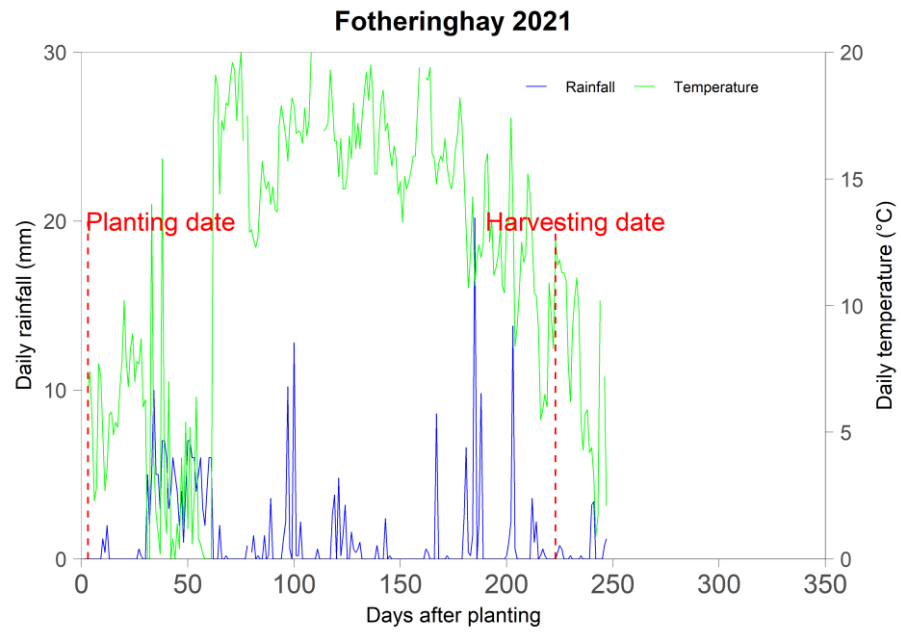
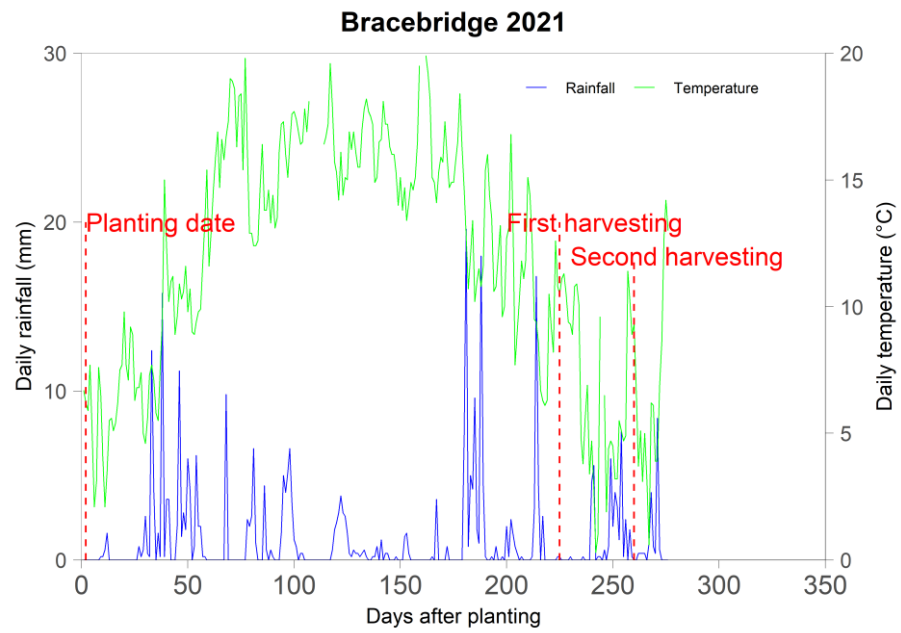


Figure 3-4: Daily rainfall and temperature distribution collected on the experimental site using a BBRO weather station at Bracebridge and Fotheringhay in 2021.

3.3.2 Varieties

Varieties used were chosen from the 2019 recommended list BBRO (2019). The list has 25 varieties however, eight (Sabatina, Hornet, Haydn, Daphna, BTS1140, BTS3325, Gauguin and Firefly) (**Table 3-2**) were selected based on availability, root yield, sugar content and breeding companies. They are all high yielding with root yield of above 100 tonnes ha⁻¹ and sugar content of approximately 18 %. They also represented all breeding companies across the UK (Betaseed, KWS UK Ltd, Limagrain UK Ltd, Strube Sugar Beet UK Ltd and SESVanderHave UK Ltd) apart from MariboHilleshog (MH).

Table 3-2: Variety characteristics

Description	Mean (for all varieties on RL)	Variety							
		Sabatina	Hornet	Haydn	Daphna	BTS1140	BTS3325	Gauguin	Firefly
Adjusted tonnes % of C = 100% ²	113.7t/ha	103.9	100.7	99.9	107.9	107.6	103.1	101.3	100.2
Sugar yield % of C = 100% ²	17.3 t/ha	104.1	100.9	99.9	108.3	108	103	102	100.4
Root yield % of C = 100% ²	95.5 t/ha	105.7	102.7	100.2	110.4	108	102.2	104	101.7
Sugar content %	18.10	17.8	17.8	18.1	17.8	18	18.3	17.7	17.9
Year first listed		2015	2014	2013	2017	-	2017	-	2016
Breeder		KWS	SV	STR	KWS	BTS	BTS	STR	SV
UK Agent		KWS	SV	STR	KWS	LG	LG	STR	SV

Note: BTS = Betaseed, KWS = KWS UK Ltd, LG = Limagrain UK Ltd, STR = Strube UK Ltd, SV = SESVanderHave UK Ltd

3.3.3 Experimental design and field management

Field trials were drilled during 2019, 2020 and 2021 seasons in the UK at Bracebridge, Lincolnshire and Fotheringhay, Northamptonshire. At both sites, one seed was drilled per planting station with an intra and inter-row spacing of 16 and 50 cm, respectively, giving a total plant population of 12.5 m⁻². The plots were six metres long and three rows were planted per experimental unit. The trial was drilled in a randomised complete block design with three blocks. At Bracebridge harvesting was done twice with the first one at physiological maturity and the second one 30 days later in order to assess the effects of sequential harvesting. At Fotheringhay, harvesting was only done at physiological maturity. Physiological maturity was determined when the crop had reached at least 1300 growth degree days (GDD) which is the minimum for sugar beet (Neamatollahi et al., 2012). GDD is defined as the number of temperature degrees above a certain threshold temperature, which

varies among crop species (1 °C for sugar beet). Planting and harvesting dates for all trials at both sites were as detailed in **Table 3-3**.

Table 3-3: Planting and harvesting dates at the experimental sites

Site	Year	Planting date	First harvesting date	second harvest harvesting date
Bracebridge	2019	01/04/2019	20/01/2020	18/02/2020
Bracebridge	2020	02/04/2020	01/12/2020	05/01/2021
Bracebridge	2021	30/03/2021	09/11/2021	13/12/2021
Fotheringhay	2019	02/04/2019	04/12/2019	-
Fotheringhay	2020	01/04/2020	05/11/2020	-
Fotheringhay	2021	01/04/2021	09/11/2021	-

3.3.4 Sampling

Power analysis which is calculation used to estimate the smallest sample size needed for an experiment, given a required significance level, statistical power, and effect size was used to determine the sample size for the damage test (Cohen, 1988). We used effect size for damage tests and other variables as detailed by Van Swaaij et al (2003). Power was estimated at 0.90 with an effect size and significance level of 0.15 and 0.05, respectively. This gave a total number of 120 plants for the whole experiment meaning 5 roots per variety were needed for the damage test. The sample size for textural analysis was reduced to 2 per plot. This was following Kleuker et al., (2019) who reported that number of roots for uniform sugar beet varieties marginally affects textural results meaning that stable results are achievable with a relatively small sample size. Sugar beet roots were systematically sampled from the middle row within each plot (**Figure 3-5**) (Ehler et al., 1997). Before sampling, 2 metres were cut off from either side of the sampling row (**Error! Reference source not found.**) to avoid the edge effects. Fifteen roots were gently lifted by hand from the sampling row. Ten uniform roots were put in storage sacks and taken to Harper Adams University (HAU) where they were stored in a cold room at 6 °C and relative humidity of 85 %.



Figure 3-5: Sampling plot

3.3.5 Texture analysis

Texture analysis was done 48 hours after harvesting and the samples were washed using water (**Figure 3-6**) before subjecting them to a texture analyser. The tests were performed according to Kleuker et al., (2019) and (Nause et al., 2020) using a texture analyser (TA.HDplus - Stable Microsystems Texture analyser, Godalming UK) (**Figure 3-7**). The texture analyser was loaded with a 500 Kg load cell. Force values obtained were converted to megapascals (MPa) for comparison's sake. This was done by dividing the amount of force by the probes and sample area for figures associated with resistance to puncture and compression resistance, respectively.



Figure 3-6: Washing sugar beet root samples



Figure 3-7: Texture analyser.

3.3.5.1 Puncture and shear resistances

The texture analyser was fitted with a P/2 cylindrical probe at a crosshead speed of 60 mm per minute (Kleuker and Hoffmann, 2019; Nause et al., 2020). The force test was performed up to a penetration depth of 5 mm (**Figure 3-8**) and the force required to penetrate the periderm was considered as the puncture resistance. While shear resistance was considered as the average force from 0.5 mm after rupture until 5 mm. Using two root samples per plot, the puncture and shear resistance tests were performed axially at the top, middle and root tip (**Figure 3-9**). The use of a lower sample number per position was justified by Kleuker et al., (2019) who observed that sample number and tests per position do not affect the results when the samples are uniform or graded. Hence, only one test was performed per each position giving a total of six tests from two roots per variety.

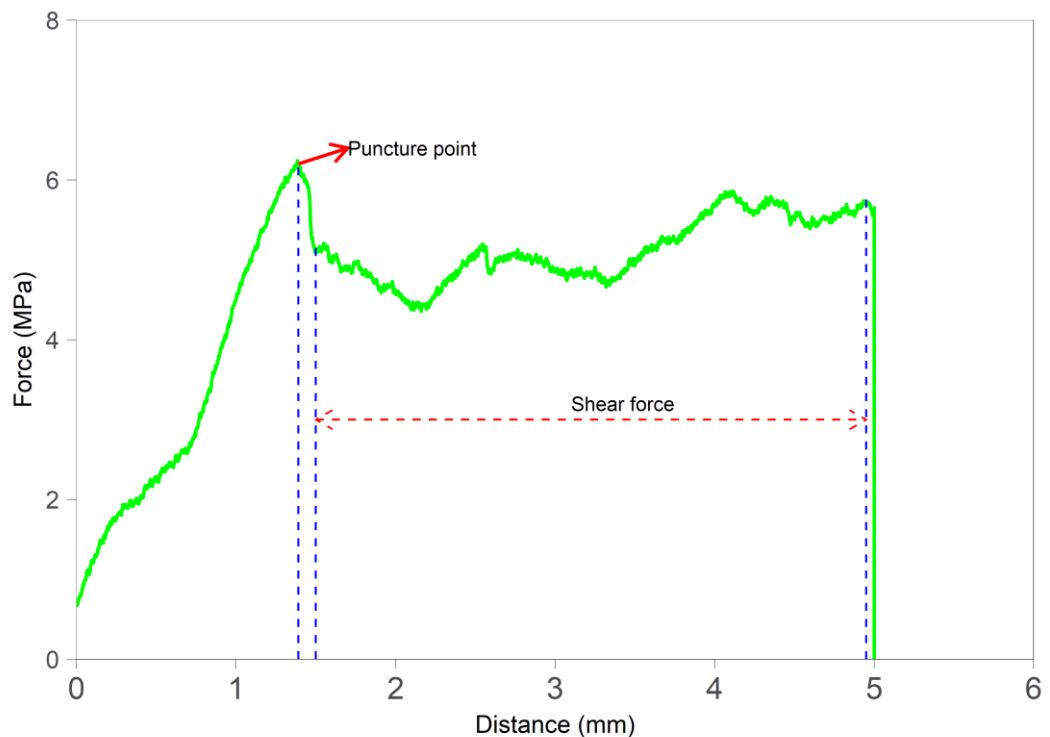


Figure 3-8: Puncture and shear points during one of the tests.



Figure 3-9: Axial distribution of sampling points for puncture test.

3.3.5.2 Compression resistance

A 25 mm high cylindrical section was cut at the widest circumference of the root and samples were taken using an 18 mm cork borer from the edge, between, and centre of the cross-section (**Figure 3-10**). In some varieties, the roots were very small for all the samples to be taken from one side, hence one of the samples was taken from the other side of the root provided they were from the edge, between and centre (**Figure 3-11**). Samples from these three positions were further reduced in height to 20 mm using a knife. Two roots were used from one plot making a total of six samples from one plot and a total of 432 for the whole study. A P/75 cylindrical probe at a crosshead speed of 60 mm/minute was used to compress the samples and the force at which the tissues ruptured was recorded (**Figure 3-12**). Kleuker and Hoffmann (2019) reported that compression resistance of sugar beet tissues significantly decreases two days after washing. Hence, all roots were compressed within two days after washing and roots from the same replication were compressed on the same day to avoid the effects of storage time. The roots used in this test were those that were used also in the puncture test.

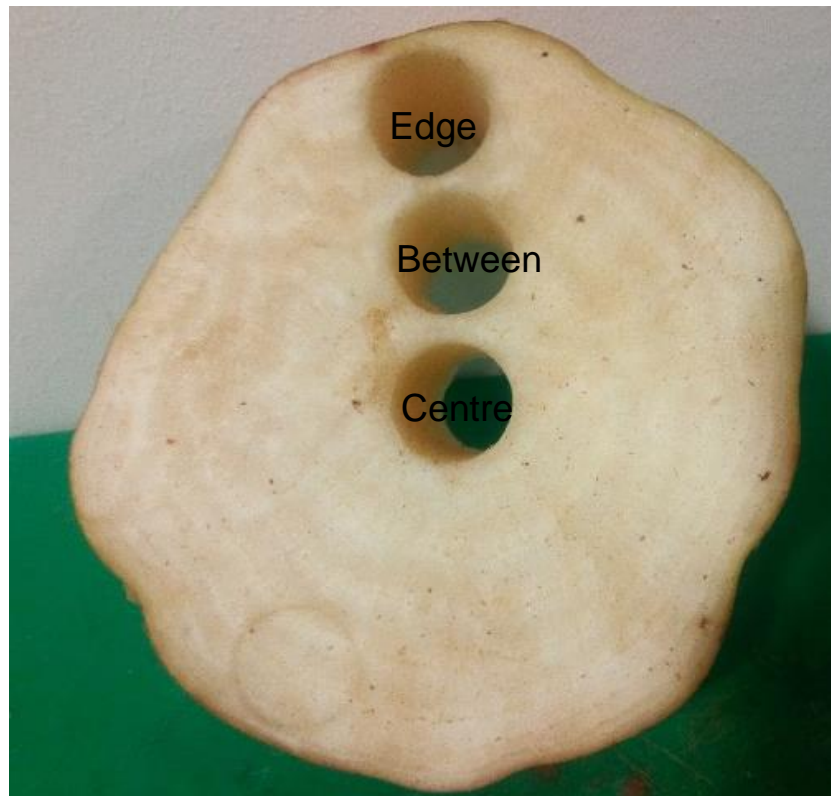


Figure 3-10: Sampling positions on a cut cross-section of a sugar beet root.



Figure 3-11: Cross sections of roots and their extracted samples

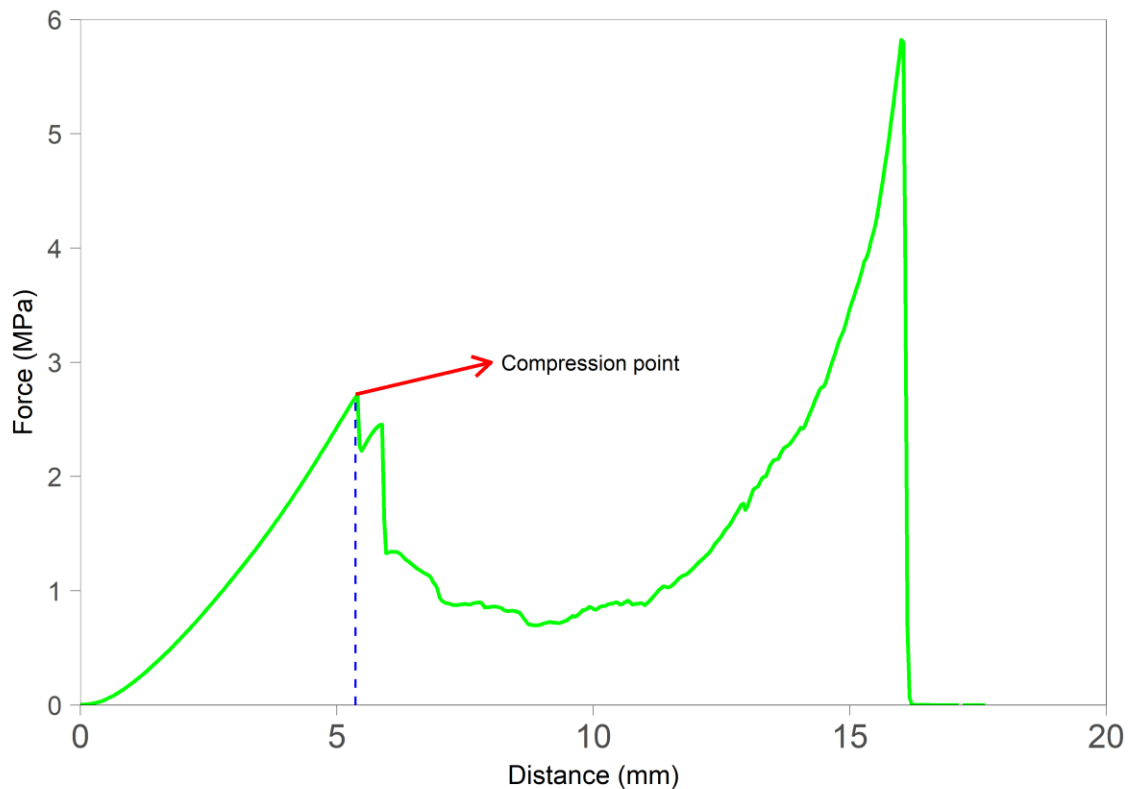


Figure 3-12: Compression resistance during one of the tests.

3.3.6 Damage test

A controlled root damage assay developed after Van Swaaij et al., (2003), Kenter et al., (2006a) and Hoffmann and Schnepel (2016) was optimised by exposing hand-harvested sugar beet roots from the trial sites to controlled damage in a rotating drum. The rotating drum (**Figure 3-13**) had a diameter of 0.6 m and a length of 1 m and was set at a speed of 45 rpm. It was made of 30 mm x 30 mm metal bars spaced 6 cm apart and the sides were covered by a 2 mm metal plate (Van Swaaij et al., 2003). It was connected to a motor which was rotated by an oil pump. Ten uniform roots were selected from the sample and put in a plastic tray (**Figure 3-14**). Morphological and physiological data including length, width, weight and root tip diameter were recorded before damage. Root length was measured from the top to the tip of the root while root width was measured on the widest circumference of the root. The root tip diameter was measured at the widest circumference of the tips. The roots were then put into the rotating drum where they were cleaned for 15 seconds. After cleaning, weight and root tip diameter were measured again (**Figure 3-15**). Surface damage which resulted due to tissues being snapped from the skin of the root (**Figure 3-16**) was scored visually as a percentage.



Figure 3-13: Rotating drum.



Figure 3-14: A sample of ten sugar beet roots before damage.



Figure 3-15: Ten samples of beets after damage



Figure 3-16: Tissues snapped from roots.

3.3.7 Data analysis

Data analysis was done using a linear mixed model (Luke, 2017) in R version 4.2.2 within the *lmerTest* package (Kuznetsova et al., 2017) with replication as a random factor. Before the analysis, each variable was subjected to ANOVA assumptions using the Shapiro-Wilk test and where the populations were not normally distributed, the data was log-transformed. Data from each site was analysed separately where variety and season were fitted as fixed factors while replication was treated as a random factor. When comparing the effects of harvesting time at Bracebridge fixed factors included: harvest date, season, and variety using first and second harvest data sets. In both analyses, a fourth fixed factor, position, was added when analysing textural data i.e., compression, puncture, and shear resistance to determine the effect of position. A post hoc analysis was performed in *emmeans* R package (Russell et al., 2023) using Tukey's Honest Significance Difference (HSD) test at a significance level of 0.05. A linear regression was done in R where Pearson's correlation was calculated to check relationship strength among morphological, physiological, and textural properties at a significance level of 0.05.

A principal component analysis was also performed using data from Fotheringhay and the first harvest at Bracebridge to check traits that contribute to variation among varieties. Using data from the first harvest at Bracebridge and Fotheringhay, cluster and silhouette analyses (Liu et al., 2022) were conducted using a complete linkage method to check variety clusters and dominant traits within and across clusters. Graphing was done using *ggplot2* (Wickham, 2009) R package and where box plots have been used, black dots represent the mean value. Treatments with different superscripts mean they are statistically different from each other.

3.4 Results

3.4.1 Effects of site, variety, season and their interactions

3.4.1.1 Morphological features

Morphological traits measured in this study included length, width and root tip diameter before damage. At both sites, root length was affected by variety ($p < 0.001$). Sabatina produced shortest roots at both Fotheringhay (21 cm) and Bracebridge (21 cm) and the trend was similar for other varieties where Haydn was relatively shorter at both sites (**Figure 3-17**). Other varieties were statistically longer than Sabatina with BTS1140 registering 29 cm at Bracebridge and Gauguin 28cm at Fotheringhay. Variety only influenced root width at Fotheringhay ($p < 0.001$) while at Bracebridge, statistical differences were not observed. Sabatina was observed to be the widest at Fotheringhay (13 cm) while the rest were not different. The diameter of the root tips before damage was dependent only on the variety ($p < 0.01$) at Bracebridge only where Haydn and Sabatina had wider tips before damage while Gauguin, Daphna, Firefly, Hornet, BTS3325 and BTS1140 had smaller tips (**Figure 3-19**). There was a negative correlation between root tip diameter before damage and the length of the roots at both Bracebridge ($R^2=0.63$, $p=0.018$) while at Fotheringhay the relationship was weak and not significant (**Figure 3-20**).

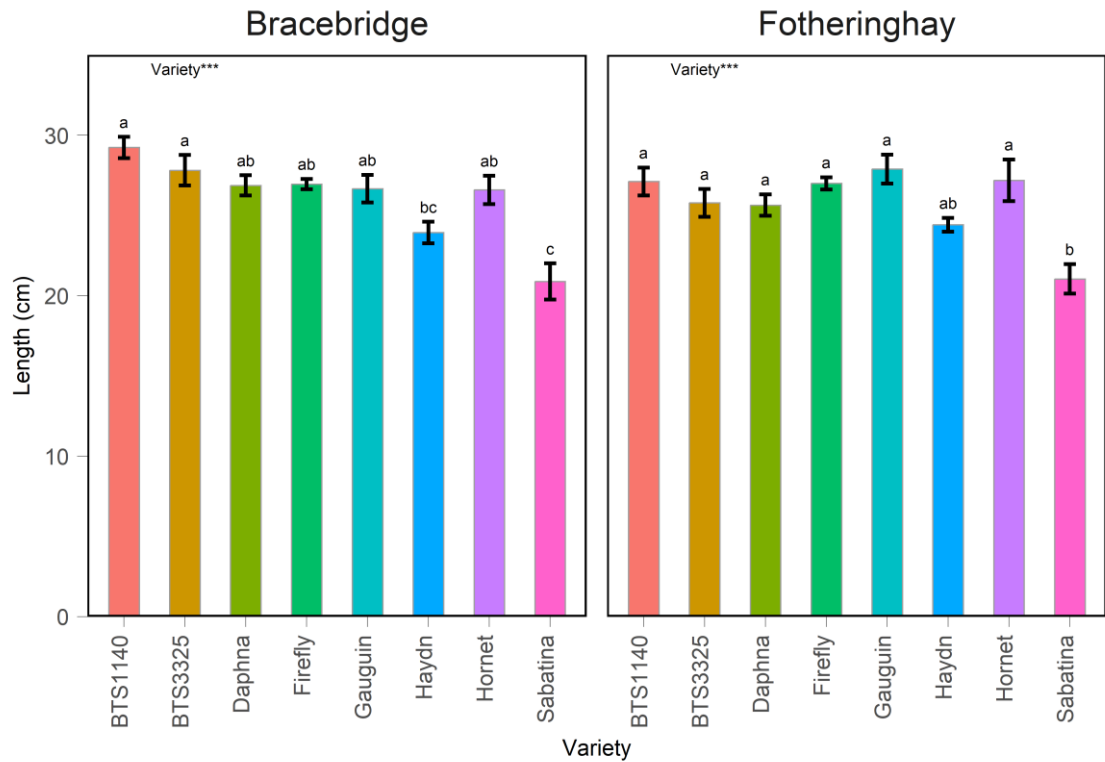


Figure 3-17: Effects of sugar beet variety on length at Bracebridge and Fotheringhay. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. For Bracebridge n=67, for Fotheringhay n=68, seasons=3, error bar=standard error.

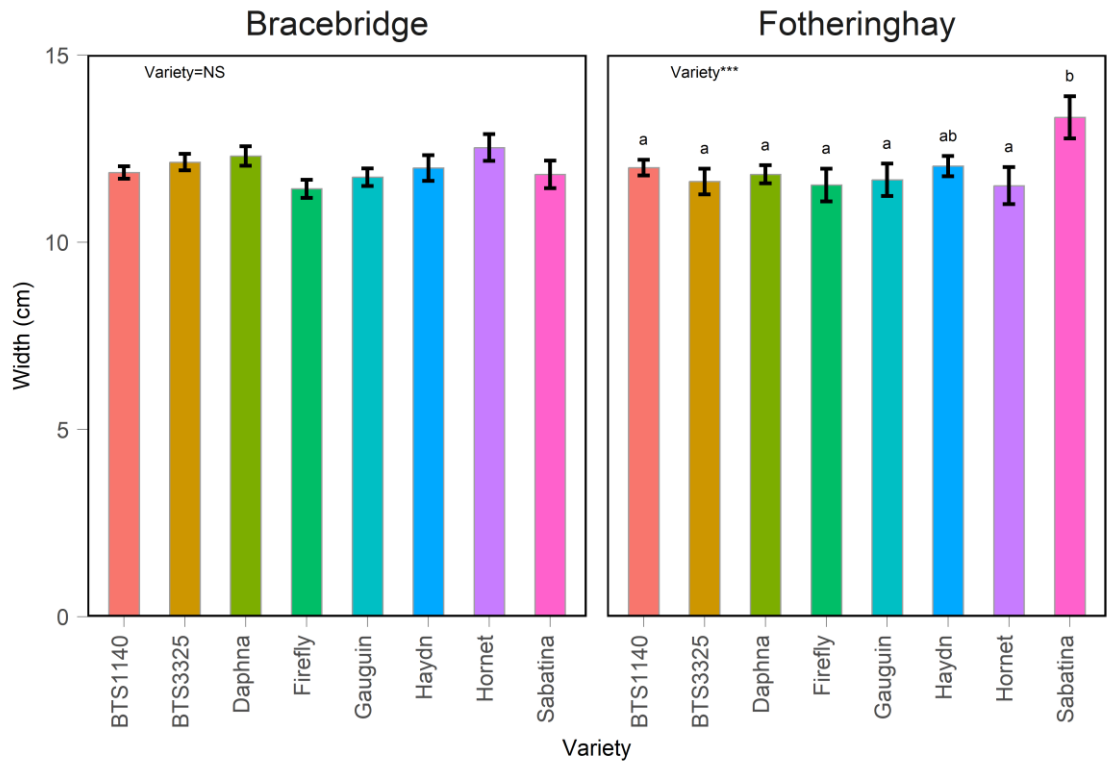


Figure 3-18: Effects of sugar beet variety on width at Bracebridge and Fotheringhay. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. For Bracebridge n=68, for Fotheringhay n=64, seasons=3, error bar=standard error.

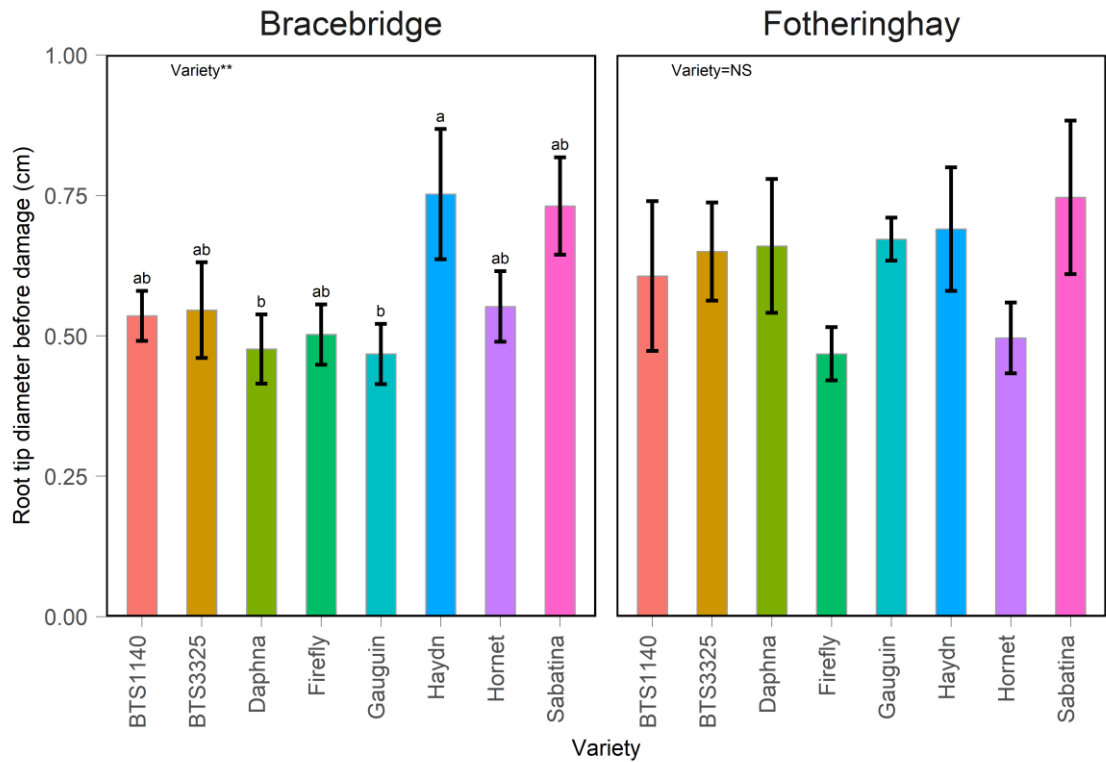


Figure 3-19: Effects of sugar beet variety on root tip before damage at Bracebridge and Fotheringhay. Treatments with different superscripts at Bracebridge mean they are statistically different from each other at that particular site. For Bracebridge n=70, for Fotheringhay n=43, Bracebridge seasons=3, Fotheringhay seasons=2, error bar=standard error.

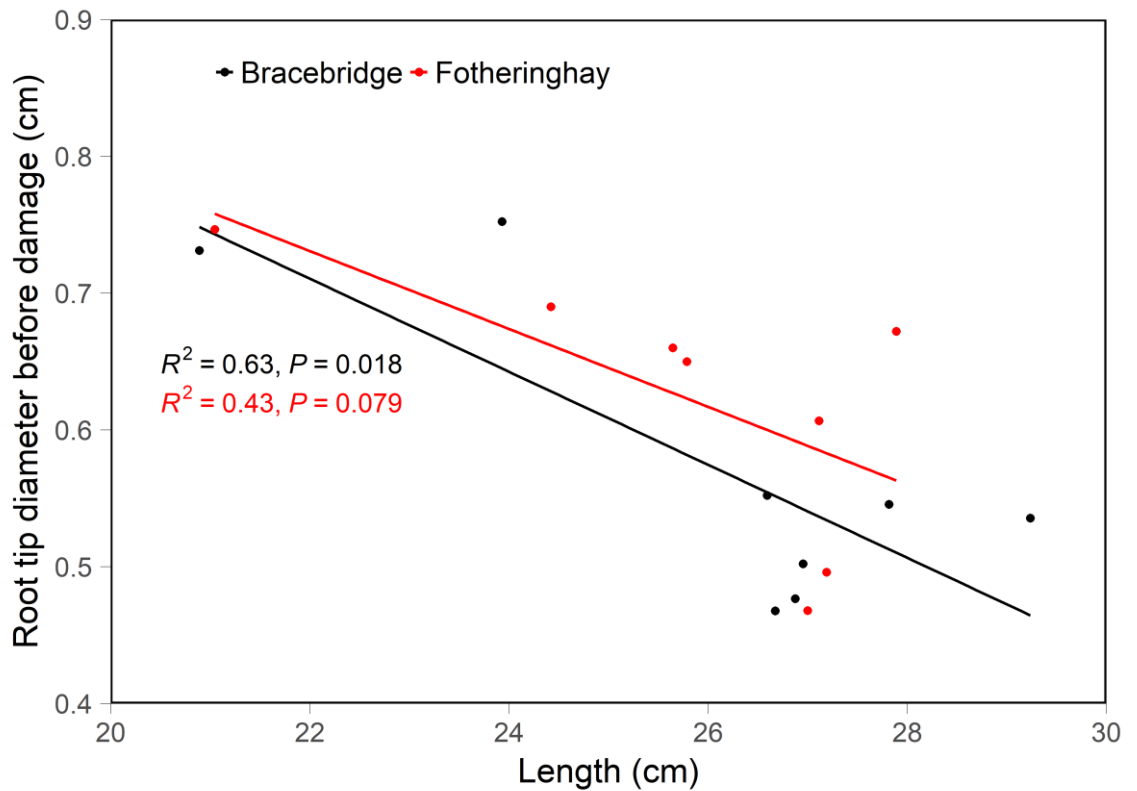


Figure 3-20: Linear regression between root tip diameter before damage and root length of eight sugar beet varieties at Bracebridge and Fotheringhay.

3.4.1.2 Root weight

Results indicate that at both Fotheringhay and Bracebridge, varieties used in this study (**Figure 3-21**) were not statistically different in terms of weight. However, average root weight for Fotheringhay plants was statistically ($p < 0.001$) heavier (1.19 Kg) than those harvested at Bracebridge (0.94 Kg). Root weight was also significantly affected by season ($p < 0.001$) at both sites. Both Fotheringhay and Bracebridge produced lighter roots (0.51 and 0.59 Kg, respectively) in 2020. While in 2019 and 2021, Bracebridge produced relatively heavier roots (1.07 and 1.15 Kg, respectively). Fotheringhay produced the heaviest roots in 2019 and 2021 (1.47 and 1.58 Kg, respectively). There was no relationship at both Bracebridge ($R^2=0.16, p=0.32$) and Fotheringhay ($R^2=0.12, p=0.39$) between root length and weight (**Figure 3-23**) while an increase in width at both Bracebridge ($R^2=0.61, p=0.022$) and Fotheringhay ($R^2=0.69, p=0.01$) increased root weight (**Figure 3-24**).

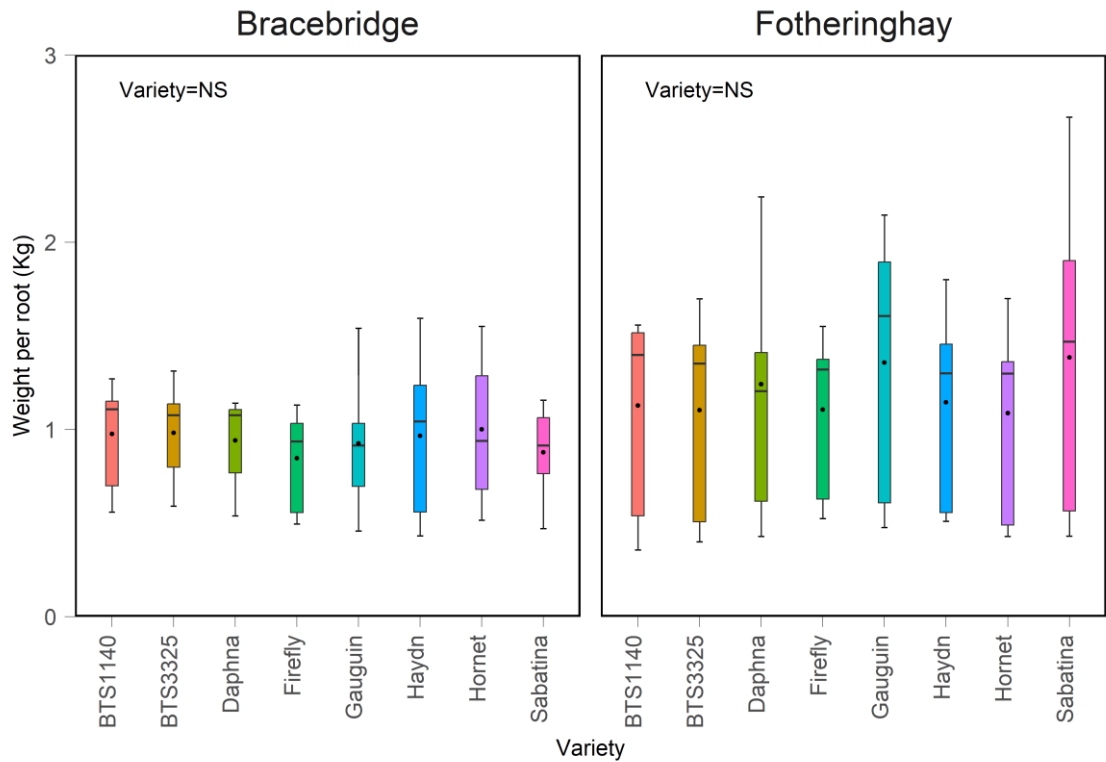


Figure 3-21: Effects of sugar beet variety on weight per root at Bracebridge and Fotheringhay. For Bracebridge n=70, for Fotheringhay n=70, Bracebridge seasons=3, Fotheringhay seasons=3. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

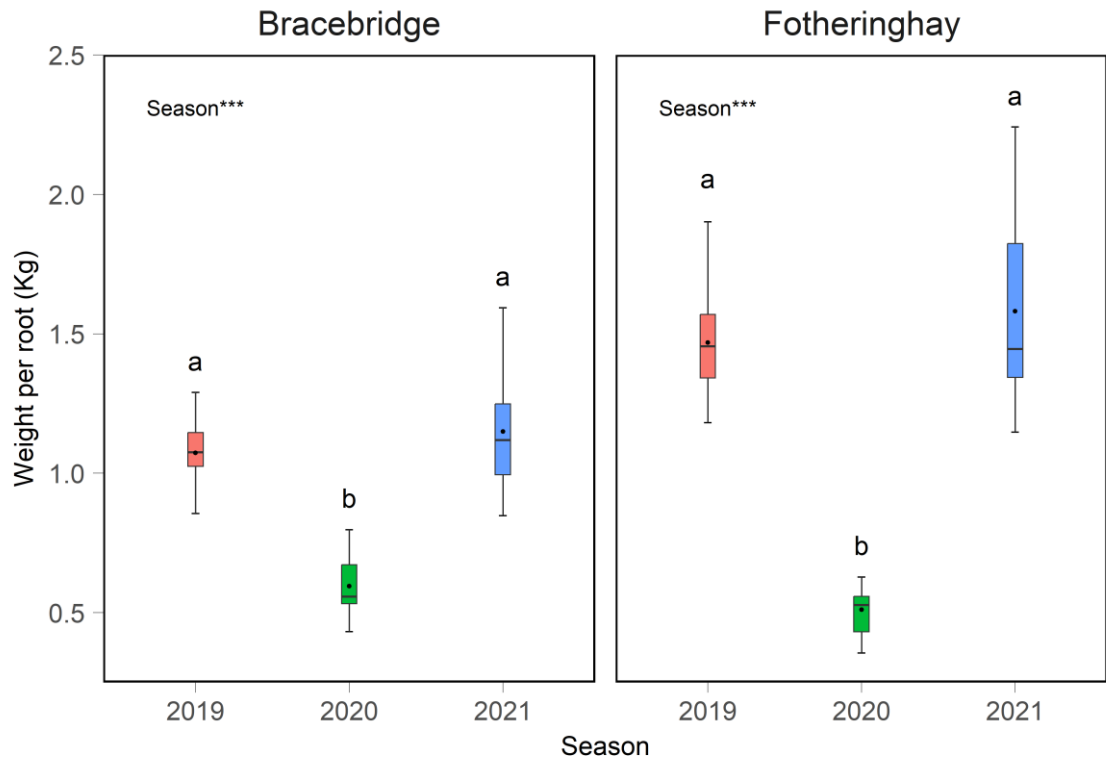


Figure 3-22: Effects of season on weight per root at Bracebridge and Fotheringhay. For Bracebridge n=70, for Fotheringhay n=70, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

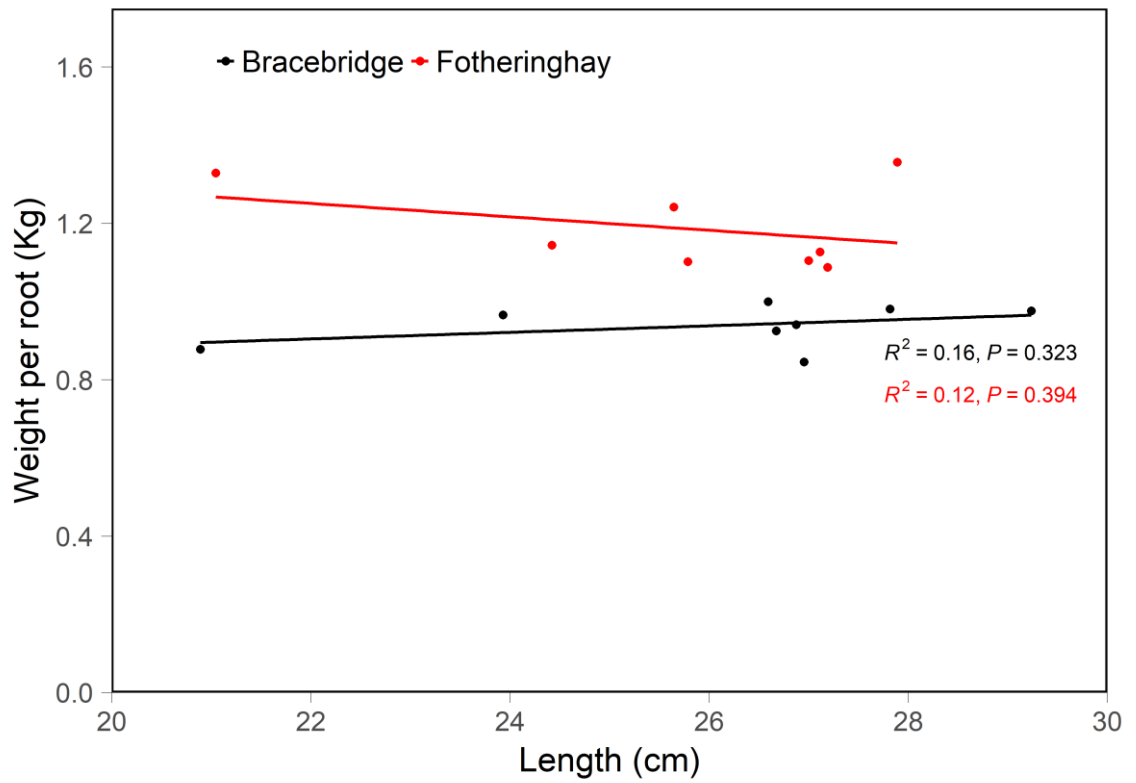


Figure 3-23: Linear regression between weight per root and length of sugar beet varieties at Bracebridge and Fotheringhay.

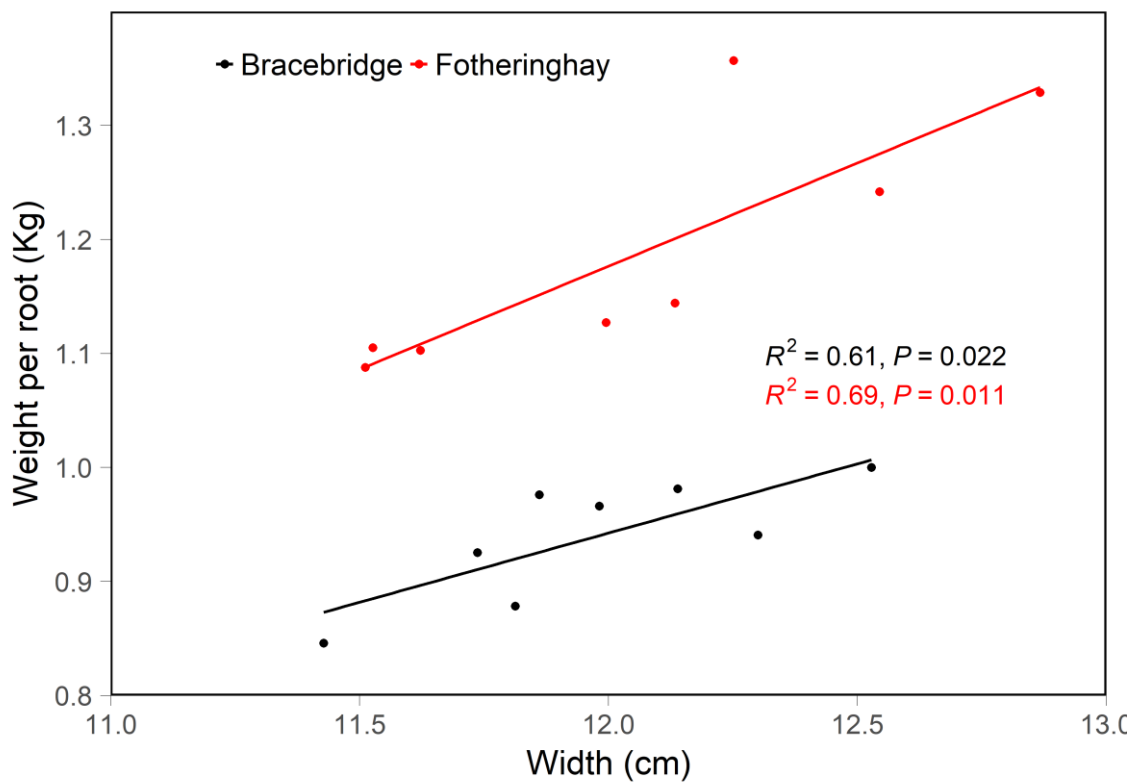


Figure 3-24: Linear regression between weight per root and width of sugar beet varieties at Bracebridge and Fotheringhay.

3.4.1.3 Textural properties

3.4.1.3.1 Puncture resistance

Puncture resistance was affected by variety ($p < 0.001$) and puncture position ($p < 0.001$). Average resistance for both sites ranged from 5.4 – 6.5 MPa and the lowest was recorded on Sabatina and Hornet (5.6 and 5.65 MPa, respectively) at Bracebridge while at Fotheringhay, lowest resistances were registered by Daphna (5.07 MPa), Sabatina (5.74 MPa) and BTS1140 (5.81 MPa). At both sites, position influenced the root's response to puncture resistance with the tips showing less resistance followed by the middle and top (**Figure 3-26**). It was also noted that despite puncture resistance values for the top being statistically different from those of the middle and tips, the puncture resistance values for the middle and tips were not different.

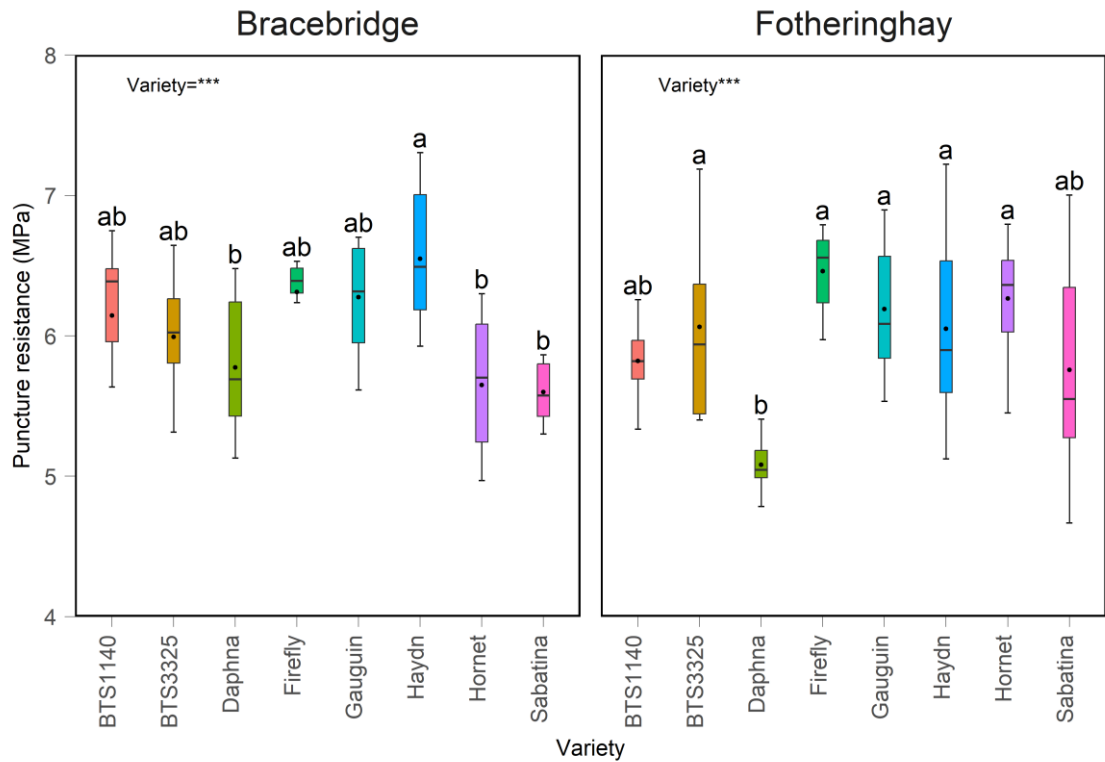


Figure 3-25: Effects of sugar beet variety on puncture resistance at Bracebridge and Fotheringhay. For Bracebridge n=64, for Fotheringhay n=62, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

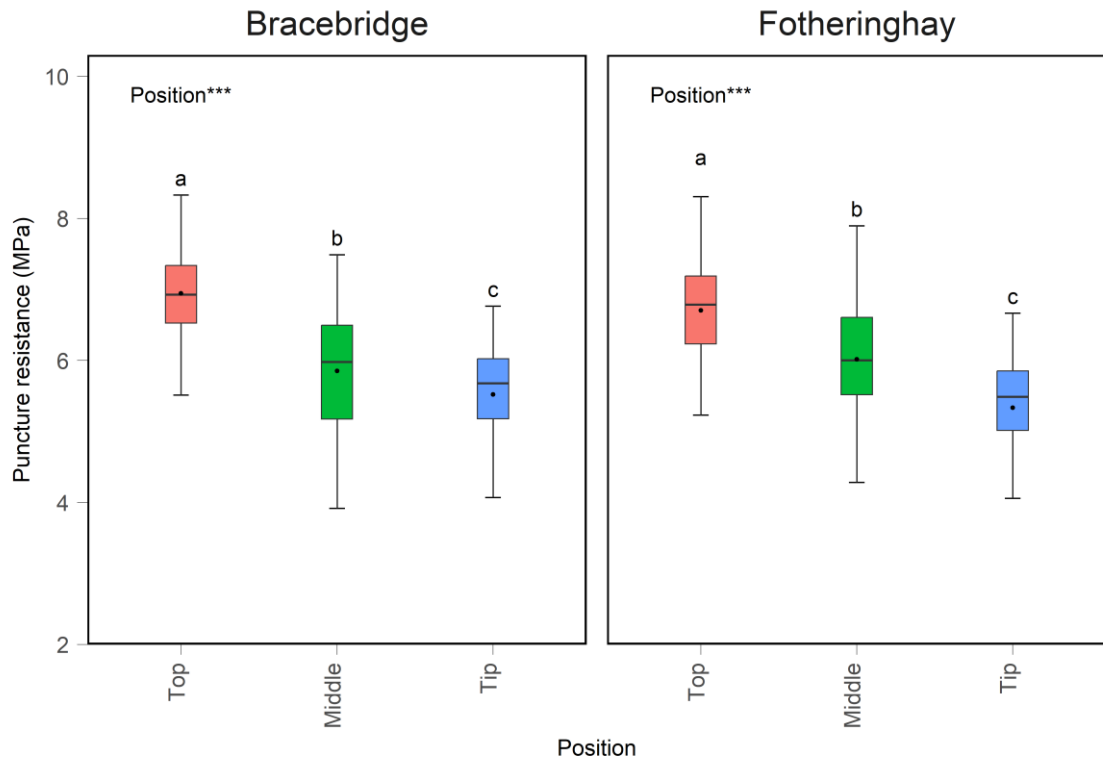


Figure 3-26: Effects of puncture position on sugar beet puncture resistance at Bracebridge and Fotheringhay. For Bracebridge n=199, for Fotheringhay n=178, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

3.4.1.3.2 Compression resistance

Resistance to compression was affected by variety (**Figure 3-27**). However, the variation was stronger at Fotheringhay ($p < 0.001$) than Bracebridge ($p < 0.05$). At Bracebridge, Daphna registered a statistically lower compression resistance (2.15 MPa) compared to BTS1140 (2.58 MPa). At Fotheringhay, lower compression resistances were recorded on Sabatina (1.98 MPa) which was statistically different from BTS1140 (2.47 MPa). At both sites, tissues extracted from the edge of the roots had higher compression resistance than tissues from the middle and the centre (**Figure 3-28**). The average compression resistances for tissues from the edge, middle and centre at Bracebridge were 7.03, 5.83 and 5.46 MPa, respectively. At Fotheringhay the edge, middle and centre registered compression resistance values of 6.71, 5.94 and 5.14 MPa respectively. At both sites, the compression

resistance values obtained from the middle and central positions were not statistically different.

3.4.1.3.3 Regression among textural properties

There was a significant correlation between compression and puncture resistances at both sites ($R^2=0.67$, $p=0.01$ at Bracebridge and $R^2=0.53$, $p=0.04$ at Fotheringhay) (**Figure 3-30**) while the correlation for puncture and shear resistance was only significant at Fotheringhay ($R^2=0.72$, $p=0.008$) (**Figure 3-29**). While there was no relationship between compression and shear resistance at both sites ($R^2=0.37$, $p=0.11$ at Bracebridge and $R^2=0.30$, $p=0.16$ at Fotheringhay) (**Figure 3-31**).

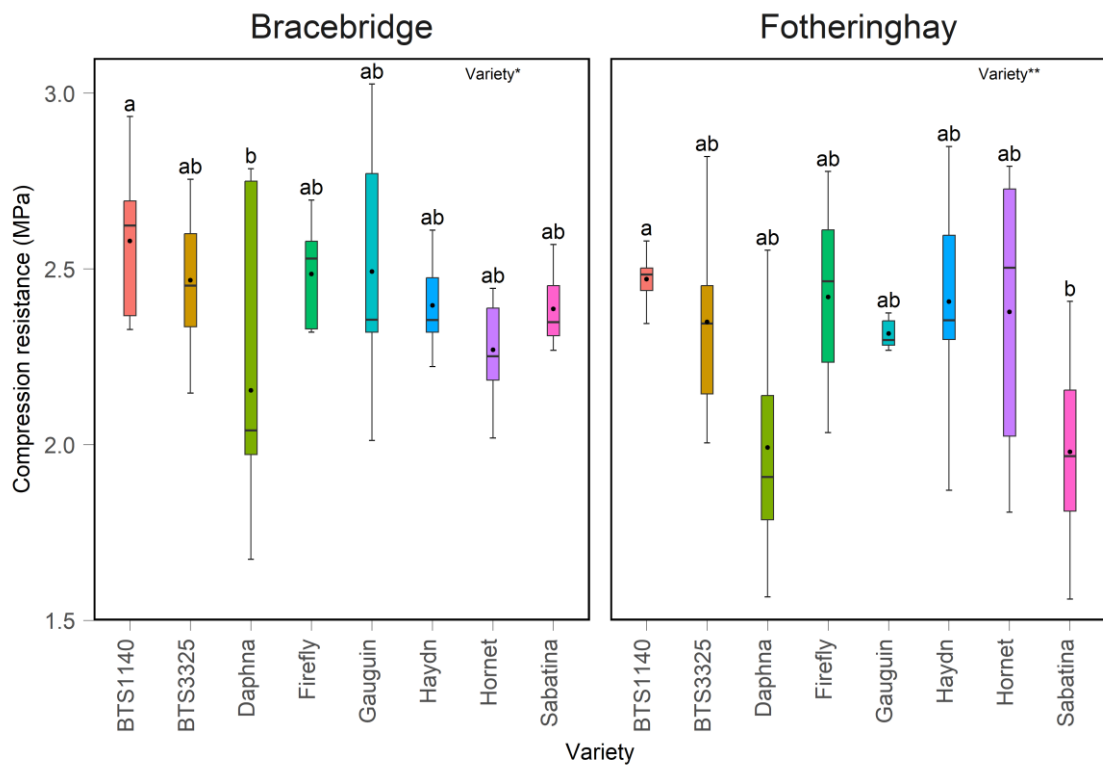


Figure 3-27: Effects of sugar beet variety on compression resistance at Bracebridge and Fotheringhay. For Bracebridge $n=55$, for Fotheringhay $n=59$, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

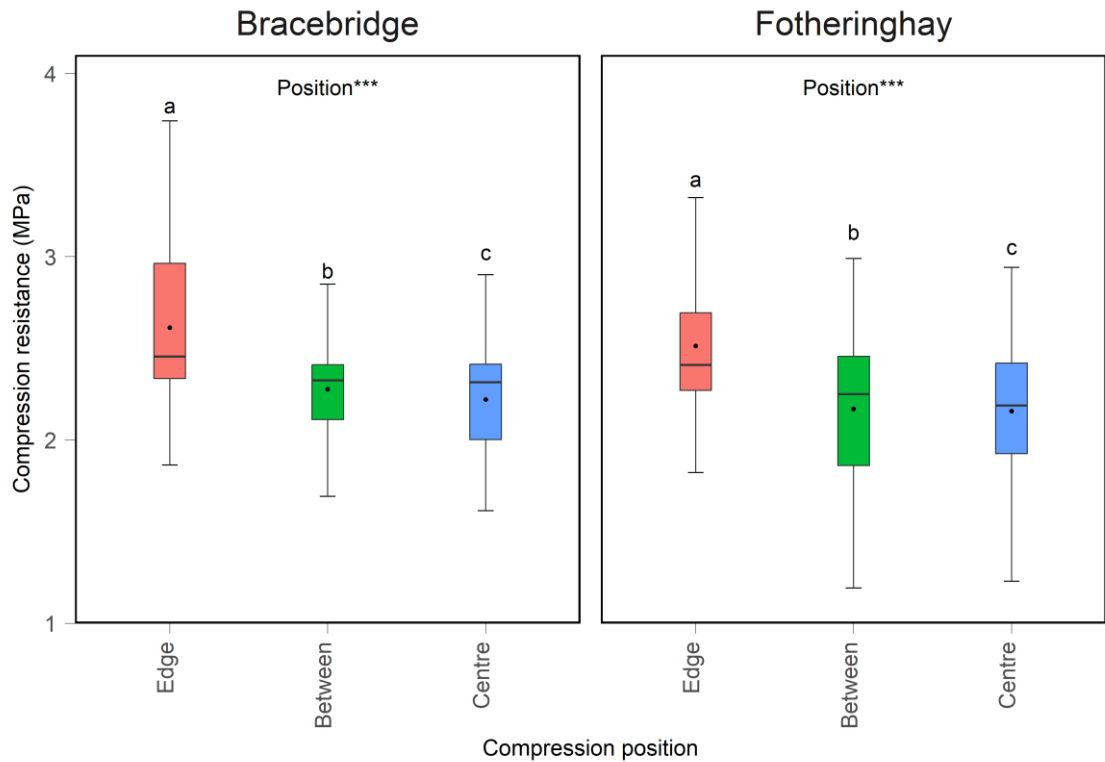


Figure 3-28: Effects of compression position on sugar beet compression resistance at Bracebridge and Fotheringhay. For Bracebridge n=213, for Fotheringhay n=189, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

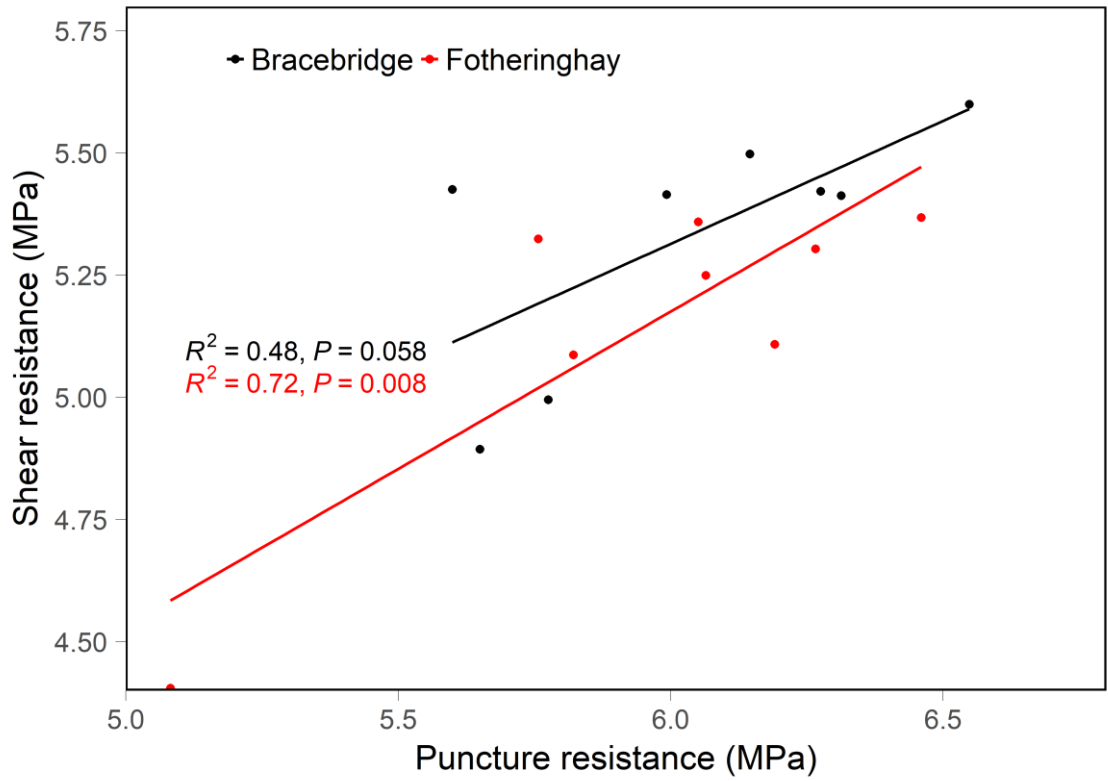


Figure 3-29: Linear regression between root tissues shear and puncture resistances of eight sugar beet varieties at Bracebridge and Fotheringhay.

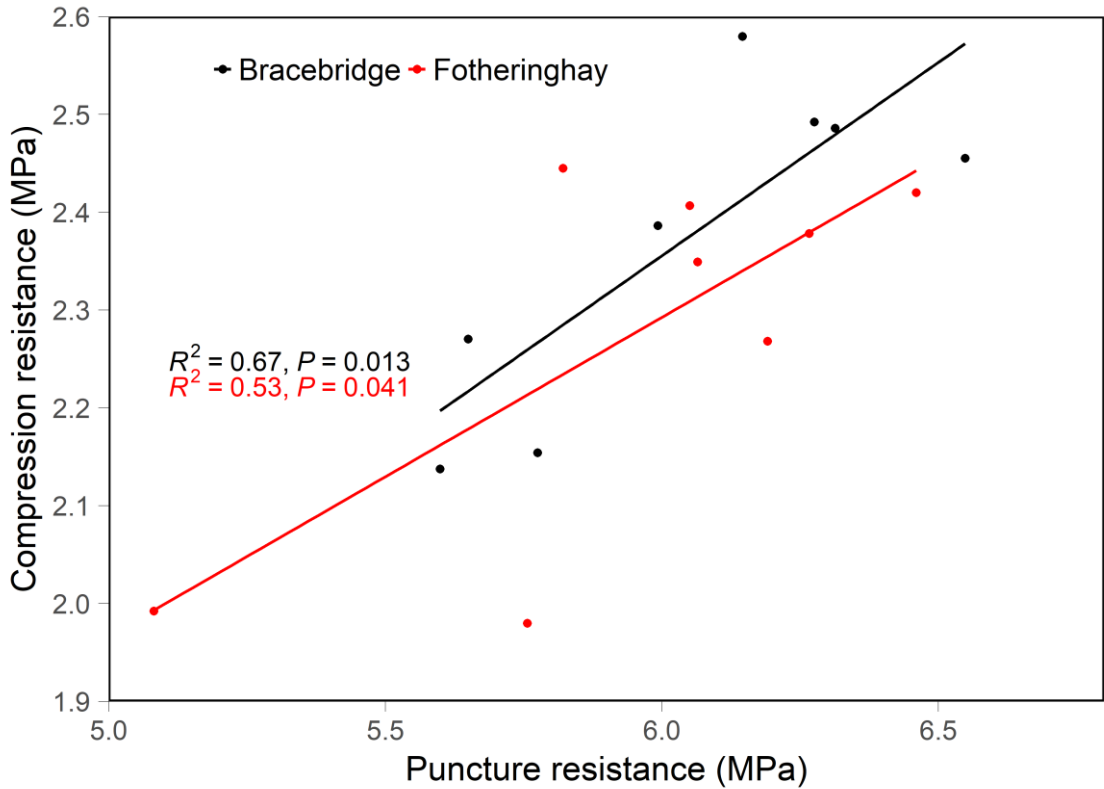


Figure 3-30: Linear regression between root tissues compression and puncture resistances of eight sugar beet varieties at Bracebridge and Fotheringhay.

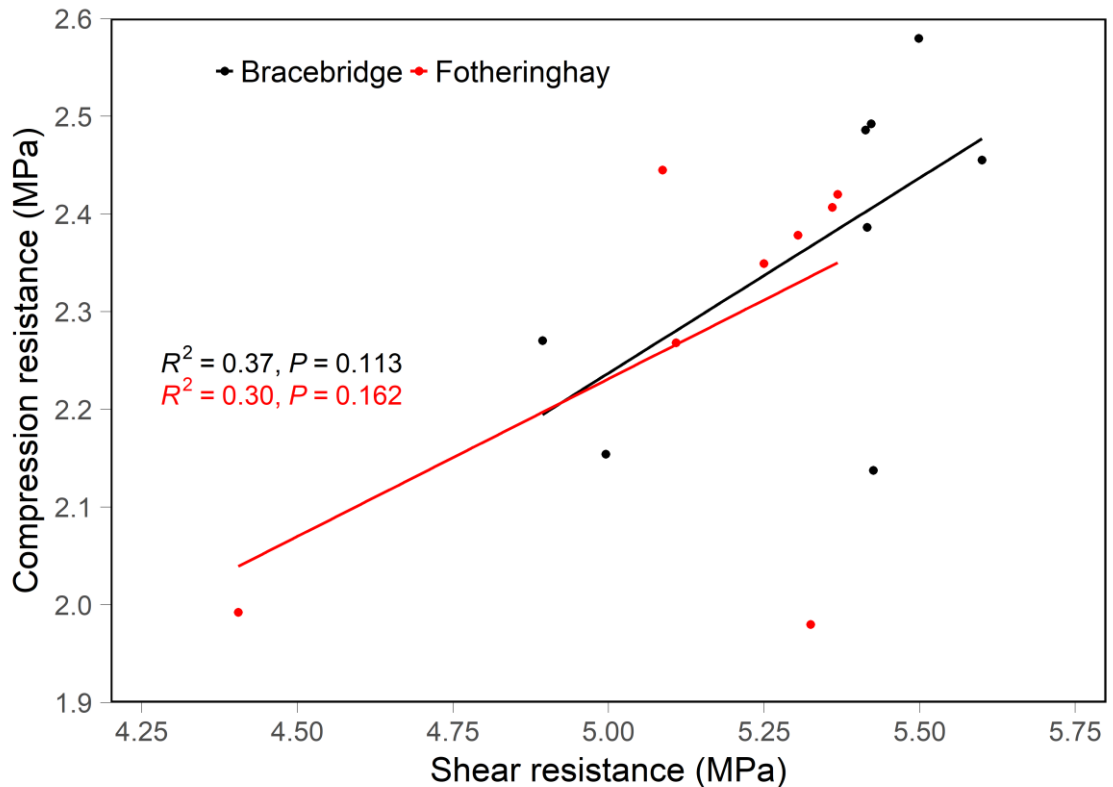


Figure 3-31: Linear regression between root tissues compression and shear resistances of eight sugar beet varieties at Bracebridge and Fotheringhay.

3.4.1.4 Root tissue damage

3.4.1.4.1 Root tip damage

Root tip damage was assessed by measuring the diameter of the broken surface at the tip of the root before and after the damage. Root tip diameter after damage was statistically affected by variety at both sites ($p < 0.05$ at Bracebridge and $p < 0.001$ at Fotheringhay). The damage ranged from 3.66-4.65 cm at Bracebridge and 3.71-5.35 cm at Fotheringhay (**Figure 3-32**). Tips Sabatina were statistically breaking the most at both Bracebridge (4.65 cm) and Fotheringhay (5.35 cm). At Fotheringhay, the root tip diameters after damage for Daphna (4.72 cm), Firefly (4.4 cm) and Gauguin (4.67 cm) were not different from Sabatina while at Bracebridge only Firefly had lower root tip diameter after damage (3.66 cm) than Sabatina. Root tip diameter after damage was positively correlated with root tip diameter before damage at Bracebridge ($R^2 = 0.52$, $p = 0.044$) while there was no relationship between the two variables at Fotheringhay ($R^2 = 0.25$, $p = 0.209$) (**Figure 3-33**). At Fotheringhay, root tip diameter after damage was also positively correlated with width ($R^2 = 0.74$, $p = 0.006$) (**Figure 3-34**) and weight ($R^2 = 0.66$, $p = 0.014$) (**Figure 3-35**). However,

there was no relationship between root tip diameter after damage and length ($R^2 = 0.38$, $p = 0.103$ at Fotheringhay, $R^2 = 0.44$, $p = 0.072$ at Bracebridge) (**Figure 3-36**). Compression showed a negative relationship to root tip after damage at Fotheringhay ($R^2 = 0.58$, $p = 0.028$) while at Bracebridge, the two variables were not correlated ($R^2 = 0.16$, $p = 0.326$) (**Figure 3-37**). A similar observation was made on puncture and shear resistances which showed no relationship with root tip diameter after damage (**Figure 3-38 and Figure 3-39**).

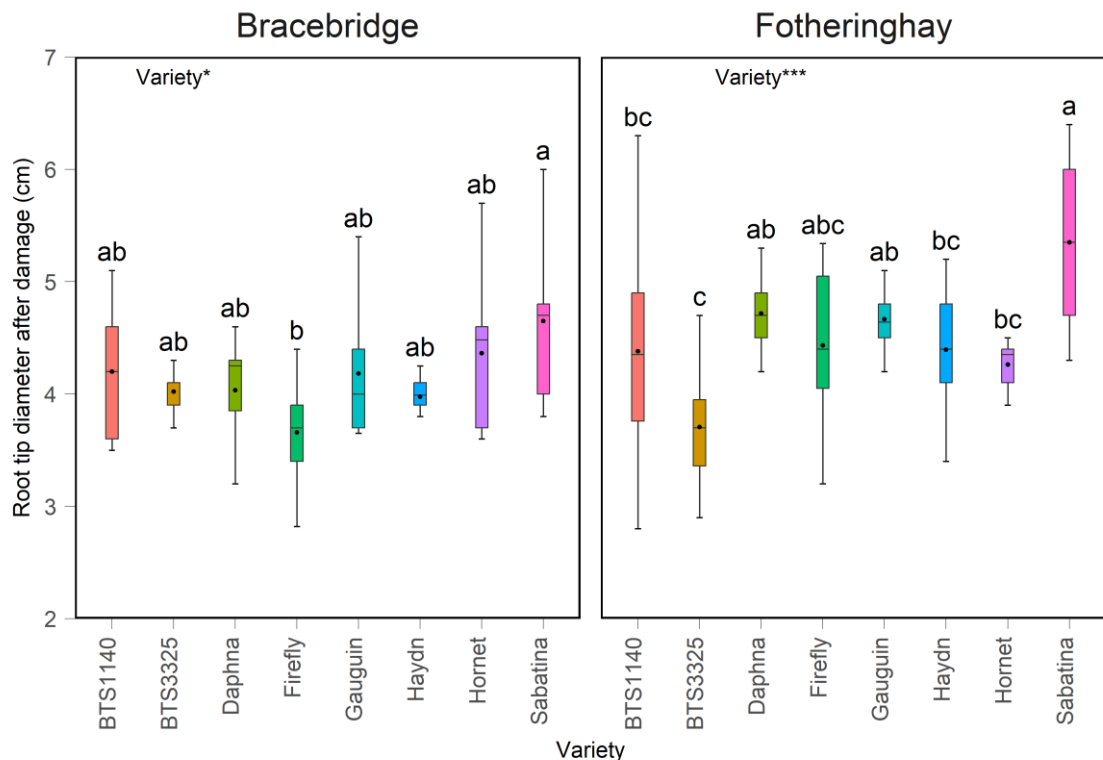


Figure 3-32: Effects of variety on sugar beet root tip diameter after damage at Bracebridge and Fotheringhay. For Bracebridge $n=70$, for Fotheringhay $n=70$, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

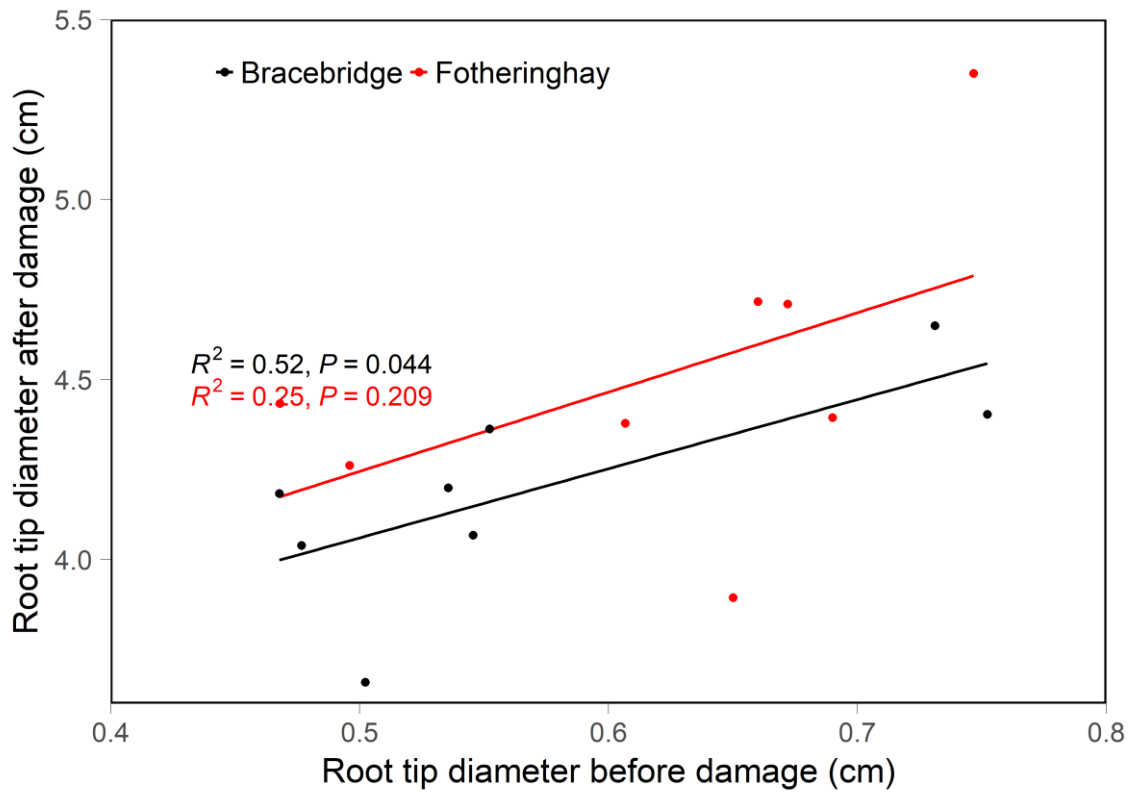


Figure 3-33: Linear regression between root tip diameter after damage and root tip diameter before damage of eight sugar beet varieties at Bracebridge and Fotheringhay.

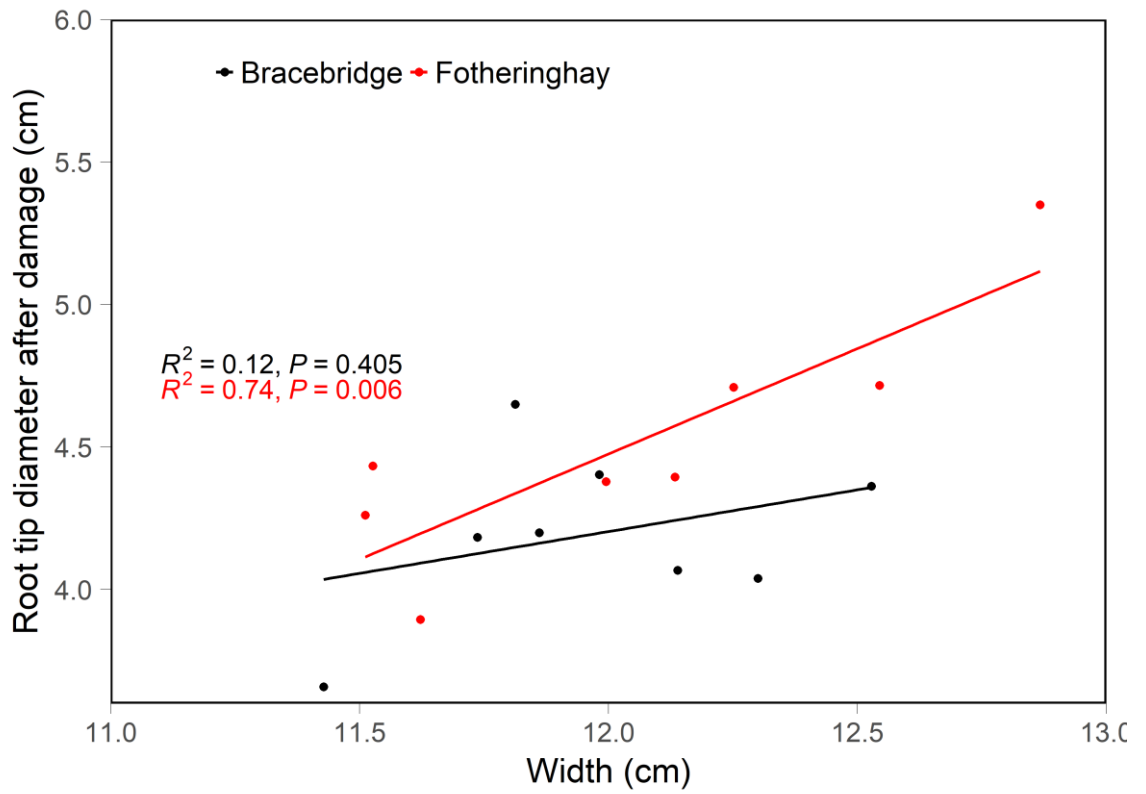


Figure 3-34: Linear regression between root tip diameter after damage and root width of eight sugar beet varieties at Bracebridge and Fotheringhay.

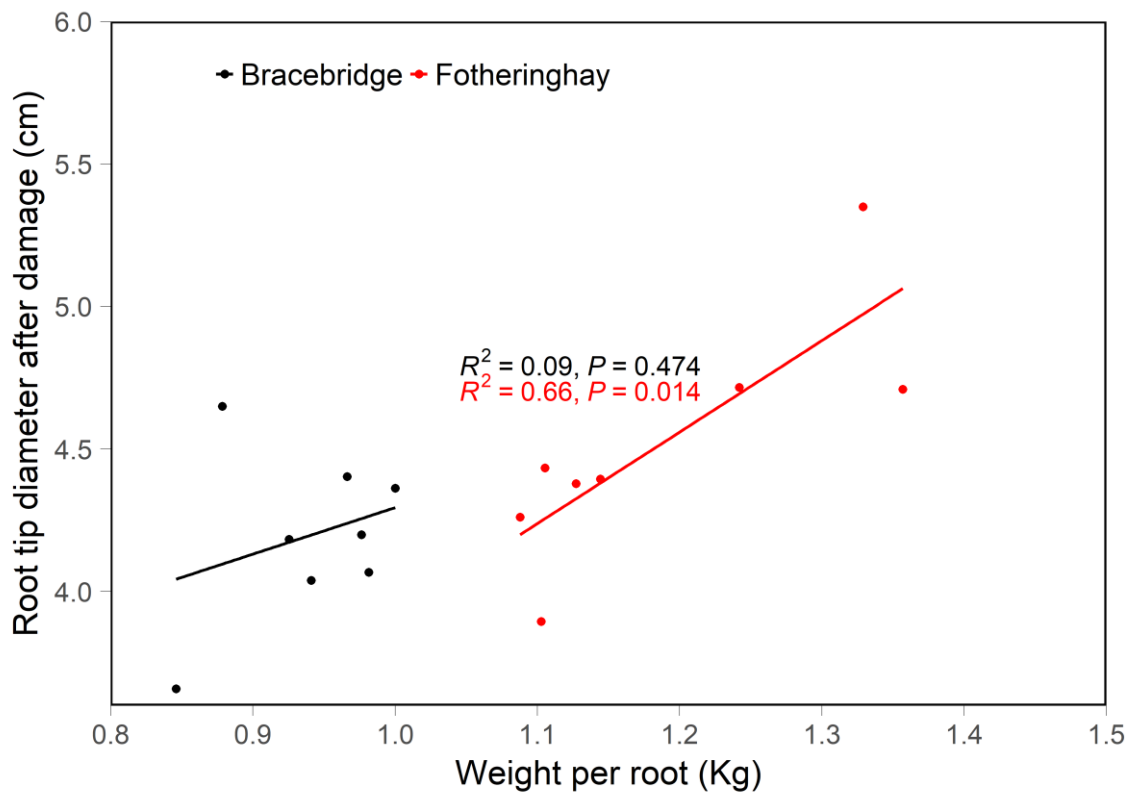


Figure 3-35: Linear regression between root tip diameter after damage and root weight of eight sugar beet varieties at Bracebridge and Fotheringhay.

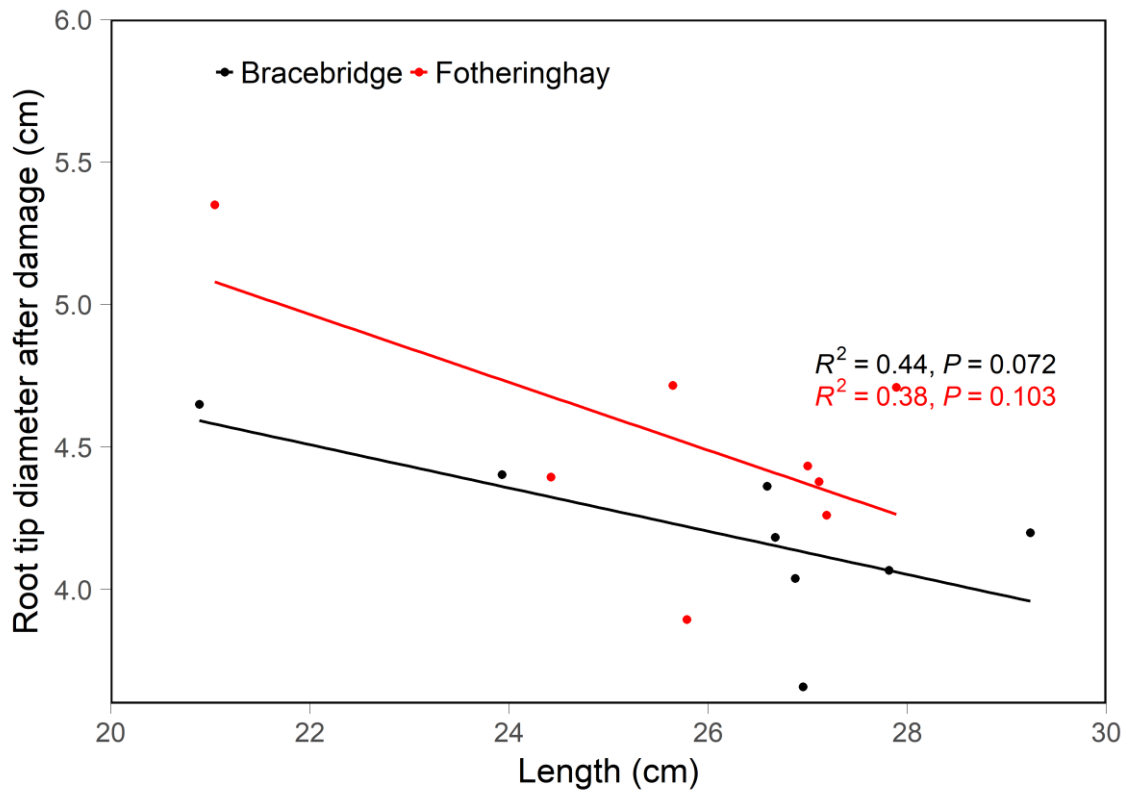


Figure 3-36: Linear regression between root tip diameter after damage and root length of eight sugar beet varieties at Bracebridge and Fotheringhay.

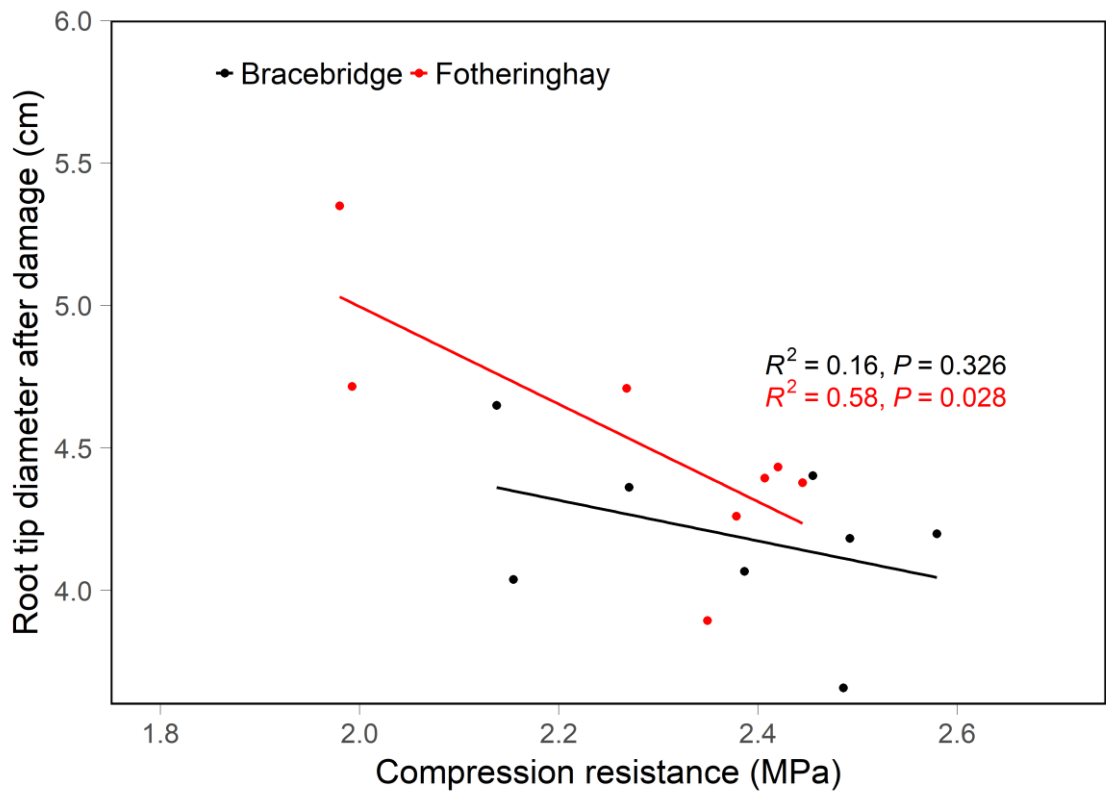


Figure 3-37: Linear regression between root tip diameter after damage and root compression of eight sugar beet varieties at Bracebridge and Fotheringhay.

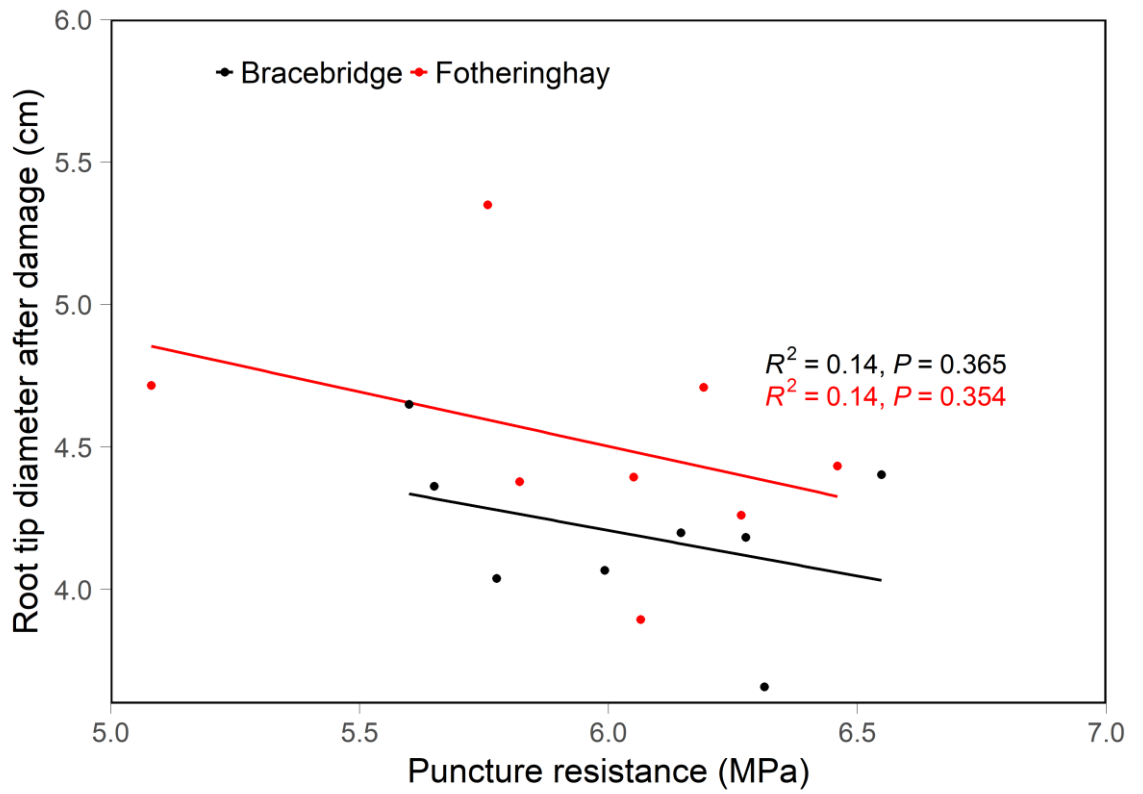


Figure 3-38: Linear regression between root tip diameter after damage and root puncture of eight sugar beet varieties at Bracebridge and Fotheringhay.

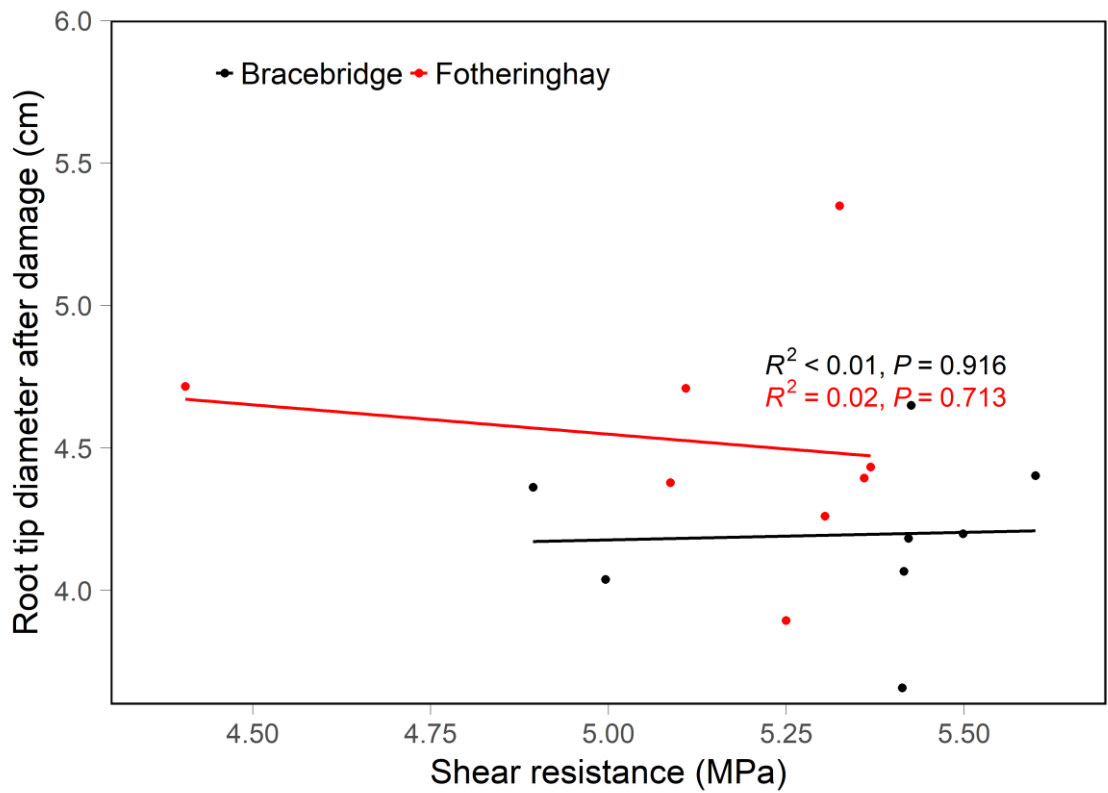


Figure 3-39: Linear regression between root tip diameter after damage and root shear resistance of eight sugar beet varieties at Bracebridge and Fotheringhay.

3.4.1.4.2 Surface damage

Surface damage was not affected by variety (**Figure 3-40**). However, seasonal effects were observed at Fotheringhay. Highest percentage of surface damage was recorded in 2019 at Fotheringhay (18%) while in 2020 and 2021, the surface damage recorded at Fotheringhay were not statistically different (**Figure 3-41**). Surface damage was negatively correlated with puncture ($R^2 = 0.56$, $p = 0.034$) (**Figure 3-42**) and shear resistance ($R^2 = 0.53$, $p = 0.041$) (**Figure 3-43**) at Fotheringhay. There was no relationship between surface damage and compression (**Figure 3-44**), width (**Figure 3-45**) and weight (**Figure 3-46**).

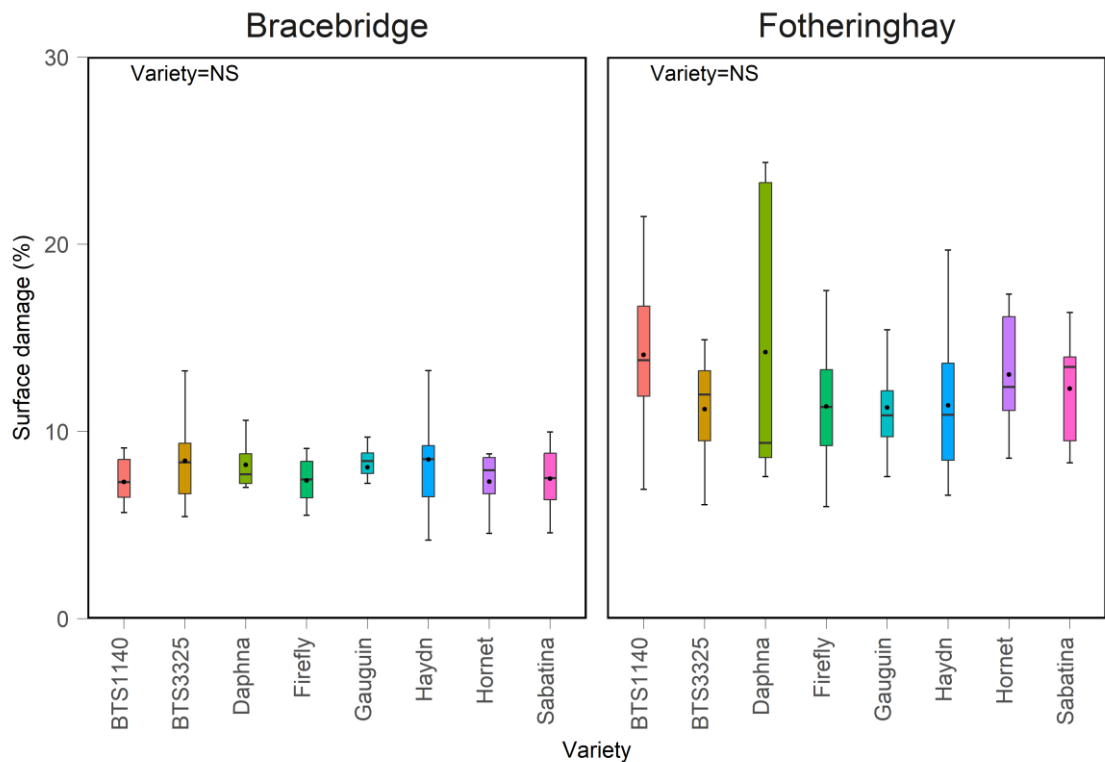


Figure 3-40: Effects of variety on sugar beet surface damage at Bracebridge and Fotheringhay. For Bracebridge $n=70$, for Fotheringhay $n=70$, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

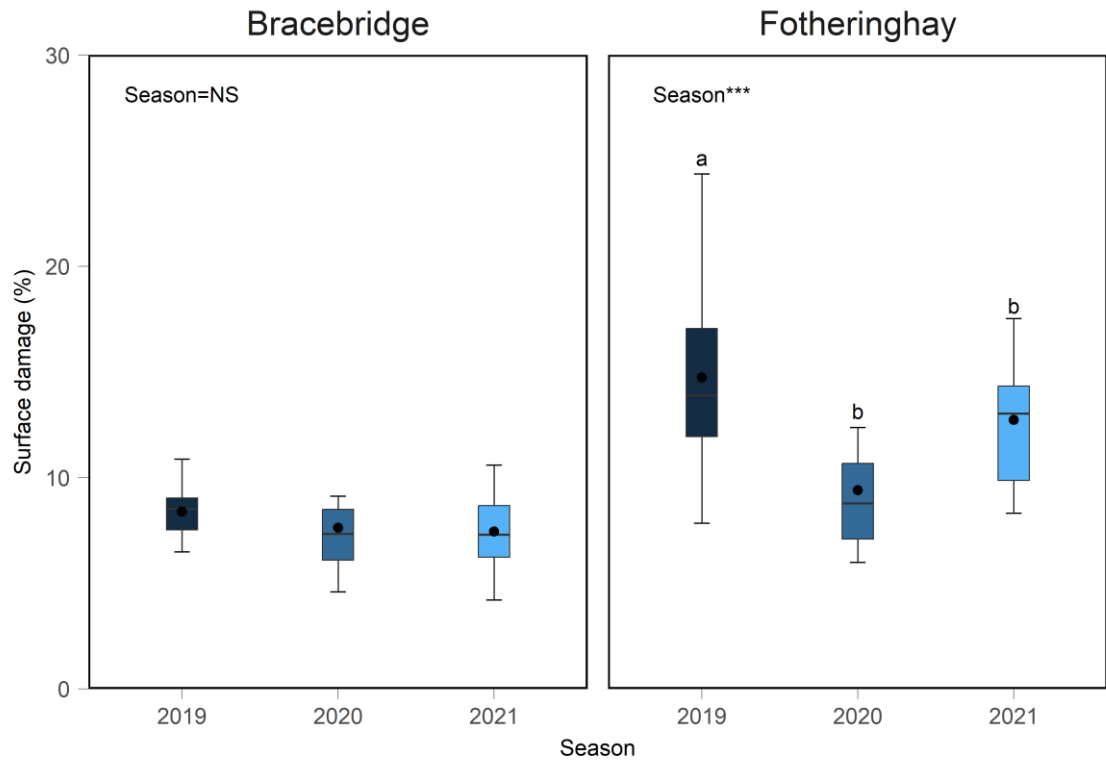


Figure 3-41: Effects of season on sugar beet surface damage at Bracebridge and Fotheringhay. For Bracebridge $n=70$, for Fotheringhay $n=70$, Bracebridge seasons=3, Fotheringhay seasons=3. Treatments with different superscripts at each site mean they are statistically different from each other at that particular site. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

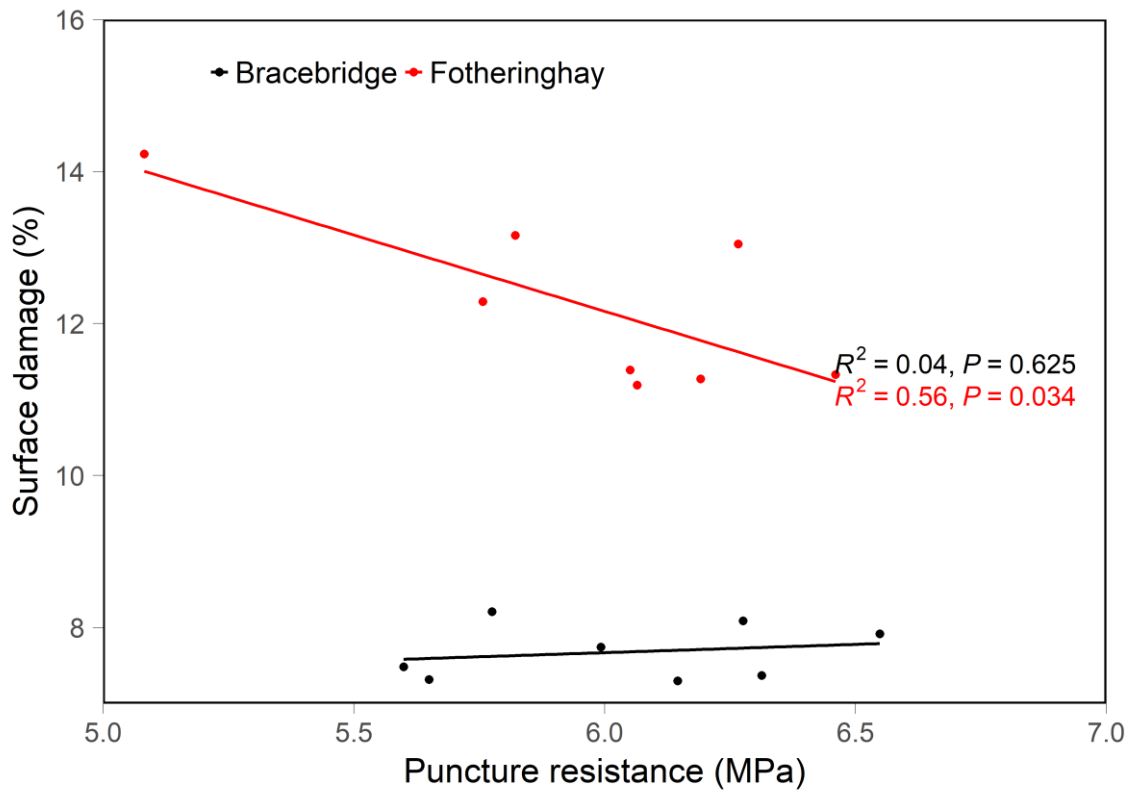


Figure 3-42: Linear regression between surface damage and puncture resistance of eight sugar beet varieties at Bracebridge and Fotheringhay.

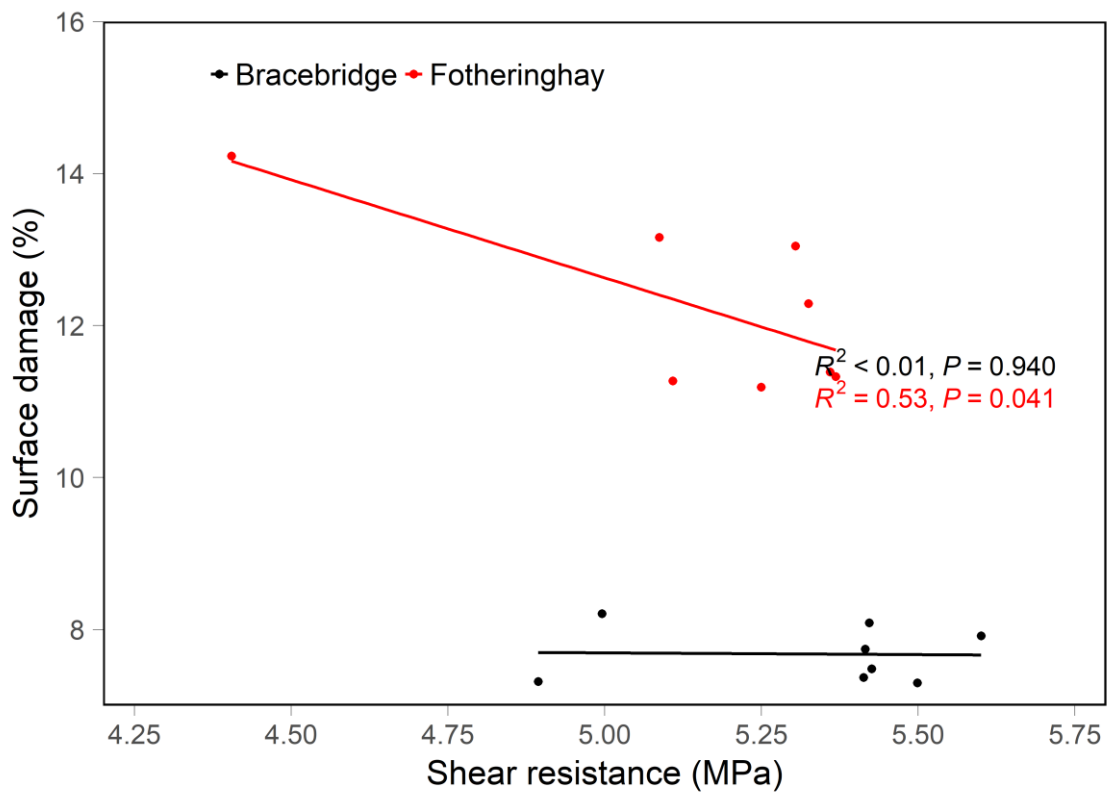


Figure 3-43: Linear regression between surface damage and shear resistance of eight sugar beet varieties at Bracebridge and Fotheringhay.

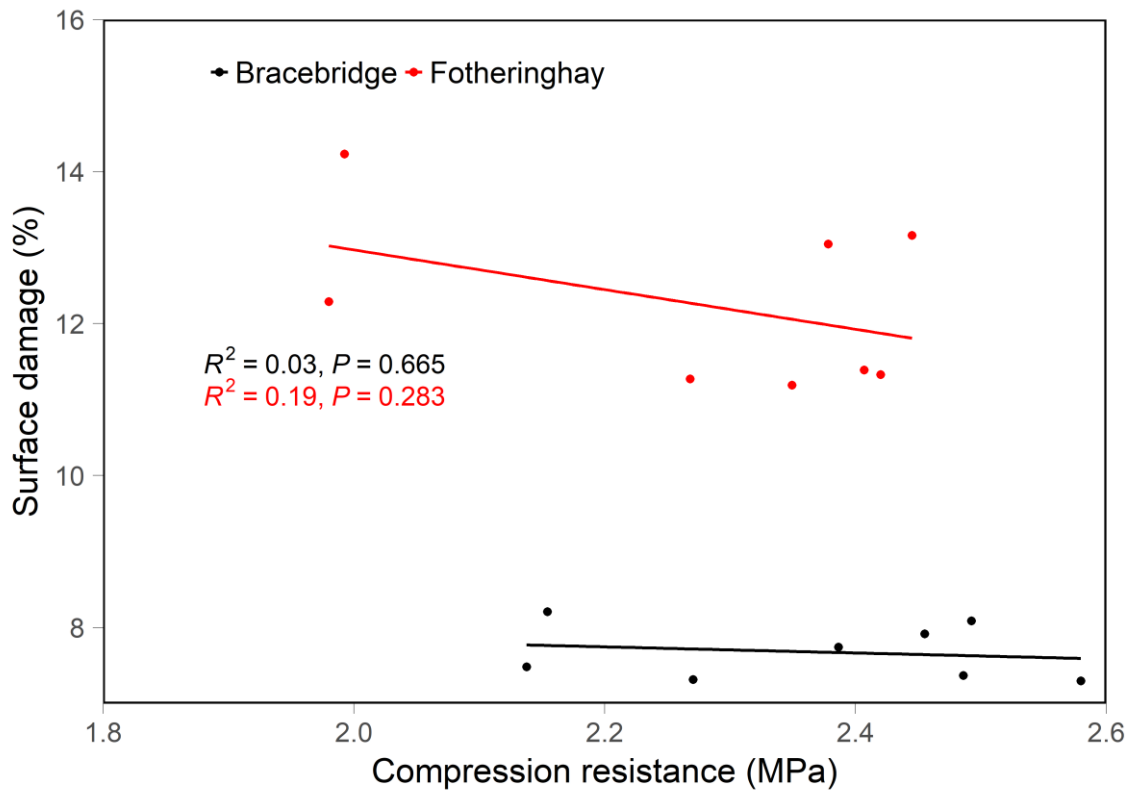


Figure 3-44: Linear regression between surface damage and compression resistance of eight sugar beet varieties at Bracebridge and Fotheringhay.

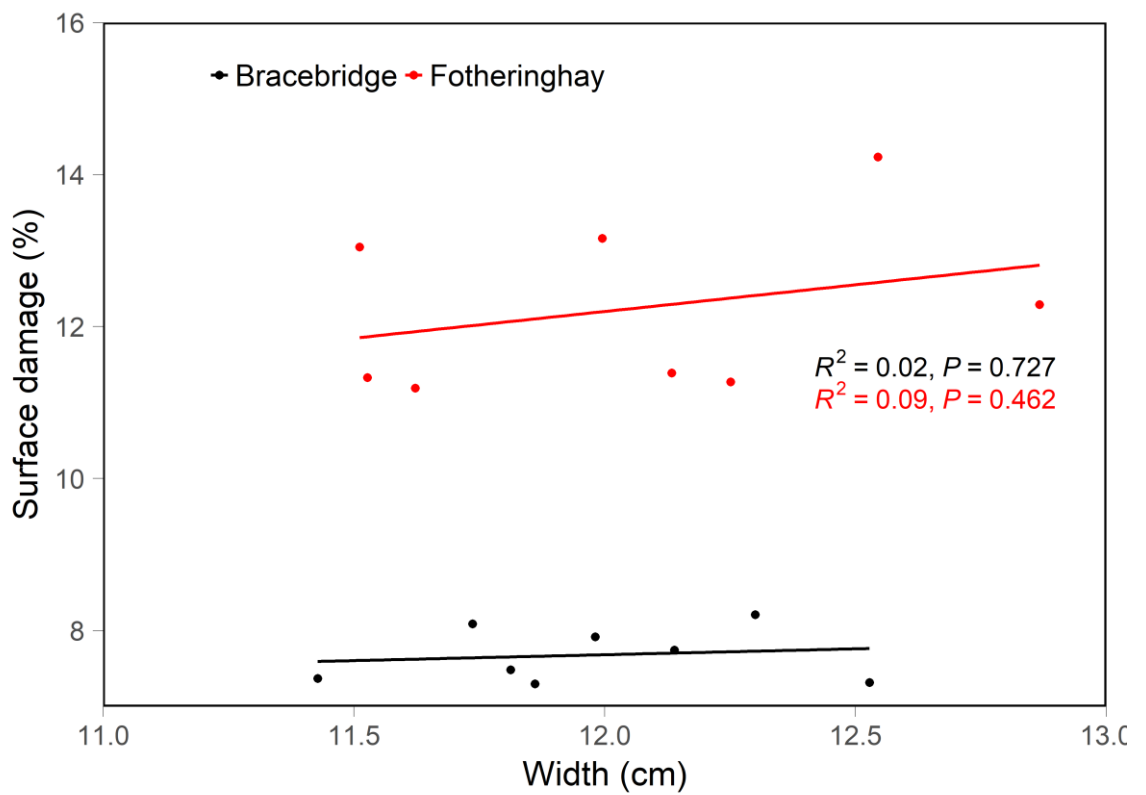


Figure 3-45: Linear regression between surface damage and width of eight sugar beet varieties at Bracebridge and Fotheringhay.

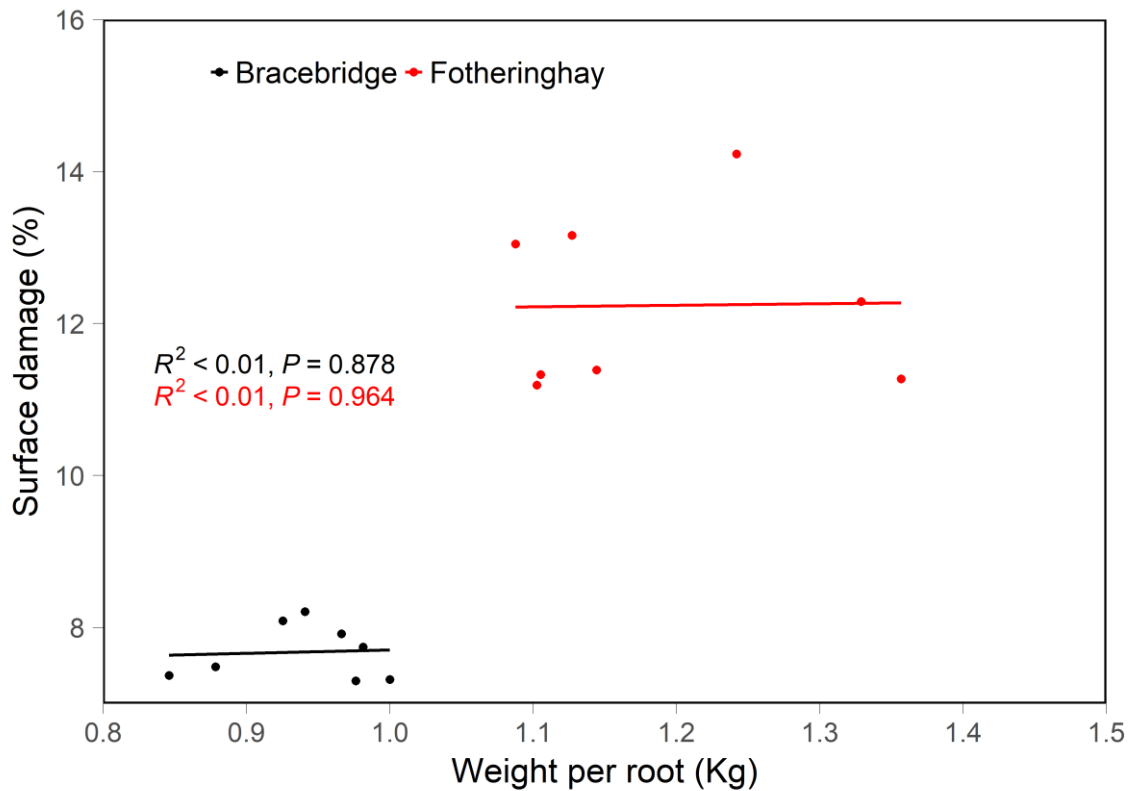


Figure 3-46: Linear regression between surface damage and weight

3.4.1.5 Principal component analysis

A principal component analysis (PCA) was performed using raw data for root tip diameter after damage, width, surface damage, root tip diameter before damage, weight, puncture, and compression resistance to determine factors that can help to improve the varieties (**Figure 3-47**). Variables that were highly correlated with one of the loaded traits were removed to avoid multicollinearity. Principal components (PC) 1 and 2 had eigenvalues of 1.50 and 1.14 respectively, while the rest had less than 1. PC1 and 2 explained 31.9 and 18.6 % of the variation, respectively, totalling 50.5 %. PC1 was affected by width, weight, root tip diameter before damage and root tip diameter after damage. This suggests that principal component one was mainly affected by morphological factors while PC2 was mainly affected by textural properties (puncture and compression). PCA also indicates that roots harvested in the 2021 season were associated with high weight and width but also high surface damage which was the opposite of 2020 (**Figure 3-48**). A PCA with sites as factors shows that Fotheringhay was associated with roots that were wide at the base and had high root tip diameter after damage. Breeding company 3 was associated with high root tip diameter after damage but also wide tips before damage while the rest of the breeders were performing averagely in all traits.

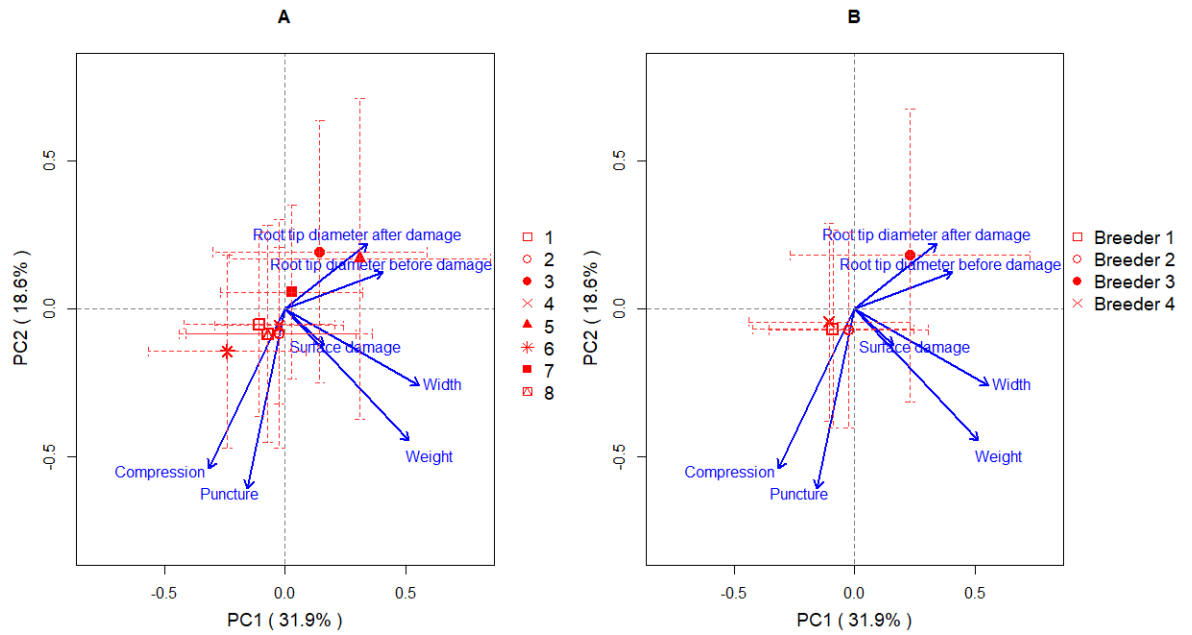


Figure 3-47: Principal component analysis for varieties (A) and breeding companies (B) when weight, root tip diameter after damage, root tip diameter before damage, width, tissue puncture resistance and tissue compression resistance were loaded. Varieties 1,2,3,4,5,6,7 and 8 are BTS3325, Gauguin, Daphna, Haydn, Sabatina, Firefly, Hornet and BTS1140, respectively.

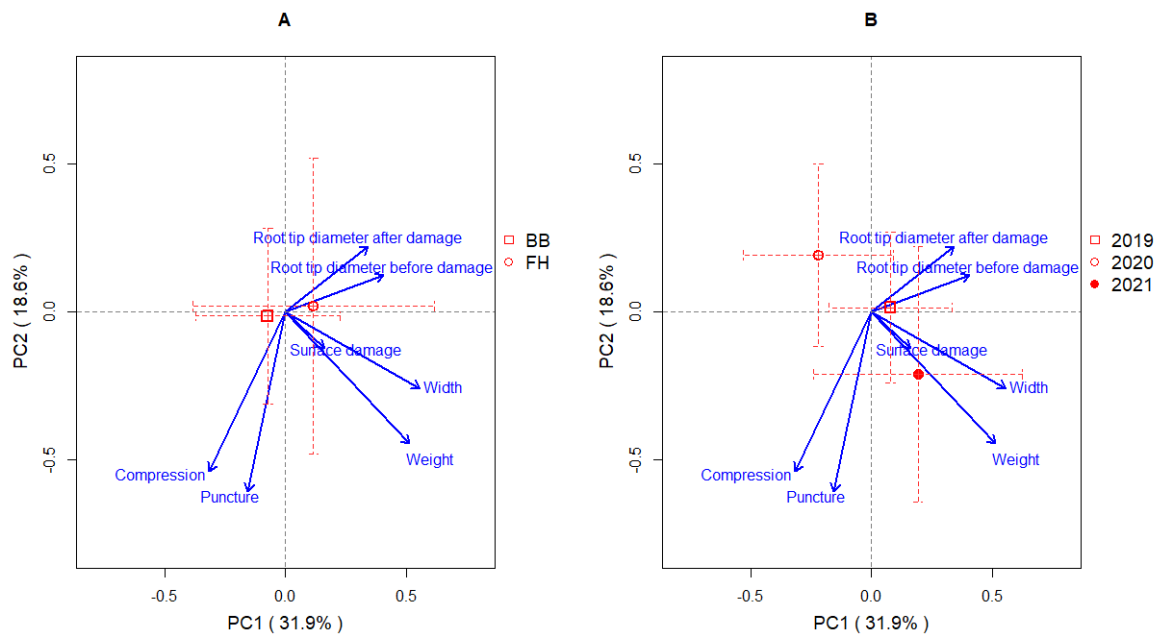


Figure 3-48: Principal component analysis for sites (A) and season (B) when weight, root tip diameter after damage, root tip diameter before damage, width, puncture resistance and compression resistance were loaded. BB means Bracebridge and FH is Fotheringhay.

3.4.1.6 Cluster analysis

Cluster analysis (Liu et al., 2022) was conducted using a complete linkage method on 9 quantitative traits, namely root tip diameter after damage, surface damage, root length, root width, root tip diameter before damage, weight, compression resistance, shear resistance and puncture resistance. The 8 sugar beet varieties were divided into 2 groups when the Euclidean height was 7. The first cluster contained varieties 1,2,4,6,7 and 8 while the other consisted of varieties 3 and 5 (**Figure 3-49**). It was also noted that varieties 3 and 5 which were in one cluster were from the same breeding company. A silhouette analysis which is a cluster analysis that measures how close each point in one cluster is to points in the neighbouring clusters was done to determine within-cluster relationships for varieties (Aranganayagi and Thangavel, 2007) (**Figure 3-50**). A higher average silhouette coefficient for a cluster indicates a close relationship among varieties while a low average silhouette coefficient for a cluster signifies a distant relationship among varieties in that cluster. Cluster one had an average silhouette coefficient of 0.5 while varieties in cluster 2 had an average silhouette coefficient of -0.03 indicating that varieties in cluster 1 were closely related compared to varieties in cluster 2. Aggregate silhouette coefficients were computed to account for dominant traits within and across clusters (**Table 3-4**). The higher the silhouette coefficient the more dominant the trait within or across the cluster. Varieties in cluster 1 were dominated by length, compression resistance, shear resistance and puncture resistance while varieties in cluster 2 were dominated by high root tip diameter after damage, surface damage, width, root tip diameter before damage and weight. Basing on the cluster analysis and silhouette values, a pictorial view of the varieties for the two clusters were drawn and a picture of two roots (one from cluster 1 and the other from cluster 2) representing the two clusters was taken during one of the laboratory analyses (**Figure 3-51**).

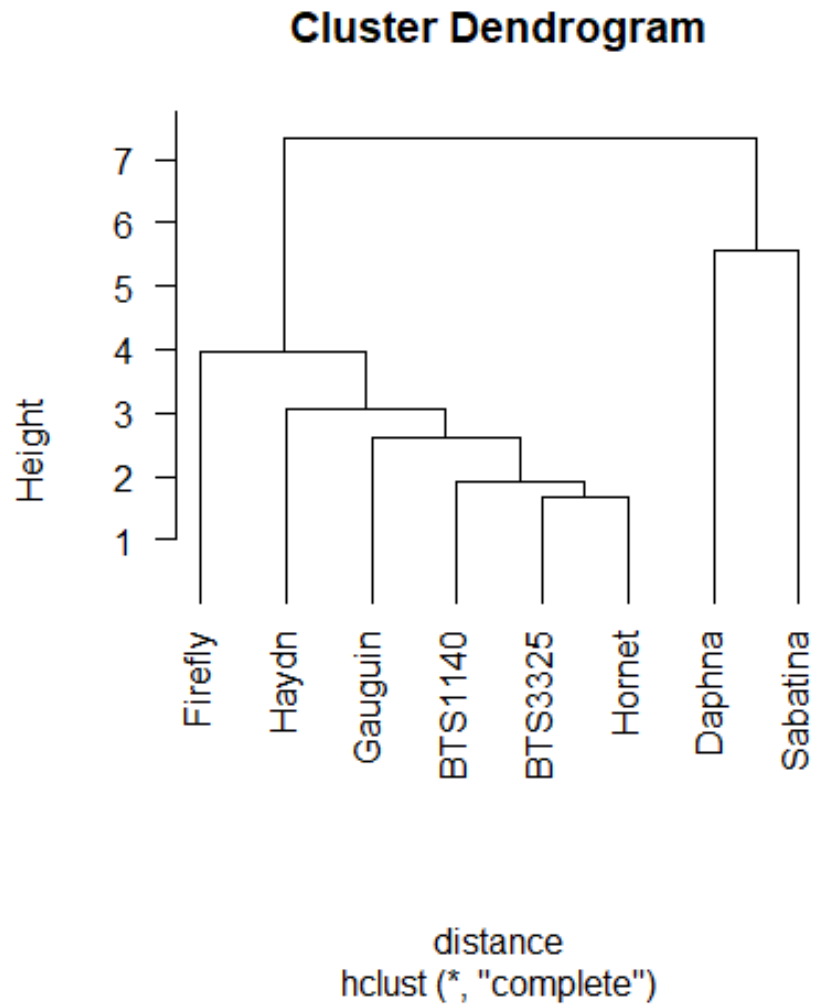


Figure 3-49: Dendrogram indicating variety cluster analysis for eight varieties when root tip diameter after damage, surface damage, root length, root width, root tip diameter before damage, weight, compression resistance, shear resistance and puncture resistance were used as traits of interest.

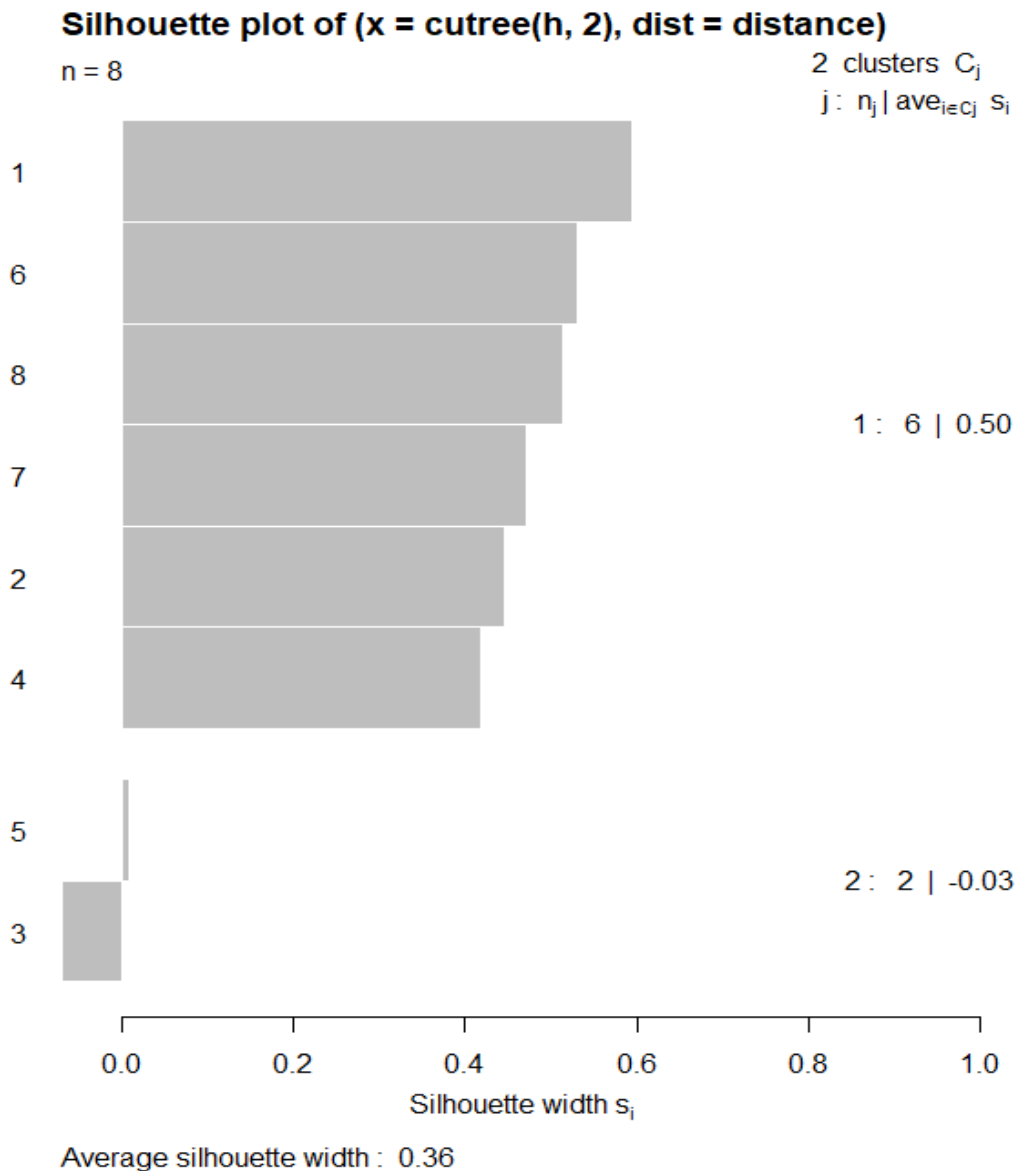


Figure 3-50: Silhouette analysis for eight varieties when root tip diameter after damage, surface damage, root length, root width, root tip diameter before damage, weight, compression resistance, shear resistance and puncture resistance were used as traits of interest. Varieties 1,2,3,4,5,6,7 and 8 are BTS3325, Gauguin, Daphna, Haydn, Sabatina, Firefly, Hornet and BTS1140, respectively.

Table 3-4: Aggregate silhouette coefficients

Cluster	Root tip diameter	Surface	Length	Width	Root tip diameter	Weight	Compress		
	afterdamage	damage			before damage		ion	Shear	Puncture
1	-0.36	-0.35	0.33	-0.44	-0.19	-0.28	0.51	0.28	0.43
2	1.08	1.04	-0.99	1.31	0.56	0.84	-1.52	-0.83	-1.29

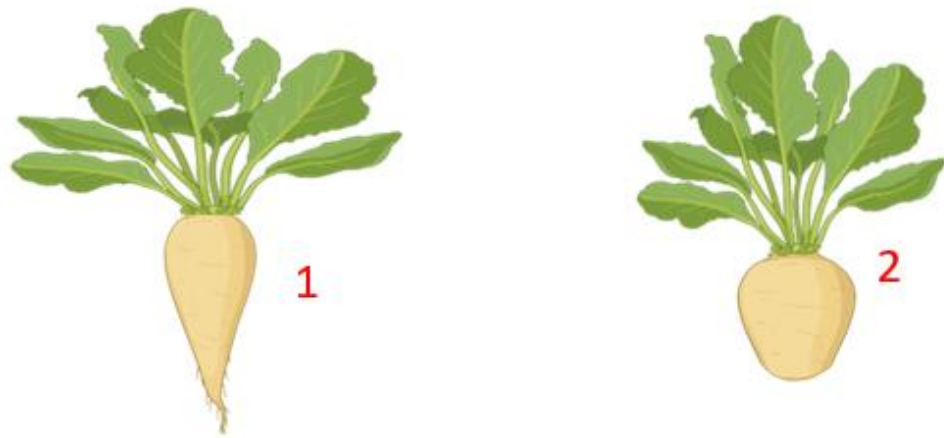


Figure 3-51: Pictorial view of morphological clusters for sugar beet. The first pictures were drawn while the second one was taken using a camera during one of the laboratory tests.

3.4.2 Effects of harvesting time

3.4.2.1 Textural properties

The textural properties under consideration in this trial were tissue puncture and compression resistance. Tissue puncture resistance were affected by variety ($p < 0.001$), harvesting time ($p < 0.01$) and the interaction between variety and harvesting time ($p < 0.001$) (**Figure 3-52**). Puncture resistances for Daphna, BTS3325, Gauguin, Firefly, Hornet and BTS1140 was stable throughout the two harvesting periods while Haydn and Sabatina had their puncture resistance values significantly reduced when harvested late (from 6.55 to 5.75 MPa and 6.2 to 5.5 MPa, respectively). Tissue compression was stable among the varieties when harvesting time was varied. This trait was only affected by variety ($p < 0.05$) (**Figure 3-53**).

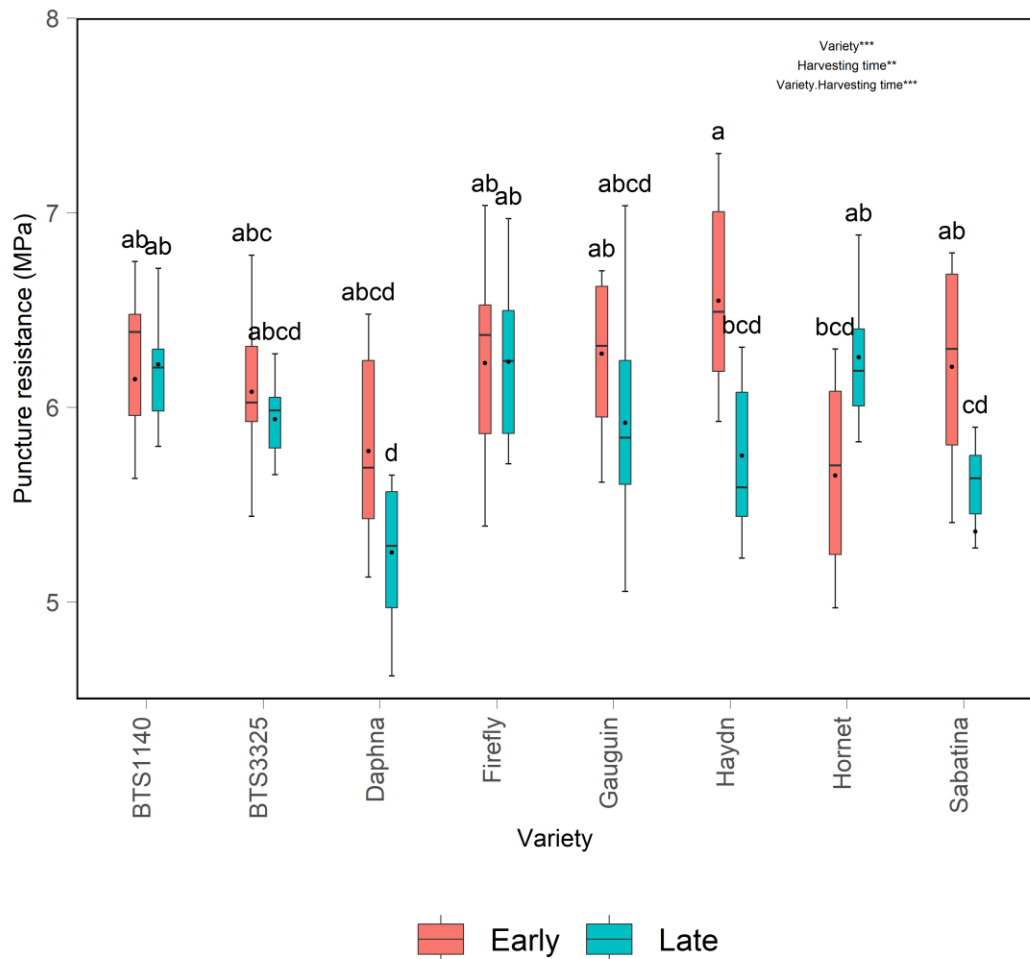


Figure 3-52: Effects of harvesting time and variety on sugar beet puncture resistance at Bracebridge. n=143, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

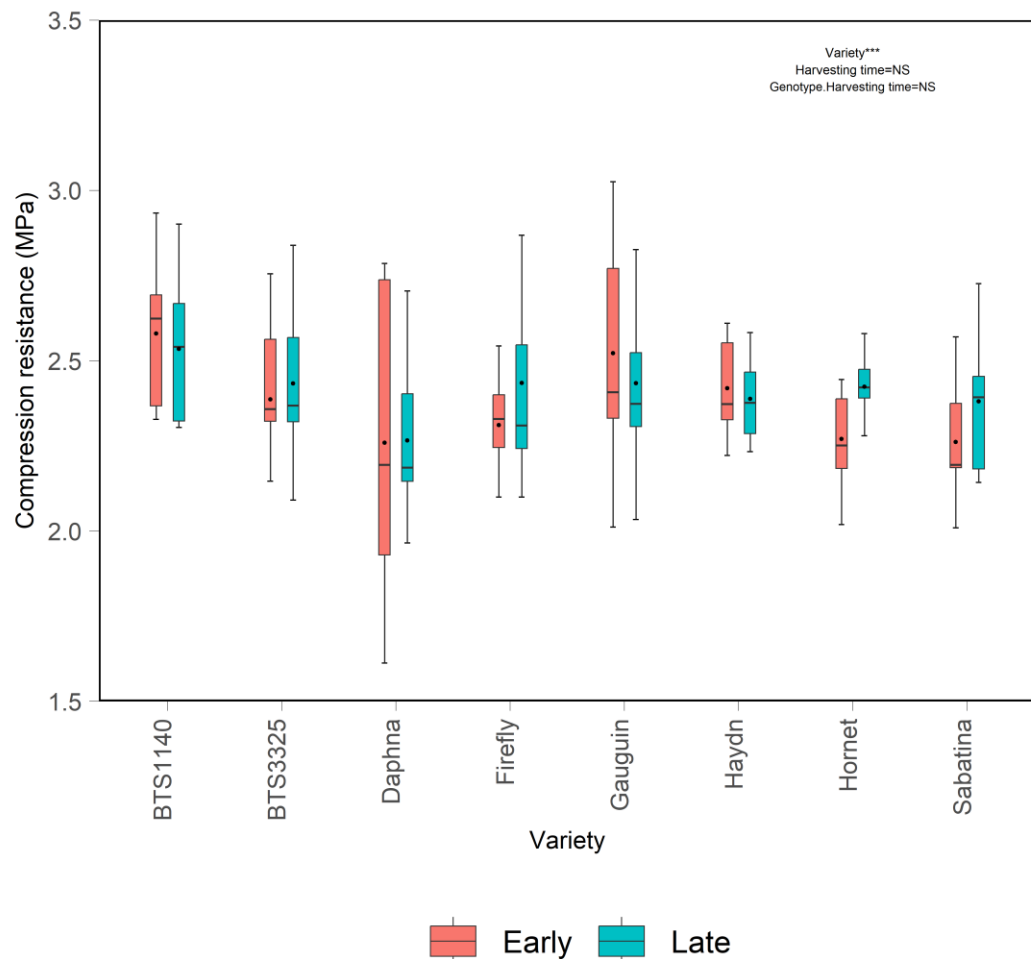


Figure 3-53: Effects of harvesting time and variety on sugar beet compression resistance at Bracebridge. $n=144$, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

3.4.2.2 Root tip damage

Delaying harvesting by one month at Bracebridge affected sugar beet root response to tip diameter after damage ($p < 0.001$). On average, root tip diameter after damage for late-harvested roots was 12 % greater than early harvested roots. However, the interaction between variety and harvesting time had an influence ($p < 0.001$) on response to root tip diameter after damage with varieties Haydn and Firefly significantly increasing their root tip diameter after damage when harvested late by 16 and 31 %, respectively (**Figure 3-54**). Other varieties were not statistically different from each other regarding root tip diameter after damage when harvested

late. There were no differences regarding root tip diameter after damage in 2019 when harvested at different times. However, during the 2020 and 2021 seasons, a significant increase in root tip diameter after damage was recorded when the roots were harvested late (**Figure 3-55**).

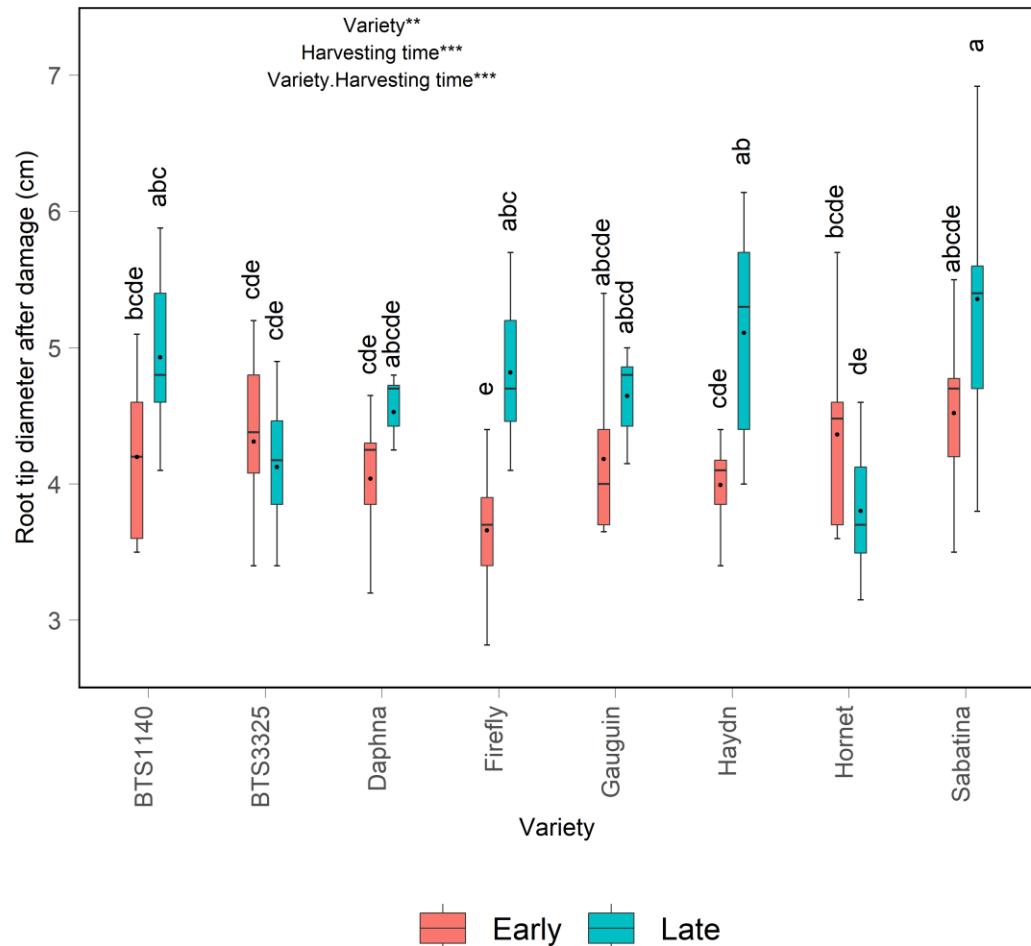


Figure 3-54: Effects of harvesting time and variety on sugar beet compression resistance at Bracebridge. n=135, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

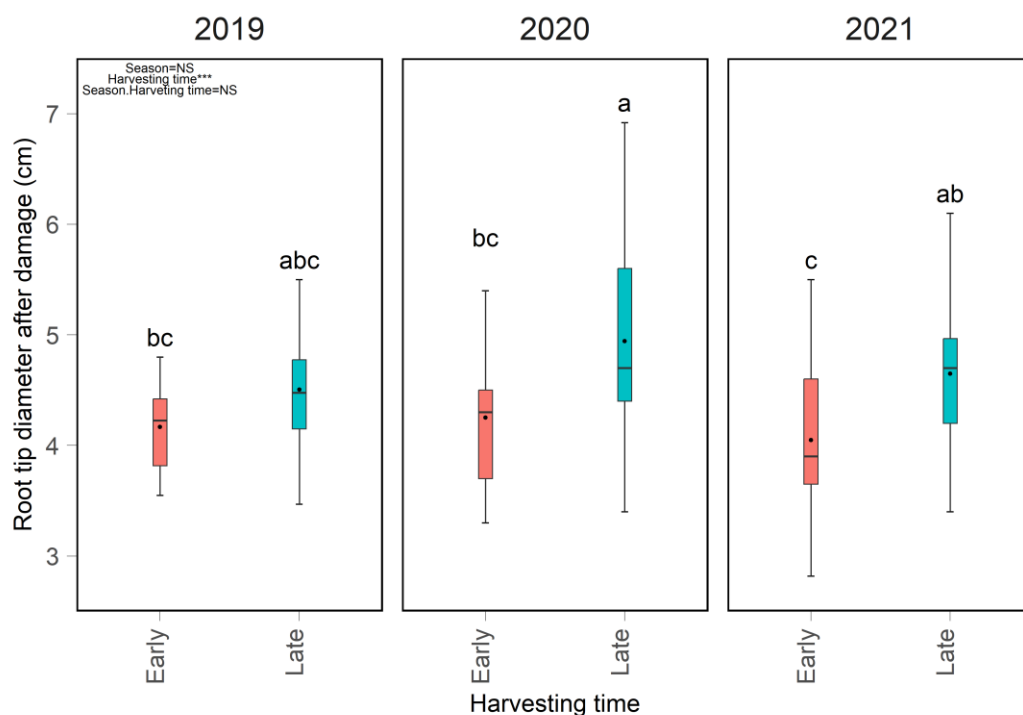


Figure 3-55: Effects of harvesting time and season on root tip diameter after damage at Bracebridge. n=135, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

3.4.2.3 Surface damage

Surface damage was not affected by harvesting time, variety and their interaction (**Figure 3-56**). However, season affected root surface damage ($p < 0.05$). Roots harvested in 2019 had a 10 and 15 % increase in surface damage compared to those of 2020 and 2021, respectively (**Figure 3-57**).

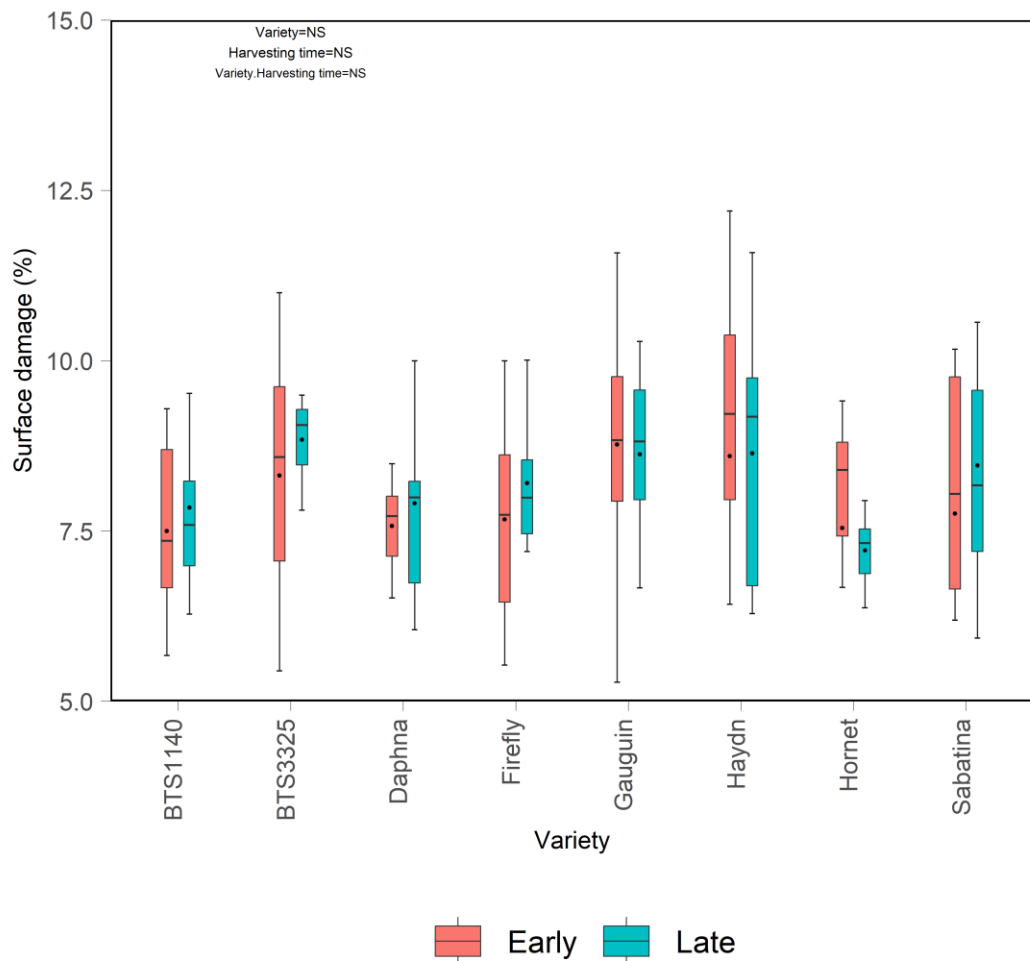


Figure 3-56: Effects of variety and harvesting time on surface damage at Bracebridge. n=135, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

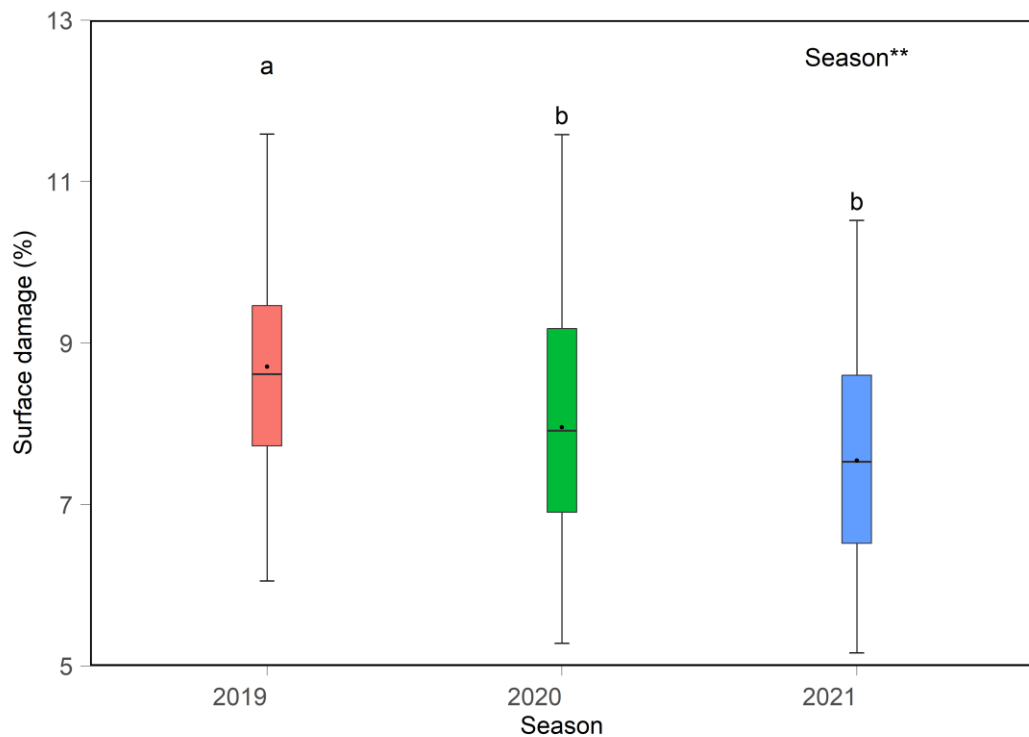


Figure 3-57: Effects of seasons on surface damage at Bracebridge. n=135, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

3.4.2.4 Regression between rainfall and damage

Accumulated rainfall in the 30 days prior to harvesting during both the first and second harvests was used as a proxy for soil moisture and was correlated with root tip and surface damage. There was no relationship between the accumulated amount of rain 30 days prior to harvesting and root tip diameter after damage ($R^2 = 0.06$, $p = 0.635$) (**Figure 3-58**). However, despite not being significant, there was a positive correlation between surface damage and the amount of rain accumulated 30 days prior to harvesting ($R^2 = 0.55$, $p = 0.092$) (**Figure 3-59**).

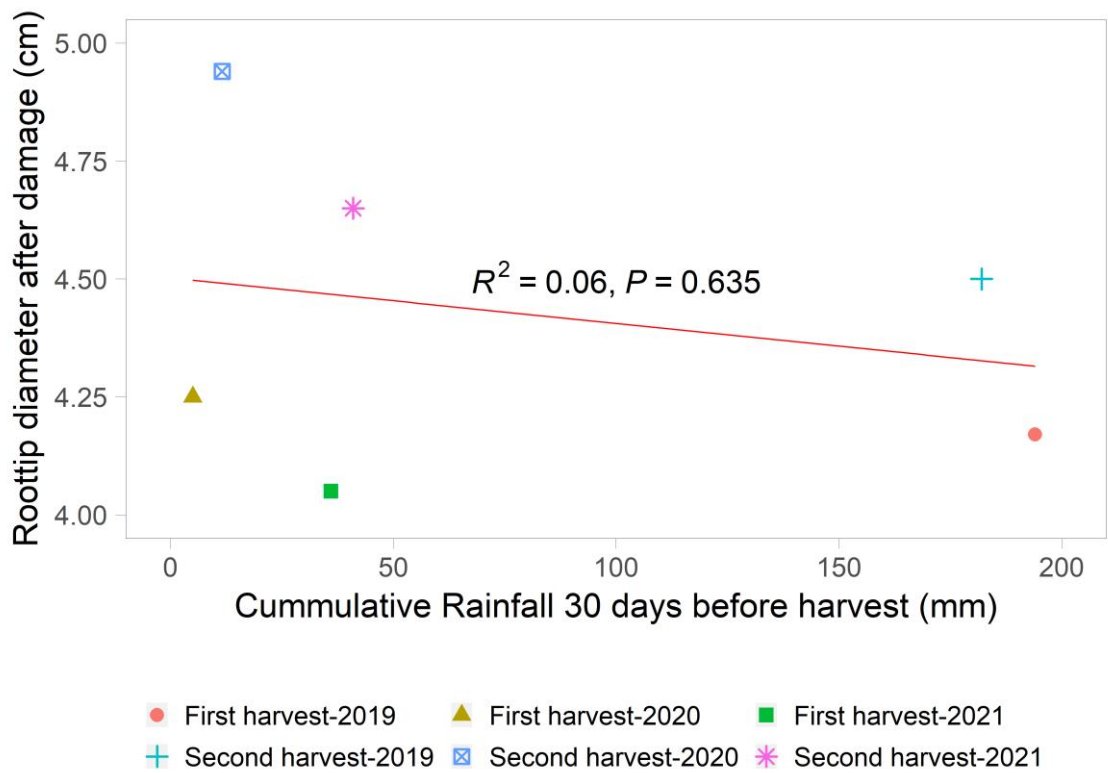


Figure 3-58: Linear regression between root tip diameter after damage and cumulative rainfall from 30 days before harvest.

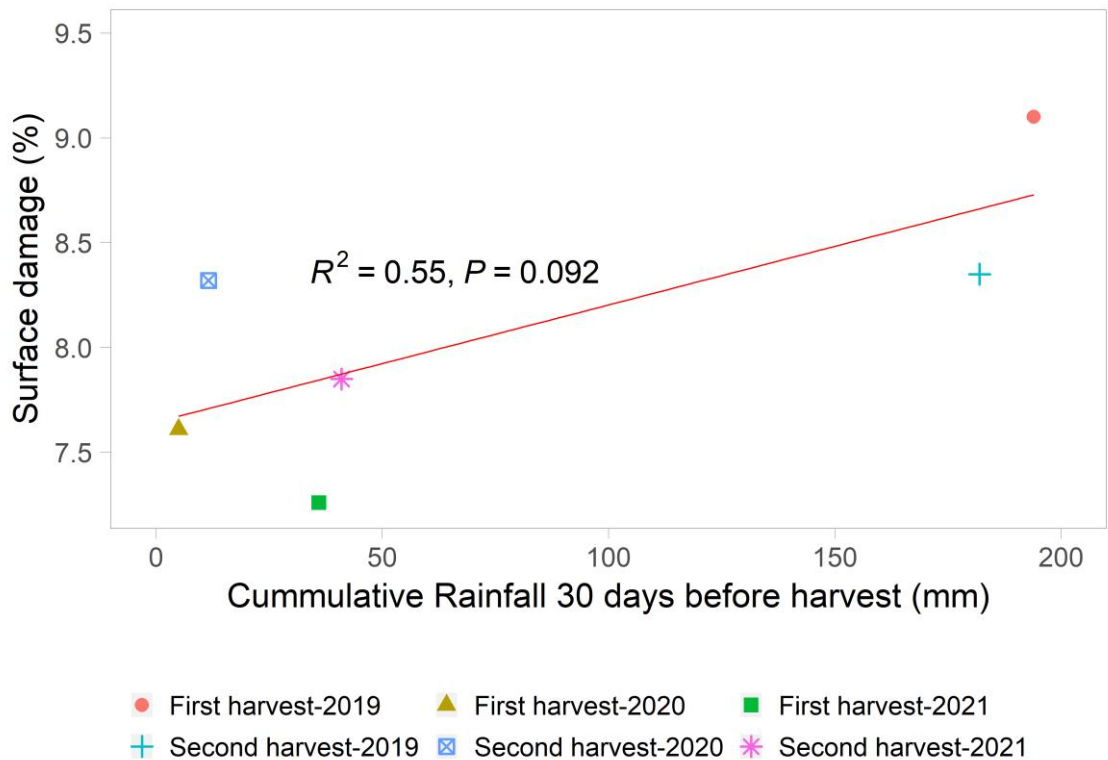


Figure 3-59: Linear regression between surface damage and cumulative rainfall from 30 days before harvest.

3.5 Discussion

3.5.1 Varieties, tissue damage susceptibility and resilience

Root tip and surface damage are marked as the most determining factors for post-harvest losses in sugar beet (Kenter et al., 2006a). Increased root tip diameter after damage in a rotating drum was well demonstrated by Hoffmann and Schnepel (2016) who reported that damaged roots averagely increased tip diameter after damage from 3 to 5 cm. Our results agree with these findings as the root tip diameters after damage were in the same range confirming that root tip breakage is inevitable. Considering varieties under the study, response to root tip diameter after damage was significant where Sabatina was extremely susceptible at both sites and BTS3325 and Firefly were resilient at Fotheringhay and Bracebridge respectively. These findings agree with those of Van Swaij et al., (2003), Hoffmann (2018) and Kleuker and Hoffmann (Kleuker and Hoffmann, 2022) who reported differences in root tip diameter after damage among sugar beet varieties. Kleuker and Hoffmann (2022) observed significant differences in root tip diameter after damage where the site that had small roots due to drought had low root tip breakages. These results mean that there is a possibility to reduce root tip diameter after damage during post-harvest handling by optimising varieties that show resilience.

Surface damage was only affected by season meaning that among the varieties used, there are no extreme varieties regarding this trait. However, Hoffmann (2018) and Hoffmann et al., (2018b) reported differences among varieties in terms of surface damage. The cited literature reported variations in root weight among their varieties which was not the case in this study. Surface damage for varieties that significantly differ in weight was well documented by Van Swaij et al.,(2003) and Akeson and Stout (1978) who attributed increased damage in heavier roots to a large kinetic energy on impact during cleaning. We therefore suggest that uniform surface damage found in this study is due to uniform weights among the eight varieties.

3.5.2 Textural properties and variety's resilience to damage

Varieties with weak textural properties are characterised by low puncture, compression and shear resistances. Kleuker and Hoffmann (2019) described sugar beet varieties with weak textural properties as those having a puncture, shear and compression resistances of equal or less than 5.98, 3.62 and 2.1 MPa, respectively. Kleuker and Hoffmann (2022) attributed this to reduced cell wall components. Our

results show that variety affected textural properties, measured as tissue puncture, shear and compression resistances, and that only Daphna and Sabatina fall within a category of varieties with weak textural properties while the rest are considered strong. Puncture resistance suggests that periderm tissues for tips are frailer compared to the middle and top parts of the roots rendering them most vulnerable to damage. Our findings suggest that tissue compression resistance decreases as you move from the peripheral to the central part of the root. Tip frailness is attributed to the maturation of root cells which are normally tender at zones of differentiation (tip) than at the middle and top. While the decrease in compression resistance on the central part is attributed to a low concentration of cambium rings (Gemtos, 1999) and higher moisture content in the parenchyma tissues. Our findings also agree with Kleuker and Hoffmann (2019) that tissue compression decreases from the peripheral of the root to the central part while puncture and shear resistance decrease as you move from the top to the tips. These results suggest that the contribution of textural properties to root damage is mainly a factor of peripheral tissues and that tips are the most vulnerable parts when exposed to external forces.

Positive correlations among textural properties (shear, puncture and compression) observed in this study agree with Kleuker and Hoffmann (2021) and imply that a puncture test is enough when determining textural strength and can be used as a proxy to predict shear and compression resistances. Kleuker and Hoffmann (2022) and Nause et al., (2020) reported strong negative correlations between textural properties and damage in environments that were not associated with drought. Root tip diameter after damage's strong correlation with resistance to compression and weak correlation with shear and puncture resistance suggest that resistance to compression is an important factor when minimising tip damage. We attribute this to the fact that unlike resistance to puncture and shear, compression resistance determines tissues resilience to external forces applied on the cross-section of the tips making it an important factor in controlling tip damage. It was also observed that at Fotheringhay, surface damage negatively correlated with puncture resistance, however, there was no relationship between the two traits at Bracebridge. This suggests that surface damage response to textural properties is site dependent. Surface damage mainly occurs on the root periderm which contributes to puncture resistance, hence a negative relationship is expected. Our results suggest that root tip and surface damage are influenced differently whereby depending on the site

root tip diameter after damage is more correlated to compression resistance and surface damage is more responsive to puncture resistance. Weak correlations between textural properties and damage at Bracebridge suggest that depending on sites, resistance to damage is not only dependent on textural properties but also morphological and physiological traits.

3.5.3 Morphological factors that affect a variety's resilience to root breakage

Morphologically, the PCA and cluster analysis suggest that the varieties used in this study can be categorised into two. The first category comprises small but long varieties with strong root tissues and small tips. The second category comprises varieties which had wide roots with weak root tissues and small tips.

Results in this study suggest that roots which are genetically characterised by weak textural properties can register smaller root tip diameter after damage when they are morphologically small and long. While varieties that are morphologically short and wide record high root tip diameter after damage. An explanation of why small beets record stronger tissues and damage less was highlighted by Gemtos (1999) who said that the sugar beet main root is composed of rings which have a relatively small inter-ring distance in smaller roots, since vascular tissues are stronger in tension forces, a smaller cross-section of the root provides greater strength. Resilience to greater root tip diameter after damage has been linked to tissue strength where varieties with a higher cell wall content have higher tissue strength and usually register lower root tip breakage (Kleuker and Hoffmann, 2022). Hoffmann et al., (2018a) and Hoffmann and Schnepel (2016) also demonstrated that weak tissue textural properties contribute to the high root tip and surface damage. And there is no information on how morphological factors of the roots affect the root tip or surface damage in both weak and strong varieties. In this study, surface damage had no relationship with morphological traits while root tip diameter after damage was discovered to be strongly correlated with morphological traits. At Bracebridge, a site that was associated with small roots by PCA, root tip diameter after damage was mainly determined by tip diameter before damage. Morphological traits that highly correlated with root tip diameter after damage included the diameter of root tips before damage, root weight per beet, length and width. Our results indicate that root tip diameter after damage response to morphological factors is more marked in varieties with weak than strong textural properties. This was

manifested when despite varieties with strong textural properties' differences in morphological features, their response to root tip diameter after damage was not statistically different. However, of the two varieties with weak textural properties, Daphna registered statistically the same root tip diameter after damage as varieties that with strong textural properties. While the other weak variety (Sabatina), was more vulnerable. We attribute the response by the two weak varieties to their morphological features. Despite Daphna having weak tissues, it was characterised by small tips and long but smaller roots hence more tensional strength from the cambium rings while Sabatina was characterised by short and wide roots but also wider tips.

3.5.4 Effect of delayed harvesting on textural properties and resilience to tissue damage.

This study shows that timely scheduling of harvesting is paramount in reducing post-harvest damage. Van Swaaij et al., (2003) reported that a delay in harvesting worsens root tip damage. Despite being significant, the increase in root tip diameter after damage found in this study due to late harvesting was small compared to the literature and that surface damage was not affected by harvesting time. We attribute this to the short window (one month) between the two harvests as compared to Van Swaaij et al., (2003) who imposed a three months delay. The positive correlation between surface damage and cumulative rainfall 30 days prior to harvesting suggest that for varieties used in this study, soil moisture 30 days prior to harvesting may be a factor influencing surface damage. However, moisture was only determined by using rainfall as a proxy hence calling for a need to institute a proper study on how soil moisture at harvest affects root tissue response to damage. An increase in root tip diameter after damage when harvested one month later as observed in this study can be explained by continued secondary growth which led to an increase in width and tips before damage. Cakmakci and Oral (2002) demonstrated that a one-month delay significantly increases root weight for sugar beets by 13 % and that the increase is also attributed to soil type. Therefore, we attribute the increase in root tip diameter after damage to the alteration of morphological features like the width and length of root tips before damage during the one month that the crop was still in the field.

Delayed harvesting did not reduce resistance to compression. This is in agreement with Nause et al., (2020) and Bentini et al., (2005) who reported that textural properties remain stable after a three months delay in harvest. However, results on puncture resistance do not agree with this literature because a one-month harvest delay significantly reduced tissue puncture resistance. Our findings therefore suggest that compared to inner tissues from the parenchyma zone, tissues forming the skin of sugar beet roots are more sensitive to soil conditions when their stay in the soil is prolonged. The decrease in tissue puncture resistance observed in this study did not correlate with root tip and surface damage suggesting that a decrease in puncture resistance alone was not enough to influence damage. This supports an earlier observation in this study that root tip diameter after damage is not significantly correlated to puncture resistance.

3.6 Conclusion

This study identified varieties that possess morphological and physiological properties which can help to address challenges associated with damage during post-harvest handling. Root tip damage is linked to tissue compression strength while surface damage is related to puncture resistance. Varieties with strong textural properties are generally small or medium in size and register low root tip diameter after damage while those with weak textural properties are wider and damage more easily. Morphology influences root tip diameter after damage in varieties with weak textural properties which register low root tip diameter after damage when they are small and long. A delay of one month in harvesting leads to relatively wider and heavier roots which are more prone to root tip damage. Surface damage is not affected by delayed harvesting but is sensitive to rainfall accumulated prior to harvesting.

Chapter 4: Effects of water and temperature at harvest on root damage and tissue integrity

4.1 Abstract

Water and temperature play a vital role in root tissue strength and damage. Root damage promotes sugar loss in storage for sugar beet through increased respiration and leakage. In the UK, sugar beet is harvested under different soil moistures and temperatures due to varying rainfall patterns, soil types and extended harvesting campaigns. However, less attention is directed towards water and temperature status at harvest and their impact on root tissue strength and damage. In response to this gap, an experiment was laid out in 2020, 2021 and 2022 to investigate the effects of water status and temperature at harvest on sugar beet's response to damage and tissue integrity. The experiment was first laid out in the field and later transferred into a polytunnel for the imposition of water treatments when the crop had reached physiological maturity. Temperature treatments were imposed by storing the roots at room temperature (10 °C) and in a cold room (3 °C) for three days after harvesting. The experiment was a 2 x 2 factorial with two temperature levels (3 and 10 °C) and two water levels (irrigated to field capacity for seven weeks after physiological maturity or not irrigated for seven weeks). Results indicate that soil water status affects relative water content (RWC) ($p < 0.001$), textural properties (puncture ($p < 0.001$) and compression ($p < 0.001$)). RWC is correlated to surface damage ($R^2=0.48$), compression ($R^2=0.70$), and tissue puncture ($R^2=0.56$). Harvesting temperatures did not affect root tip and surface damage. The high soil moisture content at harvest significantly increased surface damage ($p < 0.001$) and reduced tissue strength ($p < 0.001$). These results mean that harvesting temperatures over the range of 3 – 10 °C are not an issue of concern in sugar beet root tissue integrity and damage. However, when minimising root tip and surface damage through optimisation of root tissue strength, soil water status prior to harvest must be carefully considered.

4.2 Introduction

Sugar beet root tissue strength is influenced by several factors including cellular components, biochemical constituents, water content, cell wall composition and temperature (Nause et al., 2020). Weaker root tissues are more prone to post-harvest damage which promotes sugar losses when in storage. Water is linked to tissue strength through the maintenance of the structure and integrity of cell membranes (Gorzelany and Puchalski, 2000; Lewicki and Jakubczyk, 2004). It is an integral part of photosynthesis and regulates turgidity which is fundamental in maintaining structure and rigidity. Crops with higher parenchyma cells are more brittle and at high turgidity, protoplasts exert pressure on cell walls reducing the amount of force required to induce fracture (McGarry, 1995; Smittle et al., 1974). Studies in carrots have shown that tissue strength is inversely related to turgidity (Herppich et al., 2001; McGarry, 1993). A recent study by Lockley et al., (2021) concluded that an increase in radish hypocotyl water content was associated with an increase in splitting susceptibility due to impact and a decrease in failure force for both compression and puncture forces. Schafer et al., (2020) observed less compressive strength in root samples that had higher water content in sugar beet. However, there is no explanation of what caused an increase in water content for some samples. High tissue moisture content has also been linked with low temperatures mostly in vegetable crops. For example, the mechanical properties of dried apple tissues depend on hot air drying and at a temperature of 70 °C and below biopolymers are affected to a lesser degree than at a temperature of 80 °C suggesting that the state of water in apple slices dried at 80 °C is different in respect to that in material obtained at below 70 °C (Lewicki and Jakubczyk, 2004). Extreme temperature conditions can also denature cell wall components (Wolf and Hab, 2017) hence affecting tissue strength. Herppich et al., (2002) reported that for carrots, cutting forces are negatively correlated to temperature with the highest and lowest values at 5 and 40 °C, respectively. Herppich et al., (2005) also observed that a reduction of temperature from 20°C to 10°C increases textural strength in both carrots and radishes. Agreeing with these findings Lockley et al., (2021) reported that radish splitting susceptibility is negatively related to temperature.

Despite evidence of water and temperature's ability to manipulate root tissue mechanical properties and their resilience to damage, growers in the UK harvest sugar beet under varying moisture and temperature due to differences in rainfall

patterns and soil types (Gabarron-Galeote et al., 2019; Okom et al., 2017) and extended harvesting campaigns. Extended harvesting campaigns render growers to harvest their crops either in autumn when temperatures are mild or during winter when temperatures are relatively lower (Gabarron-Galeote et al., 2019). Variations in soil moisture and temperature conditions at harvest could be a precursor for root tissue frailness and damage. However, current research in sugar beet has focused on water and temperature's contribution to economic yield during the crop growing period. There is little information on whether water and temperature statuses at harvest manipulate sugar beet root tissues and render them prone to damage. This study therefore specifically investigated whether 1) water status at harvest affects sugar beet tissue damage susceptibility 2) the temperature of roots at harvest affects sugar beet's tissue damage susceptibility and 3) the interaction between temperature and water status affects sugar beet tissue damage susceptibility.

4.3 Materials and methods

4.3.1 Field and Polytunnel management

4.3.1.1 2020 Trial

4.3.1.1.1 Site and experimental design

During the first year (2020), the experiment was planted at HAU in a completely randomised design with three replications. It was a 2x2 factorial consisting of irrigation (irrigated to field capacity (30 %) and non-irrigation) and harvesting temperature (3 and 10 °C) as factors. Soils used in 2020 were the washed-away particles from the sugar beet delivered by growers at the Wisington sugar factory. Chemical and physical properties analysis for the soil (**Table 4-1**) was done as described on section 3.3.1. The soils were sandy loam and generally characterised by high nutrient content for most elements. Weather parameters for HAU were obtained from the HAU weather station (**Figure 4-1**) and during the time the crop was outside the polytunnel from 15th April to 29th October 2020 (197 days), the area received a total of 438 mm of rainfall which was distributed in 101 rain days. The area was characterised by some patches of drought, especially during the first 60 days. The temperature ranged from 4 to 24 with an average of 13 °C.

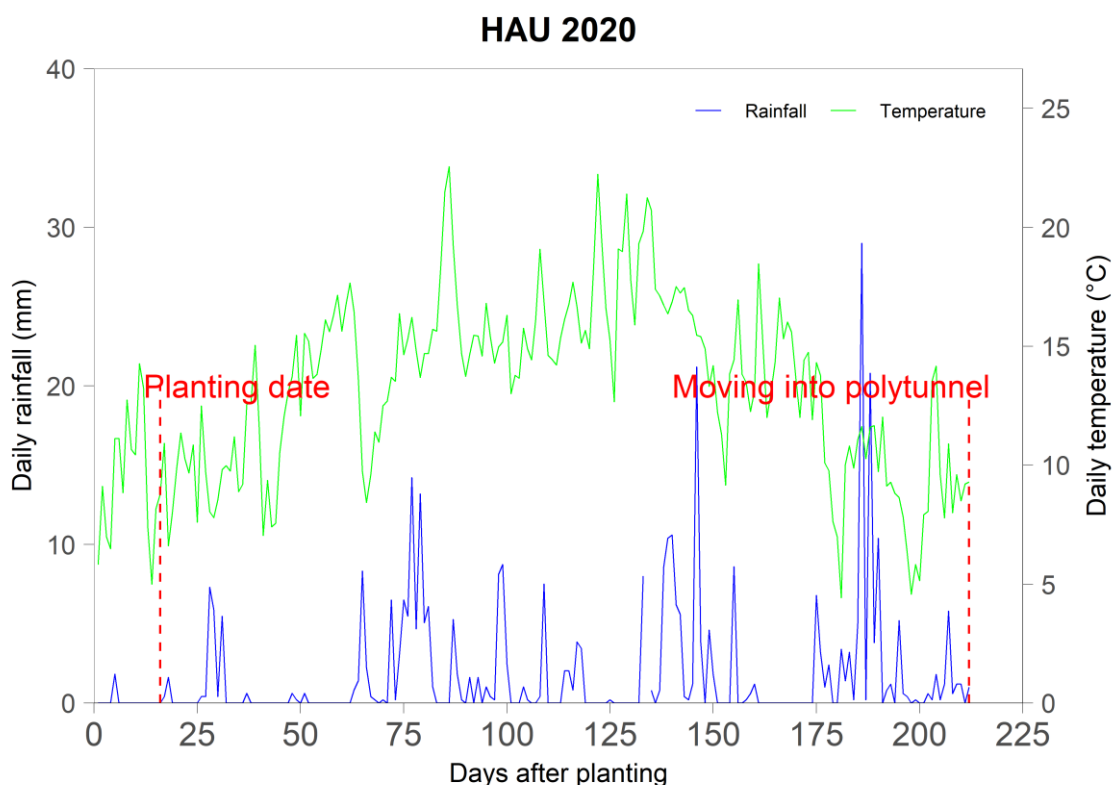


Figure 4-1: Rainfall and temperature at HAU in 2020

Table 4-1: Physical and chemical properties for soils used during the 2020 season
at HAU

Variable	Measurement
pH	7.9
P mg L ⁻¹ (Available)	63
K mg L ⁻¹ (Available)	1038
Mg mg L ⁻¹ (Available)	149
Cu (EDTA Extractable) mg L ⁻¹	5
B (Hot Water Soluble) mg L ⁻¹	2
Na (Ammonium Nitrate Extractable) mg L ⁻¹	305
Zn (EDTA Extractable) mg L ⁻¹	8
Ca (Ammonium Nitrate Extractable) mg L ⁻¹	2135
Fe (DPTA Extractable) mg L ⁻¹	146
Organic matter (LOI) %	6
S (Phosphate Buffer Extractable) mg L ⁻¹	1078
Mn (DPTA Extractable) mg L ⁻¹	12
Sand (2.00 - 0.063mm) %	65
Silt (0.063 - 0.002mm) %	20
Clay (< 0.002mm) %	15
Textural Classification	Sandy loam
Estimated CEC meq 100 ⁻¹	21
Field capacity (%)	30

4.3.1.1.2 Planting and field management

Sabatina which was categorized as weak in textural properties and vulnerable to damage from preliminary work in **chapter 3** was chosen as a test variety for the trial. The aim was to check if extreme water and temperature conditions at harvest would enhance or reduce robustness in textural properties and resilience to damage. The experiment was planted on the 15th of April 2020 in potato boxes (Fitters et al., 2018) of 1 m wide, 1.2 m long and 1 m high. Before planting, the inside part of the boxes was covered with mypex to avoid loss of water from the sides of the boxes. The boxes were then filled with soil up to the brim and left in the field for soil to settle. Leaving a space of 10 cm from the edges, three rows were drawn per box and one seed was planted per station with an intra and inter-row spacing of 16 and 50 cm, respectively (**Figure 4-2**). This resulted in 7 plants per row giving a total of 21 plants per box. To maintain natural growing conditions, the experiment was put in a field at HAU farm until the crop reached physiological maturity. After planting, a YaraBela CAN (27% N) fertiliser was applied by spreading at a rate of 100 Kg N ha⁻¹ (BBRO, 2022). A multi-B trace (Plonvint – Agri-expo, UK) was applied once a month at a rate of 3 L per hectare to supply the crop with micronutrients. To protect the crop from fungal diseases, Escolta (cyproconazole and trifloxystrobin) (Bayer – UK) was applied at a rate of 0.35 ha⁻¹ after dissolving it in water once every month. The boxes were kept weed-free by hand weeding. Moisture readings were taken every two weeks using a TDR 100 Soil Moisture Meter to a depth of 20 cm and when necessary, the plots were irrigated to avoid wilting. All other agronomic practices needed before the crop was taken into the polytunnel for the imposition of treatments were done according to (BBRO, 2022).



Figure 4-2: Sugar beet in the field one month after planting

4.3.1.1.3 Polytunnel and management

After physiological maturity, the plants were moved into a polytunnel on the 29th of October 2020 using a tractor for the imposition of water and temperature treatments. The polytunnel's temperature and relative humidity were measured (**Figure 4-4**) using data loggers (Tinytag plus 2 – GP-4505 – UK). Soil moisture at field capacity was maintained by irrigating the boxes with a water volume that was a deficit from the field capacity. That's before each irrigation, soil moisture content (**Figure 4-3**) was measured weekly in every box to a depth of 20 cm using a TDR 100 Soil Moisture Meter. Five points were chosen within the box and an average value was used as a representative value for each box. Moisture values for boxes that were irrigated were used to determine the volume of water required to maintain field capacity. The required water volume was calculated using the length and width of the boxes and a depth of 30 cm was used since the sugar beet didn't go beyond that depth. The irrigation treatment was imposed for 52 days until the experiment was harvested on the 20th of December 2020. Only the middle row was harvested (**Figure 4-5**) where two roots were used for textural tests and the remaining five for damage tests. Temperature treatments (**Table 4-2**) were imposed at harvest by

keeping the roots in a cold room with an average of 3 °C and 97 % relative humidity and others in a cold room at an average temperature and relative humidity of 10 °C and 80 %, respectively for three days before textural and damage tests.

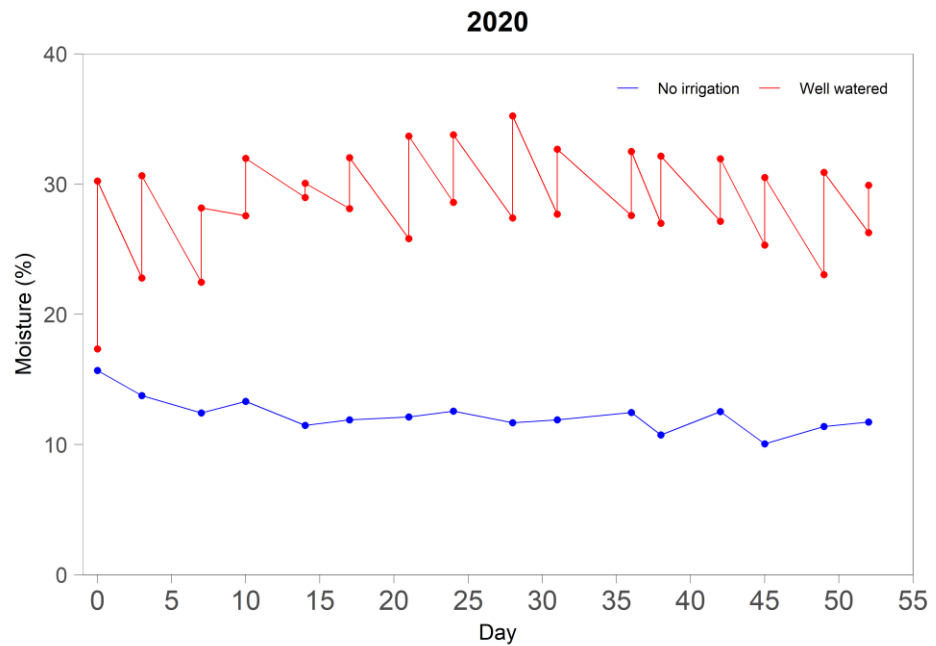


Figure 4-3: Moisture treatments in the polytunnel

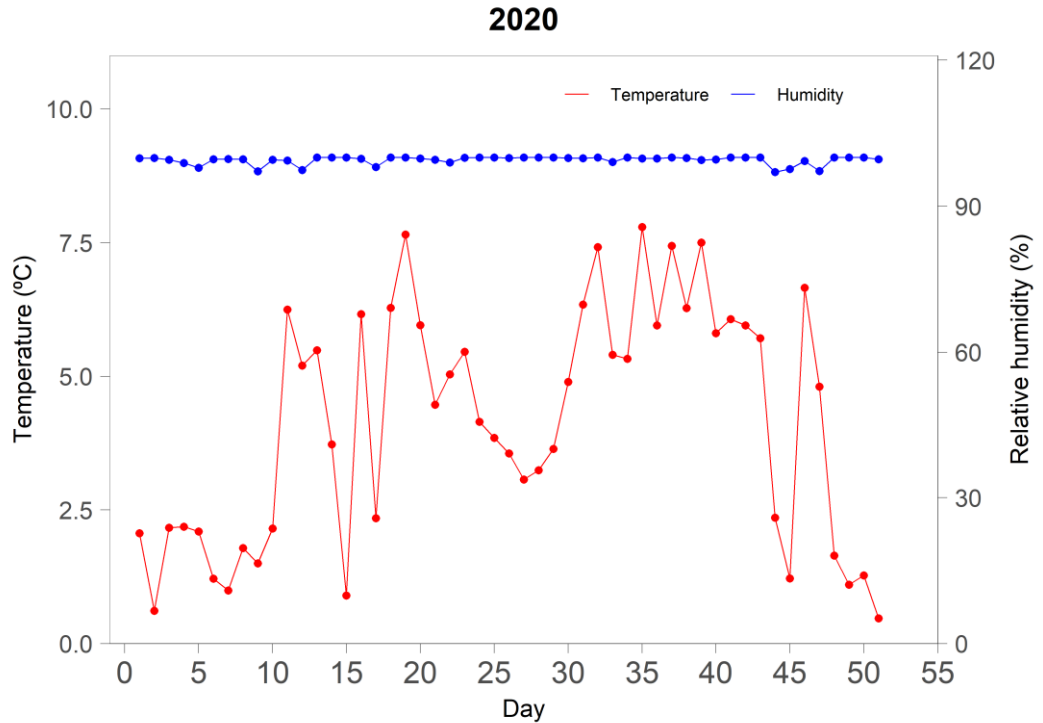


Figure 4-4: Temperature and relative humidity in the polytunnel in 2020

Table 4-2: Root temperature during textural analysis in 2020

Irrigation	Harvesting temperature (°C)	Root temperature (°C)
Irrigated	10	13±0.3
Irrigated	3	4±0.6
No irrigation	10	12±0.40
No irrigation	3	3±0.6



Figure 4-5: Harvesting in progress in potato boxes

4.3.1.2 2021 and 2022 trials

4.3.1.2.1 Field management

In 2021 and 2022, plants initially drilled at Bracebridge were transplanted into large plastic tubs instead of direct sowing in potato boxes. The same variety was maintained as the one used in 2020. The seeds were drilled on three rows of 100 m long at Bracebridge on the 1st of April in 2021 and the 2nd of April in 2022. Each row was spaced 50 cm apart and the seeds were drilled at an intra-row spacing of 16 cm giving a minimum of 100000 plants ha⁻¹. Close to four months after drilling (on 26th of July 2021 and 8th of July 2022), 20 plastic buckets of 40 and 50 cm in height and diameter, respectively were filled with soil (**Figure 4-6**) from the same plot. Two plants were gently uprooted from the middle row and immediately transplanted into each pot. The plants were immediately watered to field capacity and transported (**Figure 4-7**) to HAU. An analysis of the chemical and textural composition of the soils (**Table 4-3**) was done as described on section **3.3.1**. The analysis shows that the field used in 2021 was characterised as a sandy loam soil while in 2022 the field on which the transplants were drilled was classified as a clay loam soils. In 2022, the soils had a relatively lower amounts of K, Mg, Cu, Ca and Fe compared to the field that was used in 2021.



Figure 4-6: Pots filled with soil taken from sugar beet filled at Bracebridge



Figure 4-7: Transplanted plants

Table 4-3: Soil properties for Bracebridge

Measurement	2021	2022
pH	8.1	7.3
P mg L ⁻¹ (Available)	43	17
K mg L ⁻¹ (Available)	178	157
Mg mg L ⁻¹ (Available)	52	36
Cu (EDTA Extractable) mg L ⁻¹	8	2
B (Hot Water Soluble) mg L ⁻¹	2	2
Na (Ammonium Nitrate Extractable) mg L ⁻¹	7	11
Zn (EDTA Extractable) mg L ⁻¹	7	3
Ca (Ammonium Nitrate Extractable) mg L ⁻¹	2123	1460
Fe (DPTA Extractable) mg L ⁻¹	20	12
Organic matter (LOI) %	4	4
S (Phosphate Buffer Extractable) mg L ⁻¹	13	25
Mn (DPTA Extractable) mg L ⁻¹	6	7
Sand (2.00 - 0.063mm) %	78	50
Silt (0.063 - 0.002mm) %	10	23
Clay (< 0.002mm) %	12	27
Textural Classification	Sandy loam	Clay loam
Estimated CEC meq 100 ⁻¹	15	11
Field capacity (%)	30	32

4.3.1.2.2 HAU field management

Upon arrival at HAU, the pots were randomly put in a field for 90 days (from 26 July to 26 October in 2021 and 8 July to 8 October in 2022) for the crop to reach physiological maturity. In both years, rainfall and temperature data for the period that the crop was in the field were obtained from HAU's weather station (**Figure 4-8** and **Figure 4-9**) which was 0.64 kilometres away from the field. In 2021, when the plants were in the field, HAU received 150 mm of rain which was distributed over 23 days with a temperature range and average of 5 - 19 and 14 °C, respectively. In 2022, 132 mm of rain was received at HAU when the plants were in the field while the temperature range and average were 2 - 27 and 16 °C, respectively. Both seasons were characterised by drought hence the water was supplemented in all pots through irrigation during extended dry periods. Soil moisture content was measured weekly using a TDR 100 moisture meter.

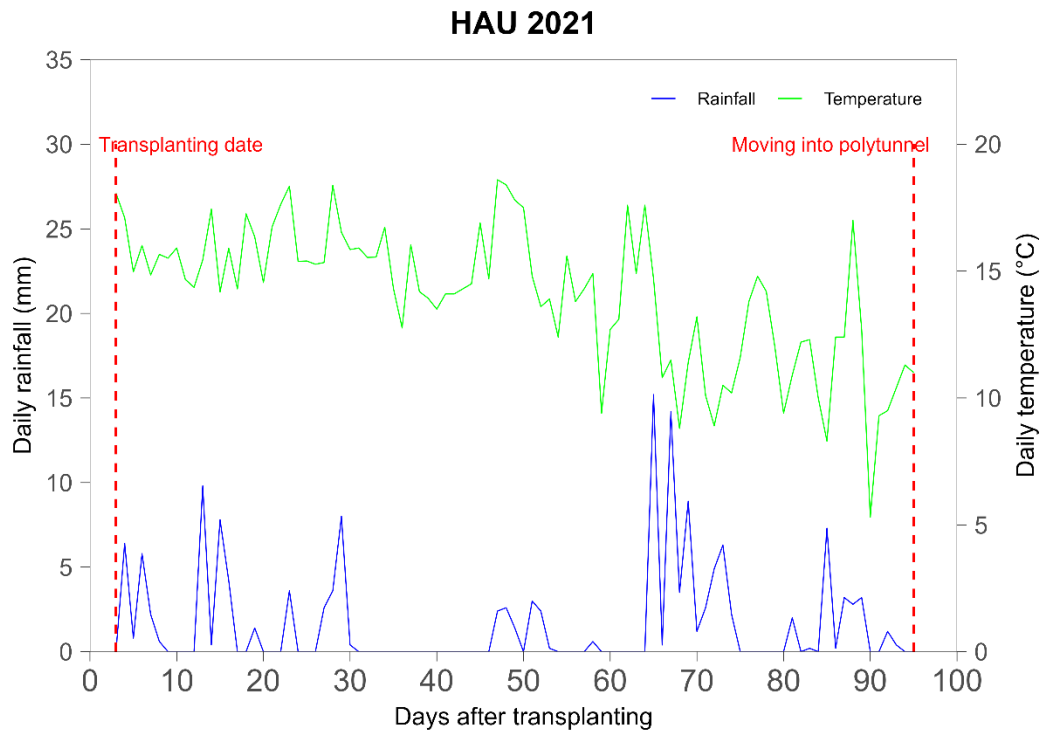


Figure 4-8: Daily rainfall and temperature at HAU when the transplants were in the field in 2021

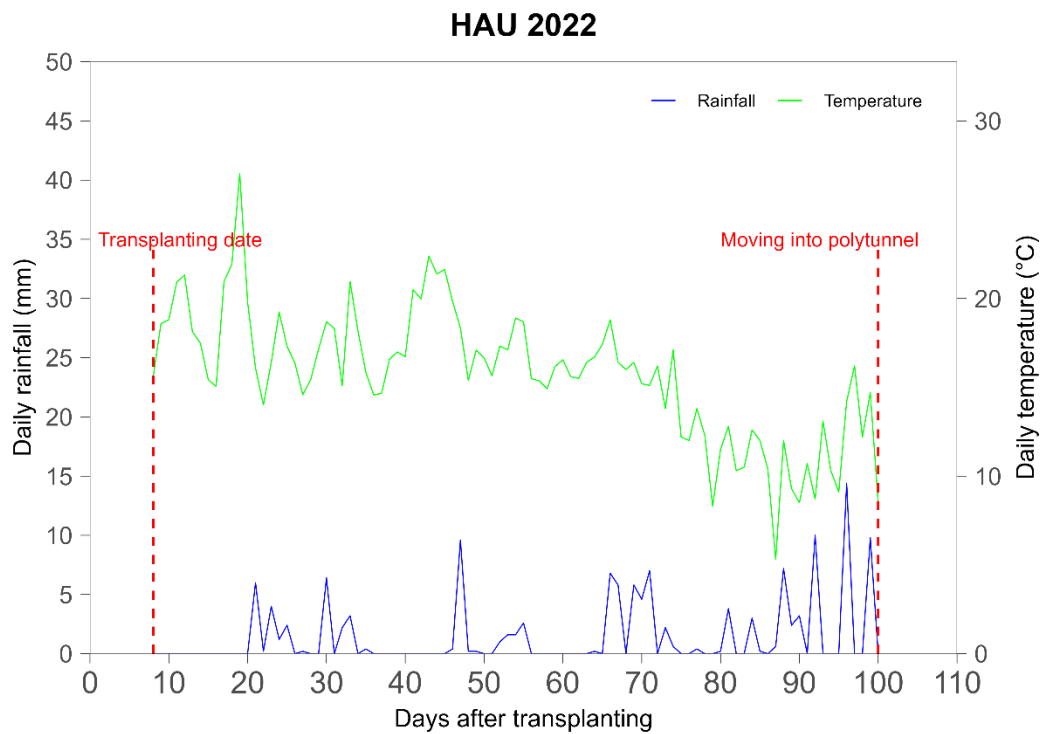


Figure 4-9: Daily rainfall and temperature at HAU when the transplants were in the field in 2022

4.3.1.2.3 Experimental design and polytunnel management

The crops were moved into a polytunnel on 26th October in 2021 and on 8th October in 2022 for the imposition of moisture treatments. In both years, the temperatures in the polytunnel were 5 °C higher compared to the field temperatures during the days leading to the polytunnel transfer. In the polytunnel, the experiment was laid in a completely randomised design with five replications. Treatments were arranged in a 2 x 2 factorial consisting of irrigation and harvesting temperature as factors. Irrigation was applied at two levels: maintaining the soil moisture at field capacity (30%) and without irrigation. Both treatments were imposed for 49 days and the experiment was then harvested for mechanical tests. Temperature treatments were imposed at harvest by keeping the roots at 3 °C and 10 °C for three days before textural and damage tests. While in the polytunnel, temperature and relative humidity were measured using data loggers (Tinytag plus 2 – GP-4505 – UK) (**Figure 4-12** and **Figure 4-13**). Soil moisture content was measured weekly to a depth of 20 cm using a TDR 100 Soil Moisture Meter (**Figure 4-10** and **Figure 4-11**). Soil moisture was measured by averaging measurements from five randomly selected points. The roots were then harvested and washed (**Figure 4-14**) (on the 13th of December in 2021 and on the 27th of November in 2022) before texture and damage tests. To impose temperature treatments, some were kept in a cold room that had an average of 10 °C and the other roots were kept in a cold room that had a temperature of 3 °C. After three days, RAW, texture (including puncture, shear and compression) and damage tests were conducted.

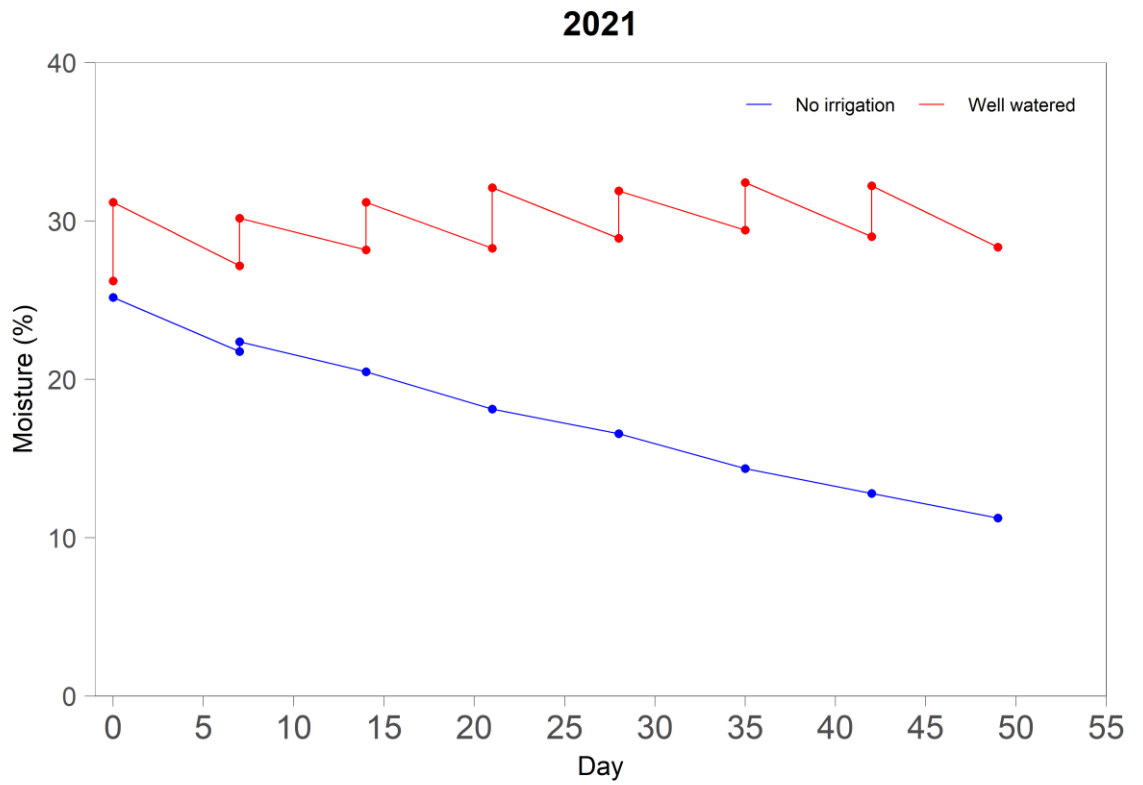


Figure 4-10: Soil moisture when the plants were in a polytunnel in 2021

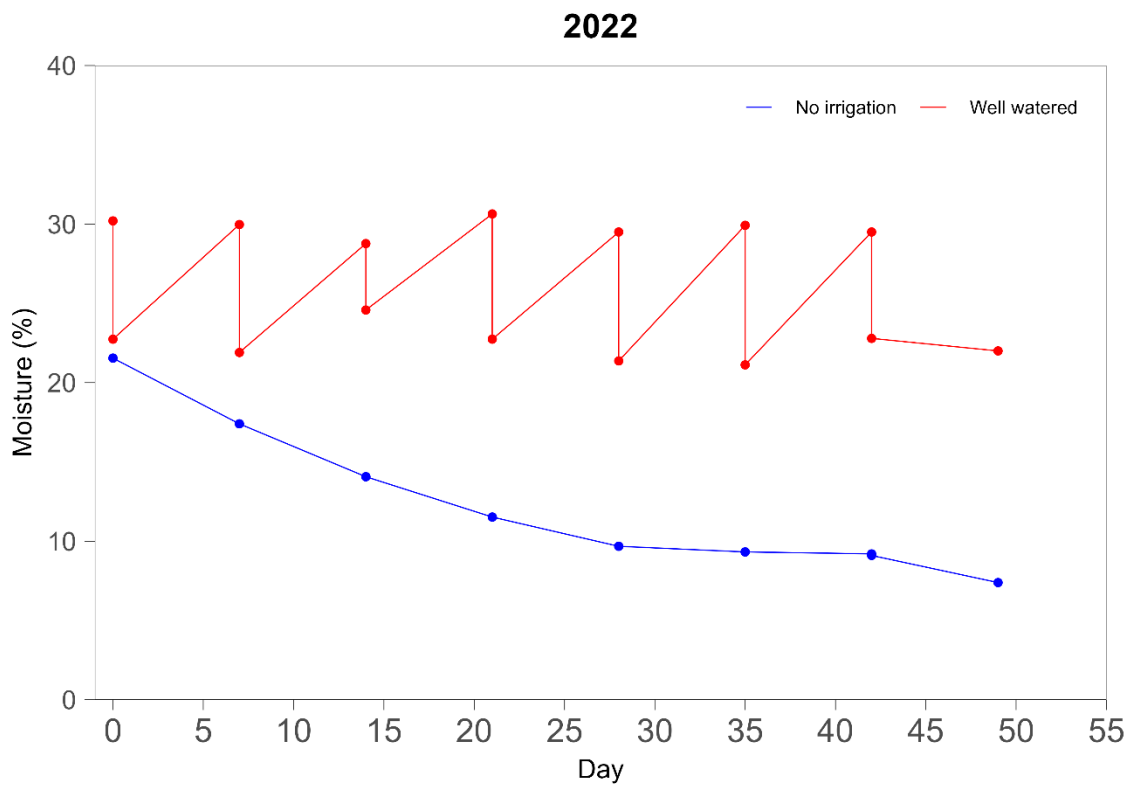


Figure 4-11: Soil moisture when the plants were in a polytunnel in 2022

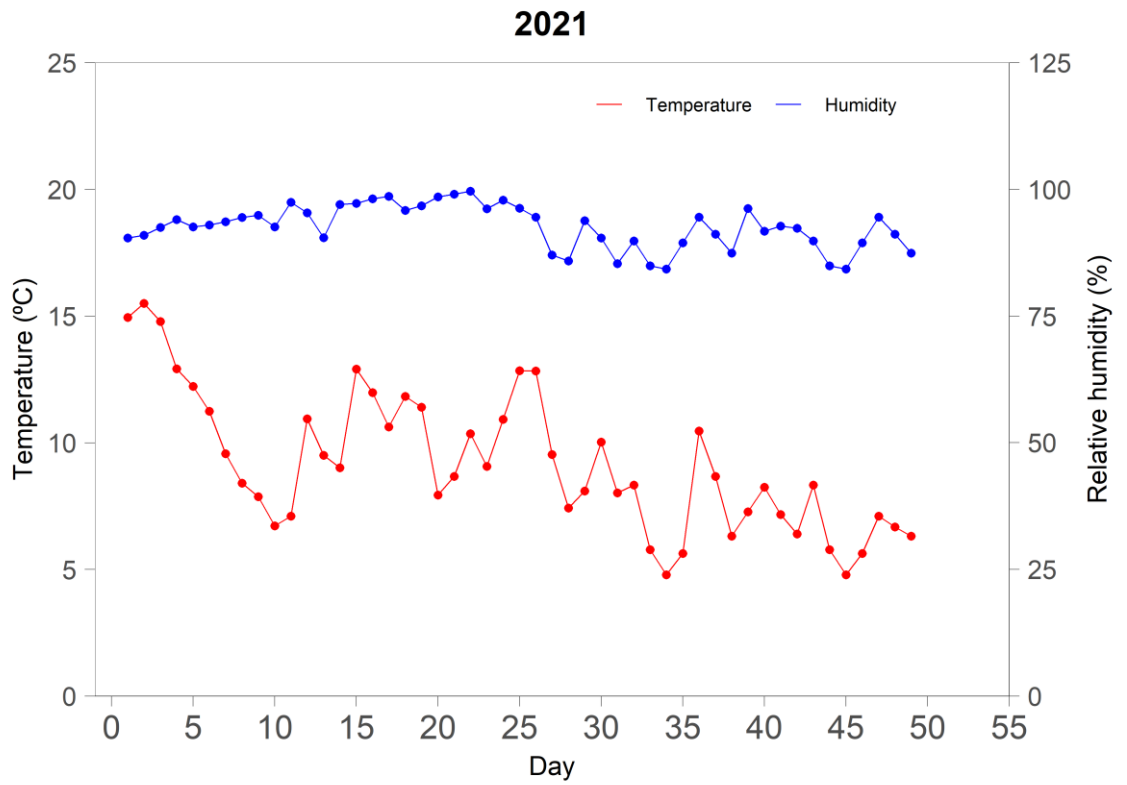


Figure 4-12: Temperature and relative humidity in the polytunnel in 2021

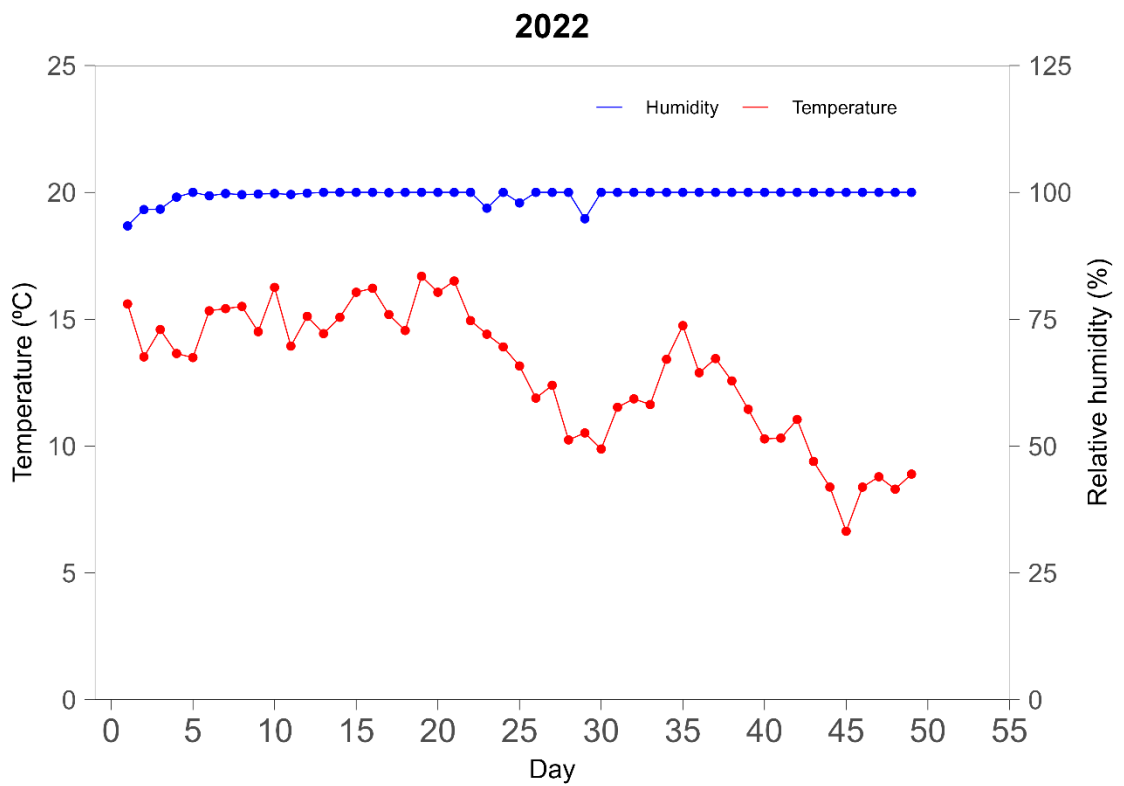


Figure 4-13: Temperature and relative humidity in the polytunnel in 2022



Figure 4-14: Harvested roots in plastic tubs

4.3.2 Soil field capacity

Field capacity is the water content at which drainage flux from a soil ceases, or becomes negligible (Meyer and Gee, 1999). In this study, field capacity was determined gravimetrically (Cassel and Nielsen, 1986). Three seed trays of 1L were weighed (W1). A composite sample was formed by mixing sub-samples of soil from each experimental unit. The composite sample was then filled into the three seed trays. The filled trays were weighed (W2). The actual weight of the soil (W3) in the seed tray was determined by subtracting W1 from W2. The soil was gradually watered until saturation point. The saturation point was considered the point when water started flowing out of the soil through the holes at the bottom of the seed trays. The top of the seed trays was covered by polythene to avoid loss of water through evaporation. The soil was left for 48 hours until the water level infiltrated due to gravitational force. At this point, the soil was considered to have reached field capacity. After 48 hours, the trays containing the soil were weighed again (W4). Wet weight at field capacity (W5) was determined by subtracting W1 from W4. The soil was then oven dried at 105°C (Salter and Haworth, 1961), for 24 hours to determine

the dry weight of the soil (W_6). Gravitational water content (GWC), Bulk density (Bd) and volumetric water content (VWC) were calculated as below.

$$\text{GWC (\%)} = \frac{(W_5 - W_6)}{W_6} \times 100$$

$$\text{Bd (g}^{-\text{cm}^3}\text{)} = \frac{W_3}{\text{Volume}}$$

$$\text{VWC (\%)} = \text{GWC} \times \text{Bd}$$

4.3.3 Leaf SPAD

To determine the general plant health, SPAD values were measured using a SPAD 502 Plus Chlorophyll Meter (Konica-Minolta, Japan) (Monostori et al., 2016). The SPAD-502 meter is a hand-held device that is widely used for rapid, accurate and non-destructive measurement of leaf chlorophyll concentrations (Ling et al., 2011). It measures the absorbances of the leaf in the red and near-infrared regions and uses these two absorbances to calculate a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. In this study, two plants were transplanted into each pot. Leaves were randomly marked on the two plants, on the base, middle and shoots of each plant making a total of six leaves per treatment and 120 leaves from the whole experiment. SPAD values were measured on the marked leaves' lamina on the first day when the plants were taken into the polytunnel and every seven days from the first measurement. Thus, six values were obtained from each plant of which two were from the bottom leaves which were almost senescing, two from the middle leaves and the remaining two were from the top leaves. An average of these six values calculated by the SPAD meter was used during the analysis. This data was only collected in the second and third years because of instrument availability.

4.3.4 Relative water content

RWC for root tissue was measured following Fiitters et al., (2018). A cylindrical section of the root was cut on the widest circumference from two roots per plot. Three samples were extracted from each root using an 18 mm probe from the middle, edge and central part of the root. The samples were cut to a height of 18 cm using a knife and weighed on a digital scale. The samples were then put in plastic containers, which were later filled with distilled water. The containers were covered and stored in a cold room at 5 °C and relative humidity of 85 % for three days so that the root tissues could absorb more water. During this time, it was expected that the tissues were turgid (**Figure 4-15**). To determine weight at turgidity, the samples

were taken out of the cold room and re-weighed. They were then put in an oven and dried at 75 °C (**Figure 4-16**). While in the oven, the samples were weighed every day until they registered a constant weight (dry weight).



Figure 4-15: Turgid root tissues

RWC was calculated using the formular below:

$$RWC = \frac{(WW - DW)}{(TW - DW)} * 100$$

Where WW tissue weight at extraction, DW tissue weight after drying and TW is tissue weight at turgidity.



Figure 4-16: Dry root tissues

4.3.5 Damage tests and Texture analysis

Traits analysed during texture analysis included puncture, shear and compression (**Figure 4-17**) resistances. Root tip damage was performed as outlined on **3.3.6** while textural analyses were done as described on **3.3.5**. However, only two roots per treatment were used in this experiment as only two plants were planted per pot. When conducting textural tests, roots of the same temperature were analysed on the same day to avoid the effects of storage time on temperature. Before each test, root temperature was measured (**Figure 4-18**, **Figure 4-19** and **Table 4-4**) using Therma 20 thermometer (Electronic Temperature Instruments Ltd, UK) to confirm whether it was at the desired temperature.

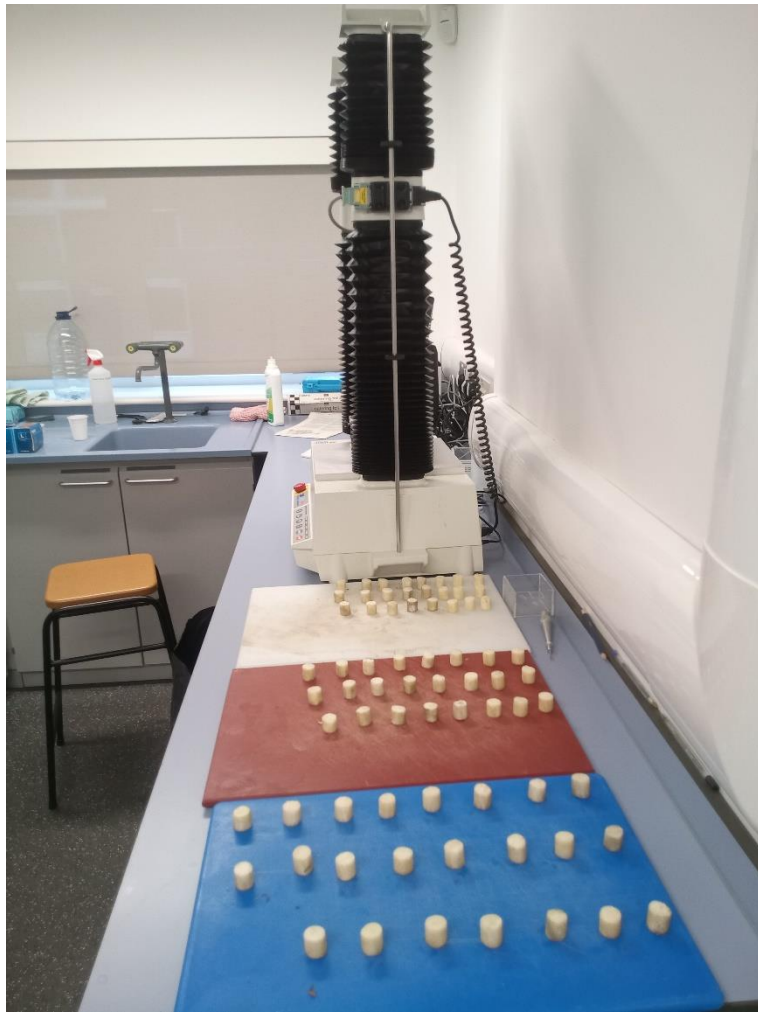


Figure 4-17: Extracted samples ready for compression test



Figure 4-18: A root at 3.2 degrees celsius

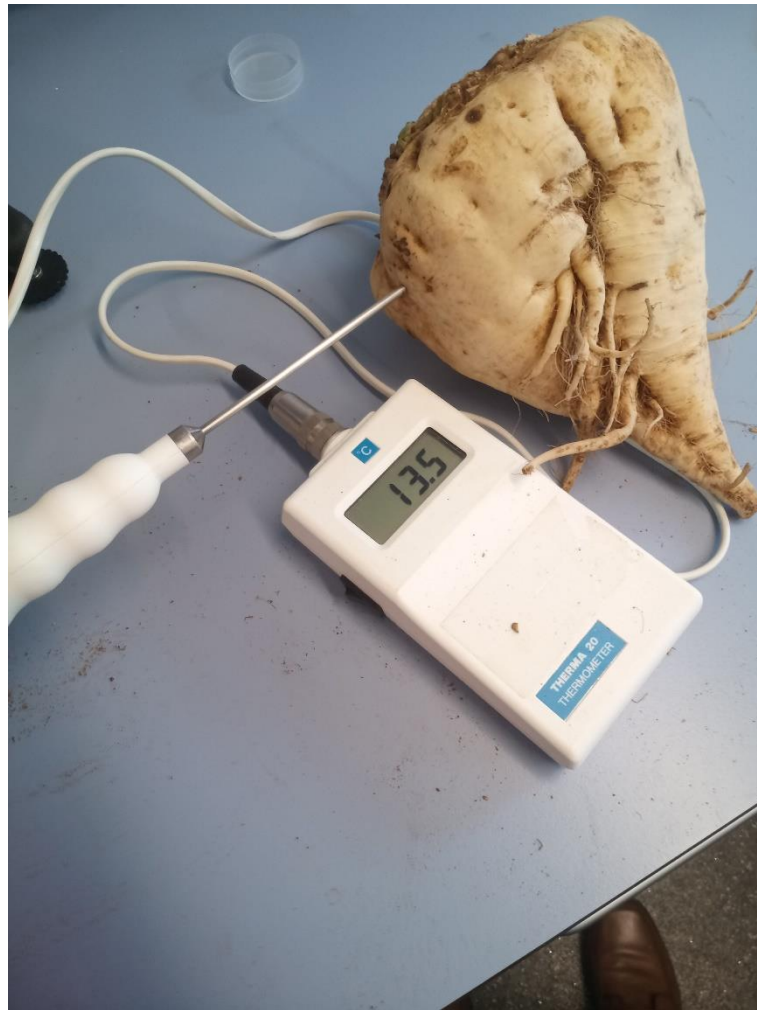


Figure 4-19: A root at 13.5 degrees celsius

Table 4-4: Root temperature during textural analysis

Irrigation	Harvesting temperature (°C)	Root temperature (°C)
Irrigated	10	12±0.4
Irrigated	3	4±0.2
No irrigation	10	12±0.4
No irrigation	3	4±0.2

4.4 Data analysis

An analysis of variance was performed using a linear mixed model in R version 4.2.2 within the *lmerTest* package (Kuznetsova et al., 2017). Before the analysis, each variable was subjected to normality using the Shapiro-Wilk test and showed normal distribution of the population. Irrigation and temperature were fitted in the model as fixed factors while year as a random factor when analysing textural properties and damage test data. When analysing morphological traits, temperature was dropped as a factor in the model since it was obvious that a three-day imposition of temperature treatment would not affect morphological features. The SPAD data was analysed by fitting irrigation and week as fixed factors and year as a random factor. Morphological factors and weight of the roots were analysed by fitting year and irrigation as fixed factors and temperature as a random factor. The temperature was considered a random factor because it was imposed while the roots had already been harvested. A post hoc analysis was performed in *emmeans* package (Russell et al., 2023) using the HSD Tukey test to compare treatment means at $p \leq 0.05$ significance level. Graphs were prepared using *ggplot2* (Wickham, 2009) R package.

4.5 Results

4.5.1 Leaf SPAD

Leaf SPAD was affected by irrigation ($p < 0.001$ where irrigated plants had a statistically higher SPAD value (45) compared to non-irrigated plants (41) (**Figure 4-20**). SPAD values for irrigated plants were not different from the first up to the seventh week. However, SPAD values for non-irrigated plants significantly reduced during the third week (**Figure 4-21**).

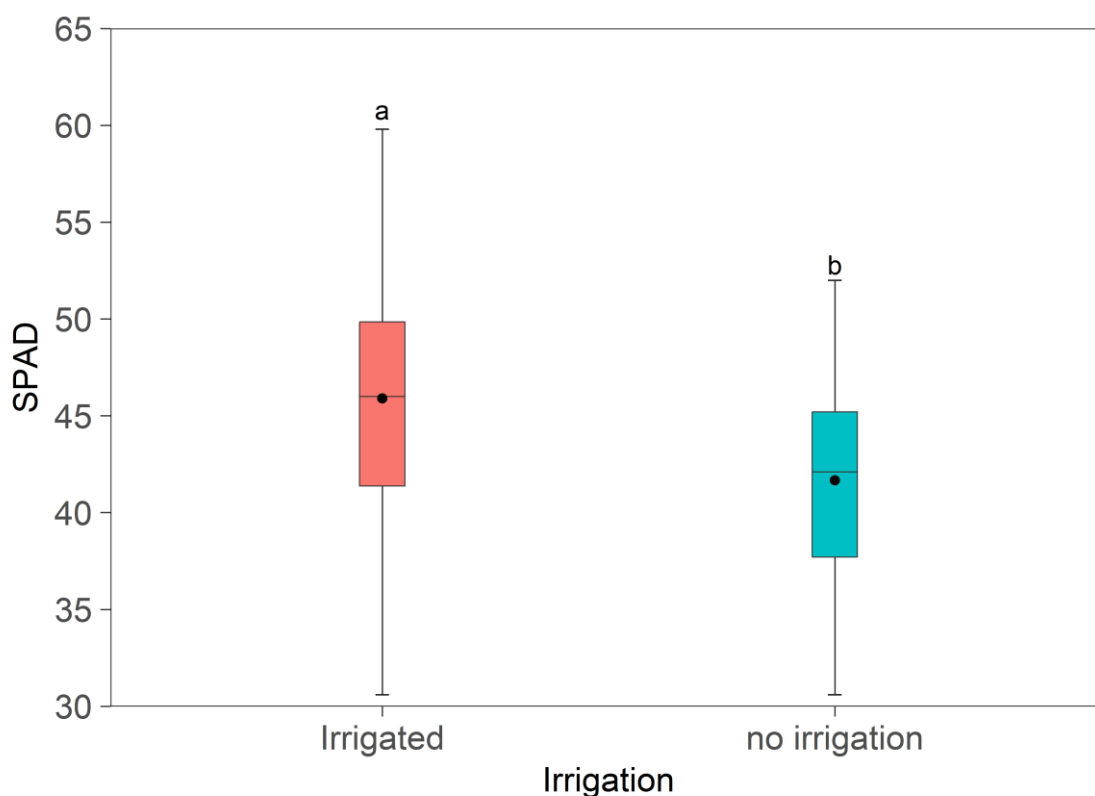


Figure 4-20: Effects of irrigation on sugar beet leaf SPAD values. $n=243$, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

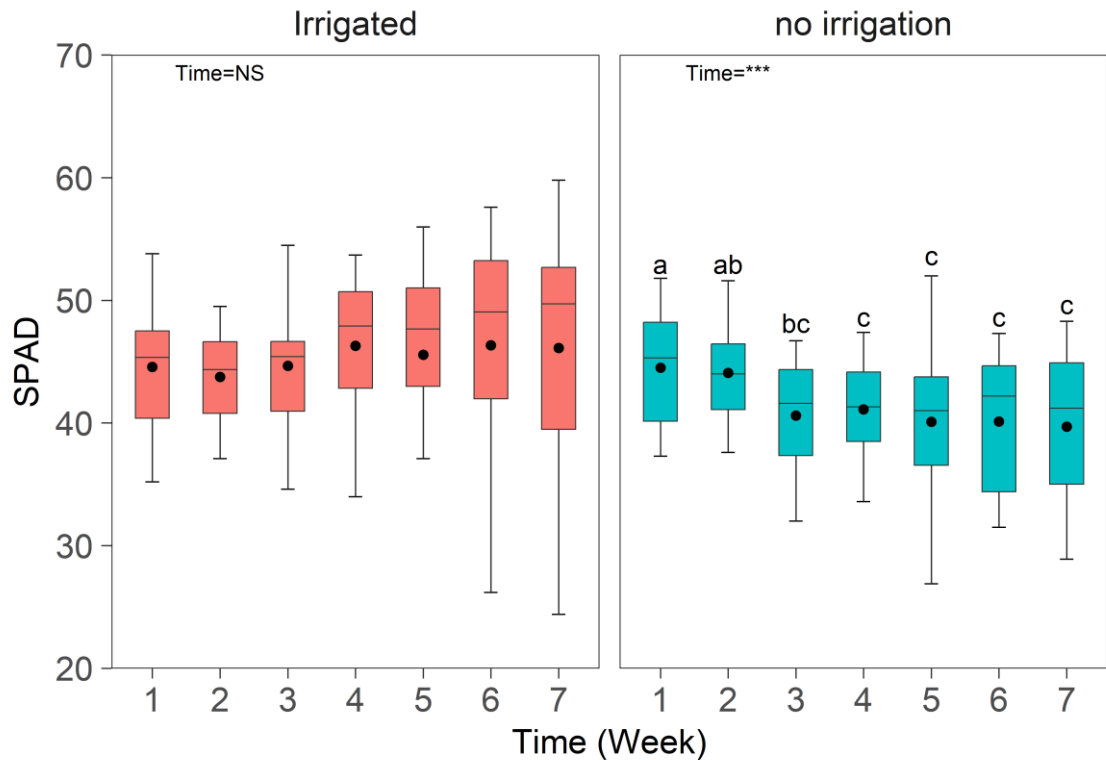


Figure 4-21: Effects of irrigation time on sugar beet leaf SPAD values. n=243, seasons=3. Treatments with different superscripts at each irrigation regime mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

4.5.2 Surface damage

Results show that surface damage was affected by soil moisture content (**Figure 4-22**) ($p < 0.001$) and not the temperature at harvest (**Figure 4-23**). Temperature and the interaction did not have an influence on root tissue's resistance to surface damage. Irrigating the crop to field capacity increased surface damage from 3.33 to 6.29. Surface damage was positively correlated with RWC with an R^2 value of 0.48 (**Figure 4-24**). While there was no relationship between surface damage and dry matter content ($R^2 = 0.12$) (**Figure 4-25**).

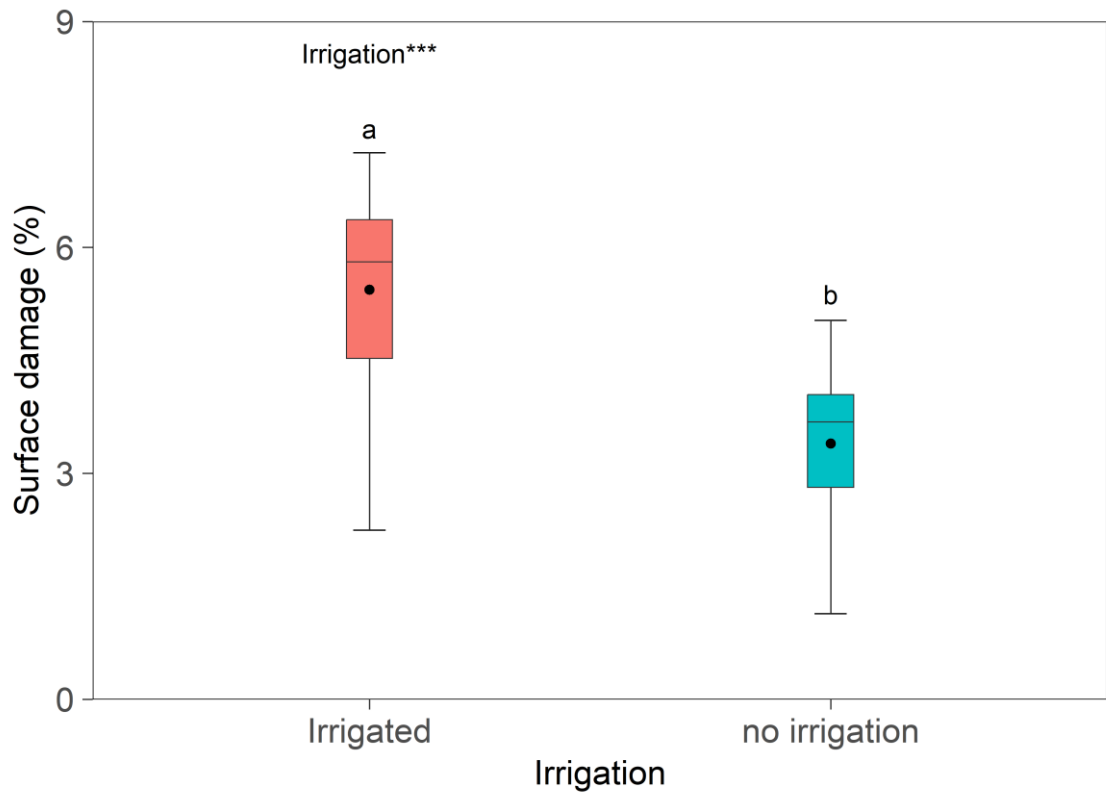


Figure 4-22: Effects of irrigation at harvest on sugar beet root surface damage. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

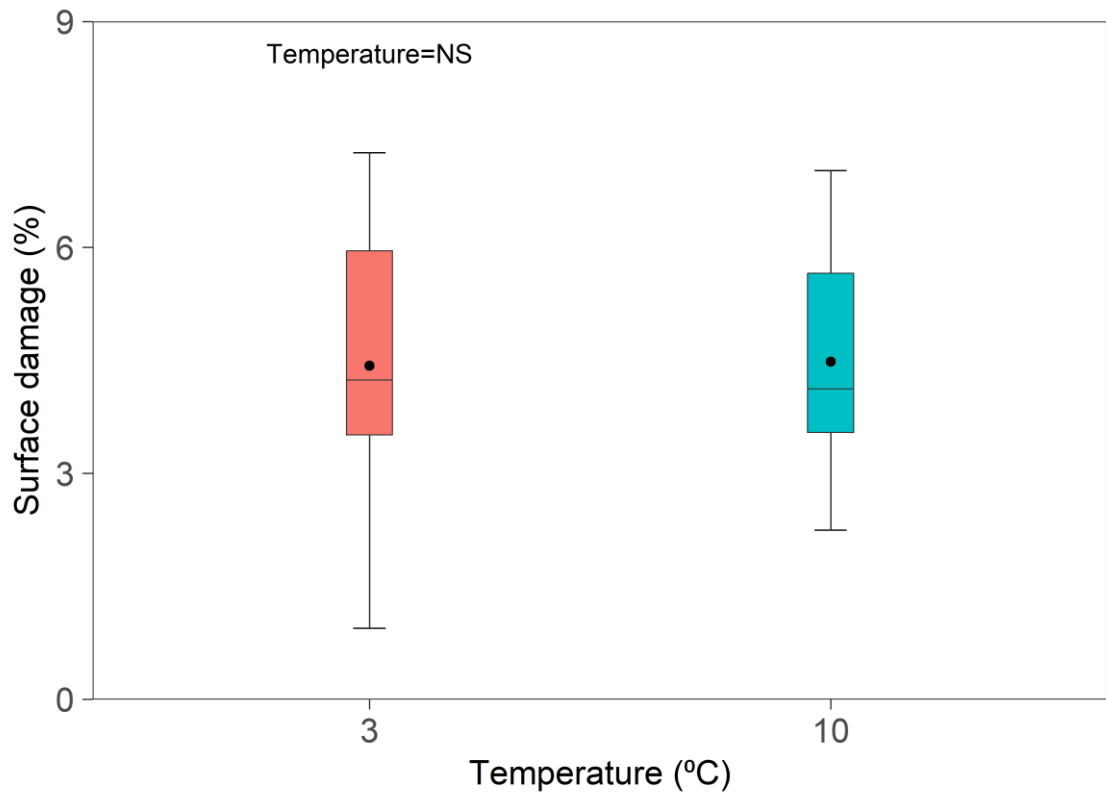


Figure 4-23: Effects of temperature at harvest on sugar beet root surface damage. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

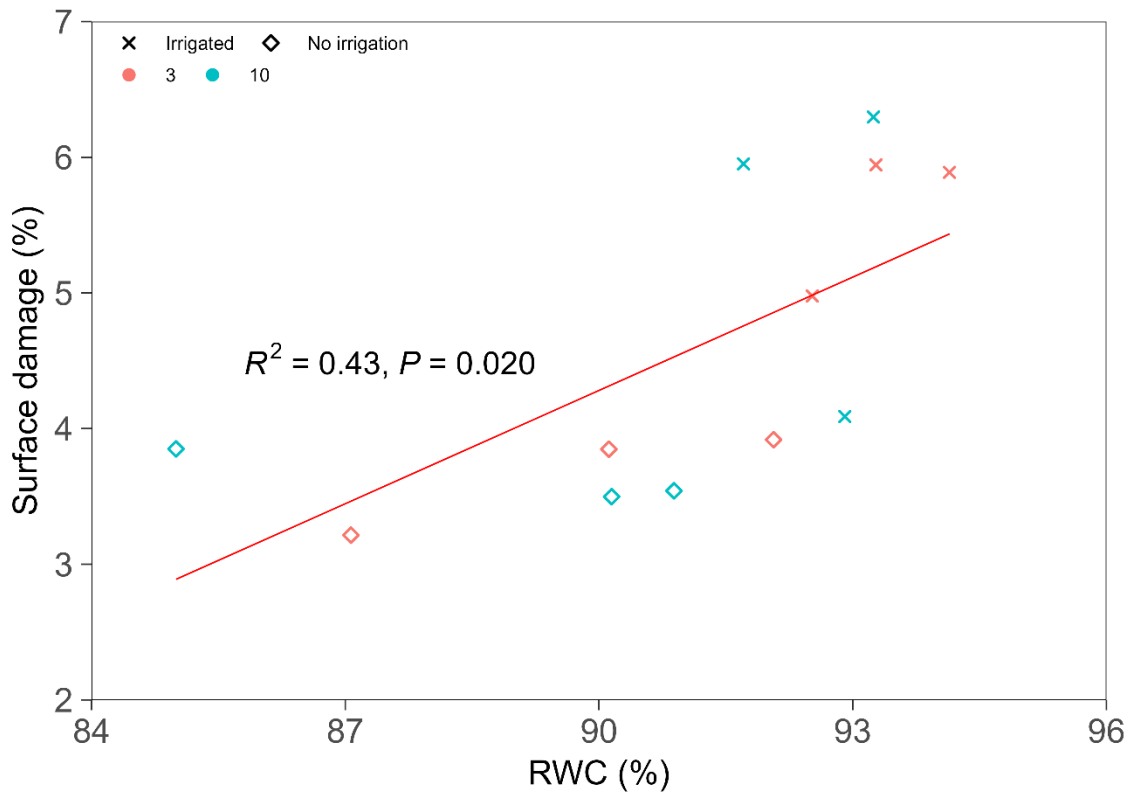


Figure 4-24: Linear regression between surface damage and RWC

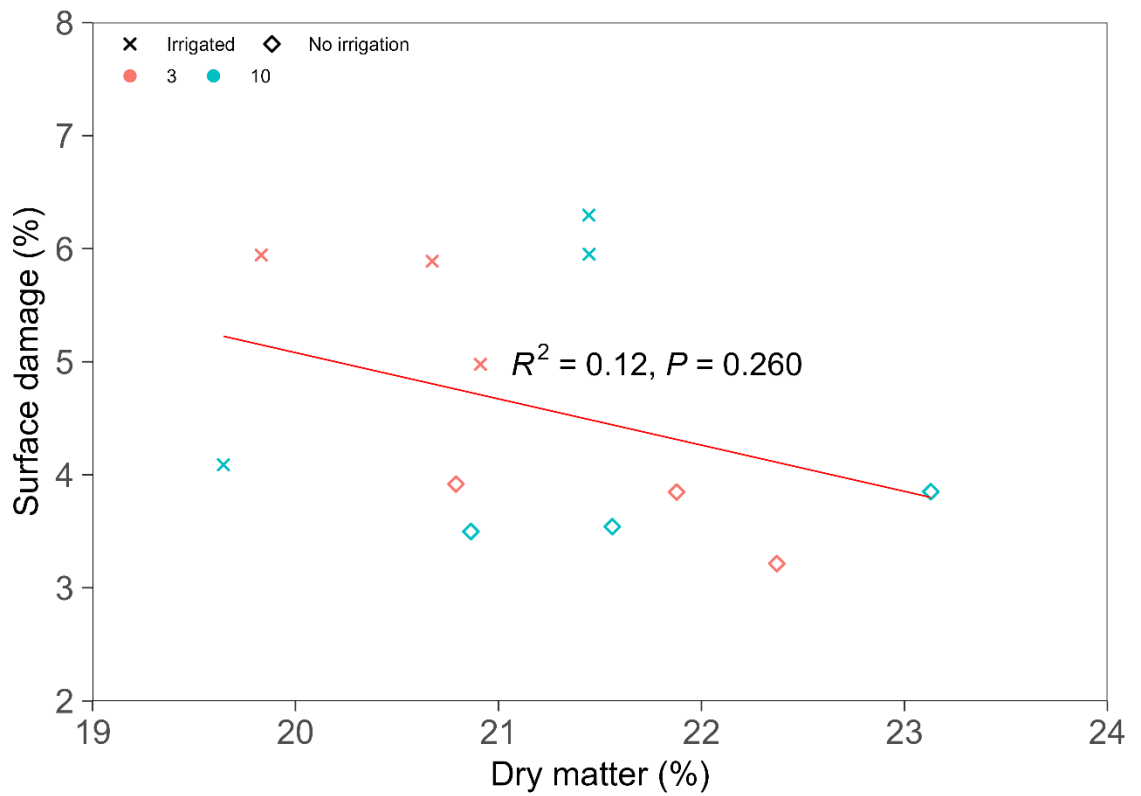


Figure 4-25: Linear regression between surface damage and dry matter

4.5.3 Root tip damage

Root tip damage was assessed by measuring the root tip diameter after damage and before damage. Soil moisture influenced root tip diameter after damage ($p < 0.05$) (**Figure 4-26**) while temperature (**Figure 4-26**) and the interaction between soil moisture and temperature did not influence root tip diameter after damage. On average, irrigated roots had a root tip diameter after damage of 4.47 cm while the non-irrigated roots had an average of 3.91 cm. There was no difference among the roots in terms of root tip diameter before damage (**Figure 4-28**). Root tip diameter after damage was negatively correlated with compression resistance ($R^2 = 0.69$) (**Figure 4-29**) and positively correlated with RWC ($R^2 = 0.42$) (**Figure 4-30**). However, there was no relationship between root tip diameter after damage and dry matter content (**Figure 4-31**).

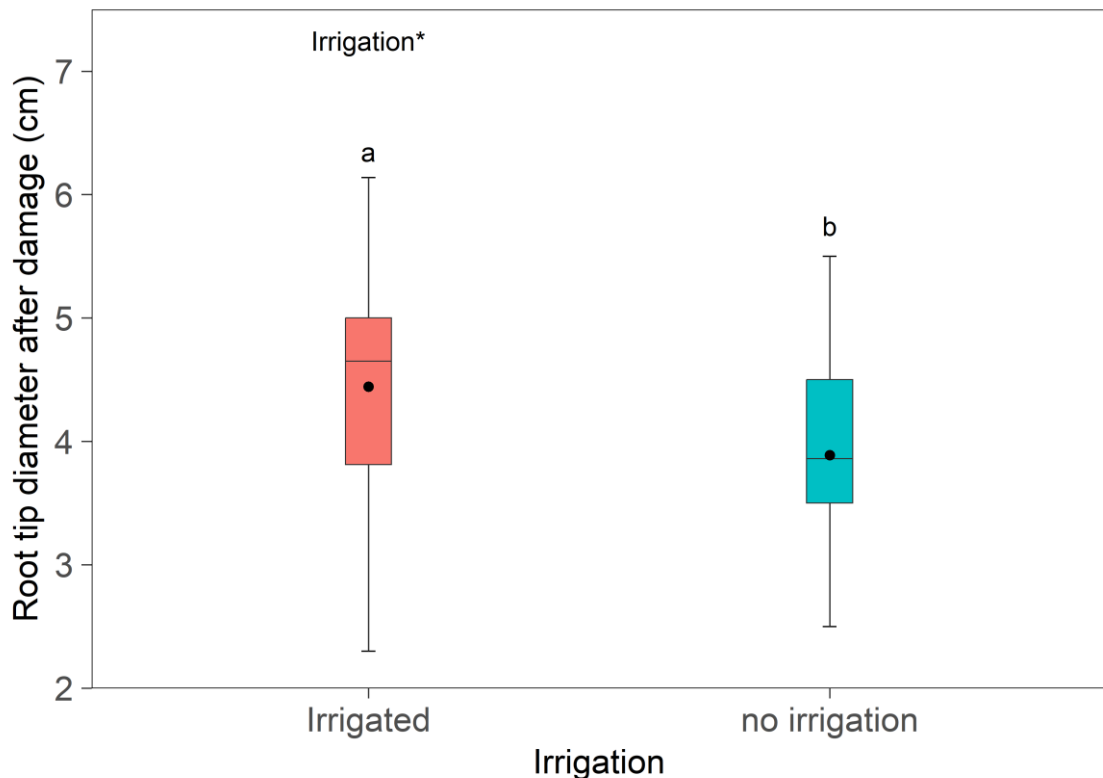


Figure 4-26: Effects of irrigation at harvest on root tip diameter after damage. $n=51$, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

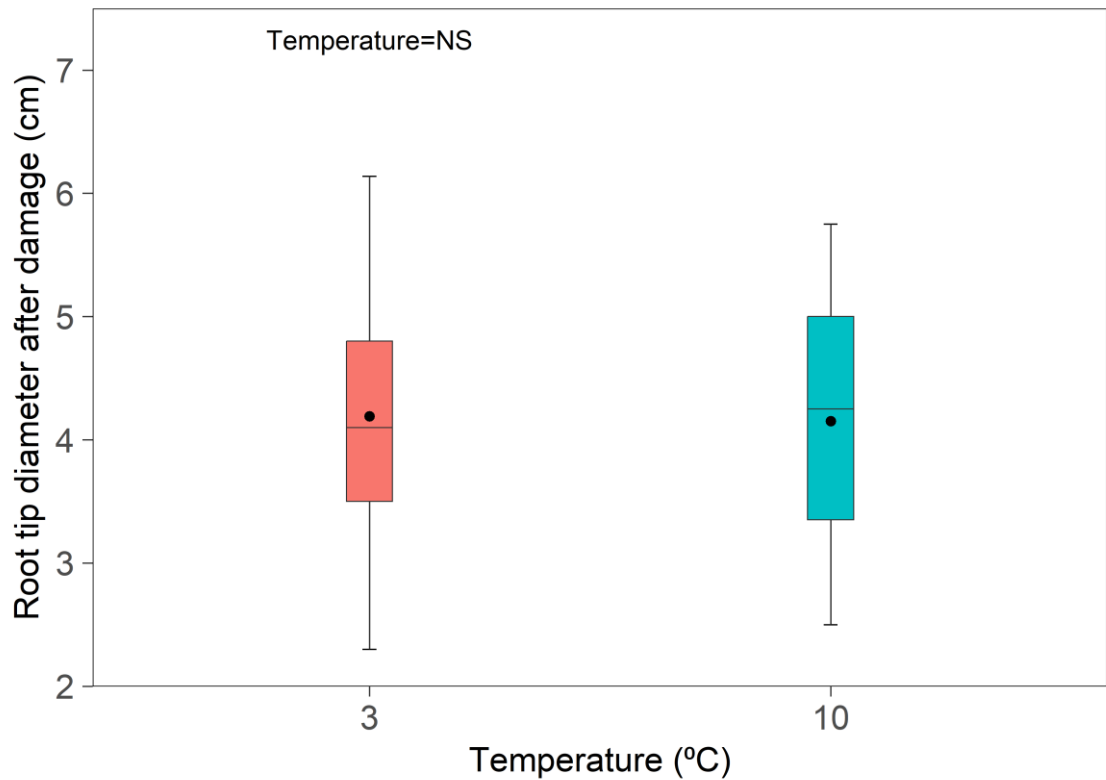


Figure 4-27: Effects of temperature at harvest on root tip diameter after damage. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

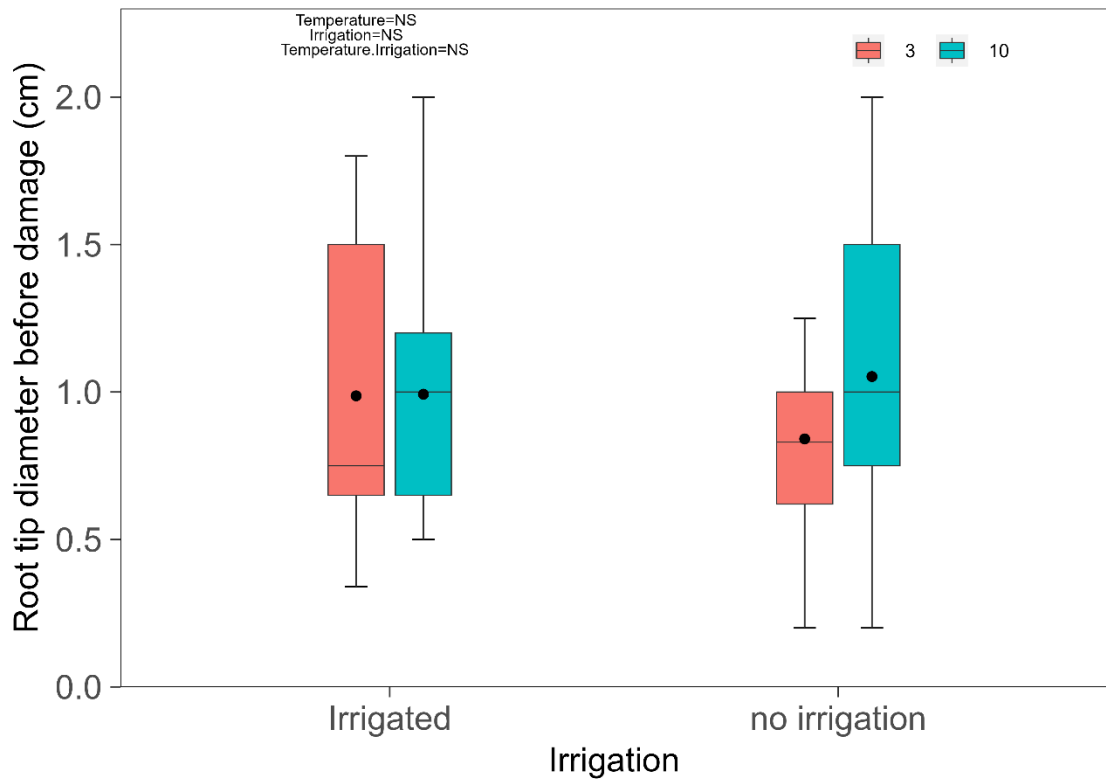


Figure 4-28: Effects of temperature and soil moisture at harvest on root tip diameter before damage. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

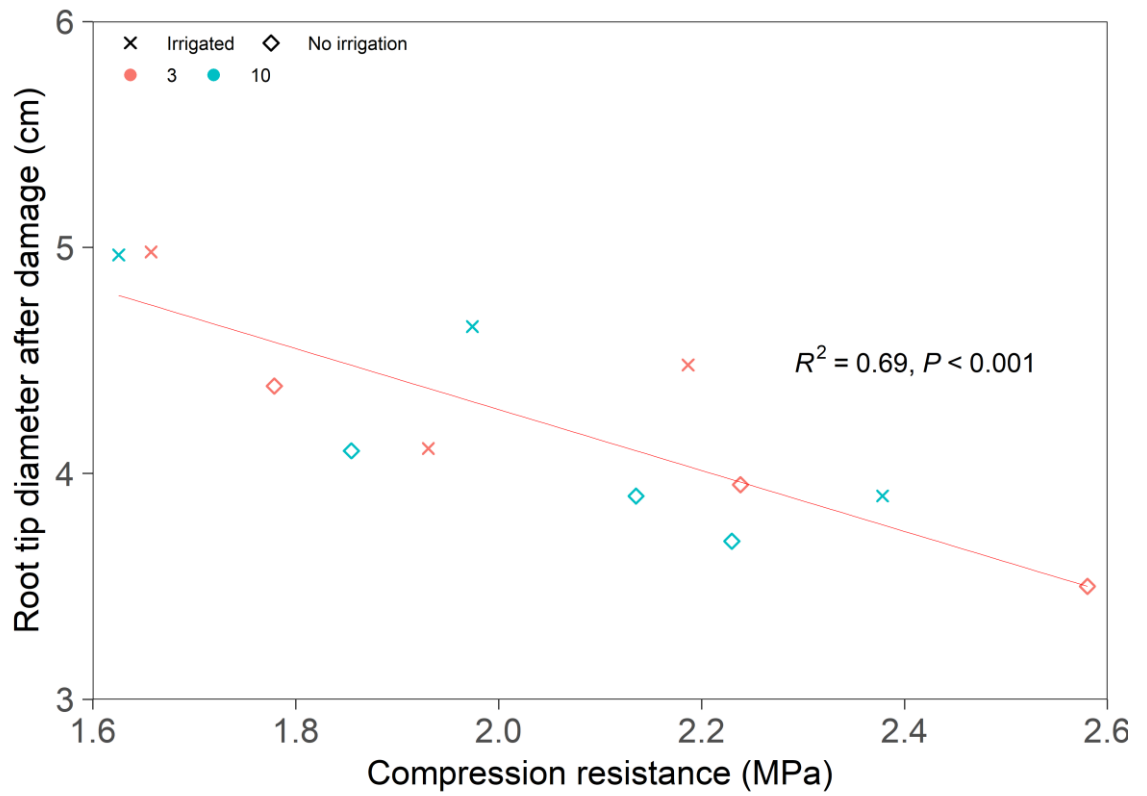


Figure 4-29: Linear regression between root tip diameter after damage and compression

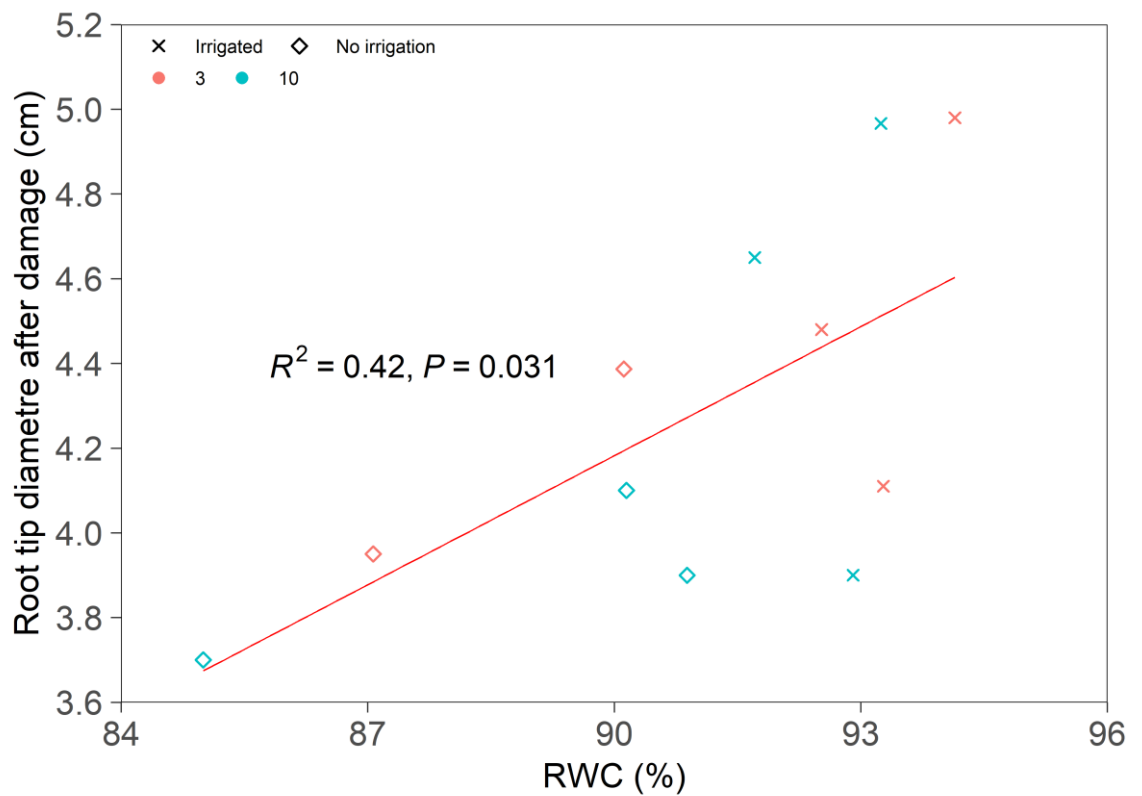


Figure 4-30: Linear regression between root tip diameter after damage and RWC

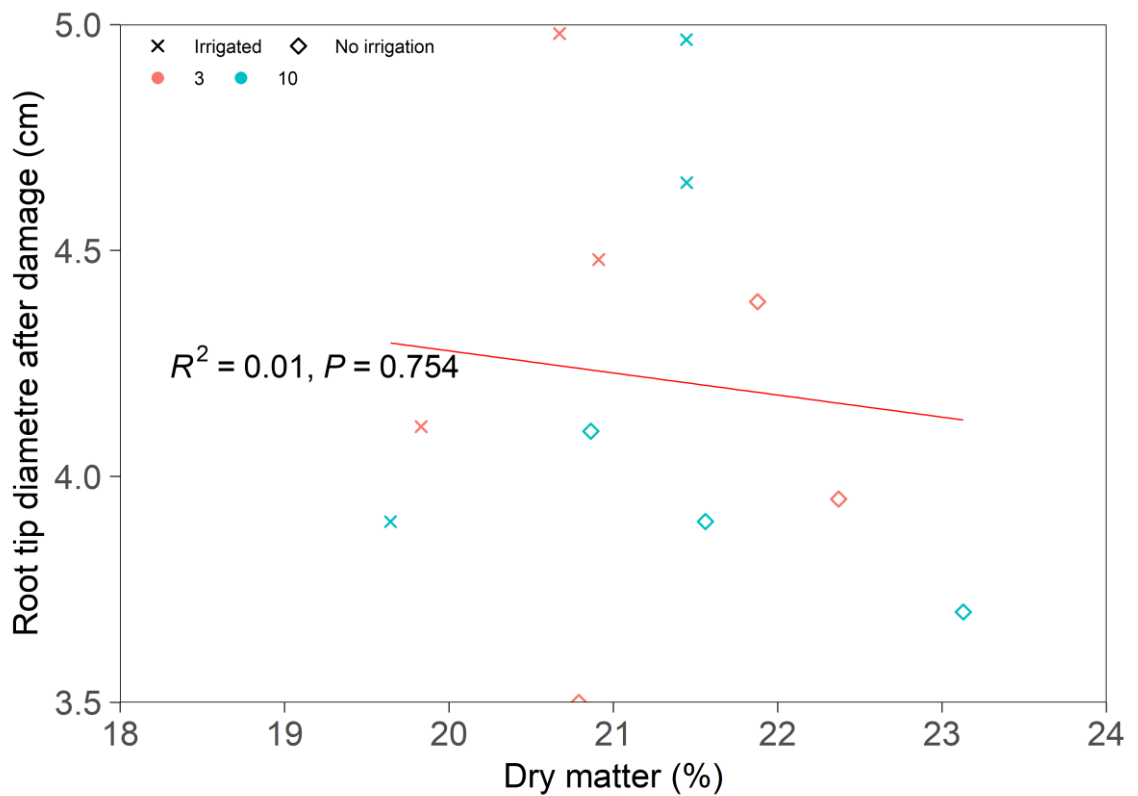


Figure 4-31: Linear regression between root tip diameter after damage and dry matter

4.5.4 Root weight

Sugar beet root weight was affected by irrigation ($p < 0.05$) (**Figure 4-32**) and year ($p < 0.001$) (**Figure 4-33**). In terms of season, in 2021 root weight per beet was not significantly different from that of 2022. However, roots harvested in 2020 had a higher weight compared to 2021 and 2022. Average weight per root for 2020, 2021 and 2022 were 1.28, 0.95 and 1.16 Kg, respectively. Non-irrigated roots were statistically lighter and on average weighed 0.93 Kg while irrigated roots weighed 1.09 Kg. Irrigation significantly increased root weight per beet from 1.05 to 1.32 Kg (representing a 26 % increase) in 2020 compared to 2021 (0.83 to 0.90 Kg) and 2022 (0.91 to 1.04 Kg) where no significant increase was recorded.

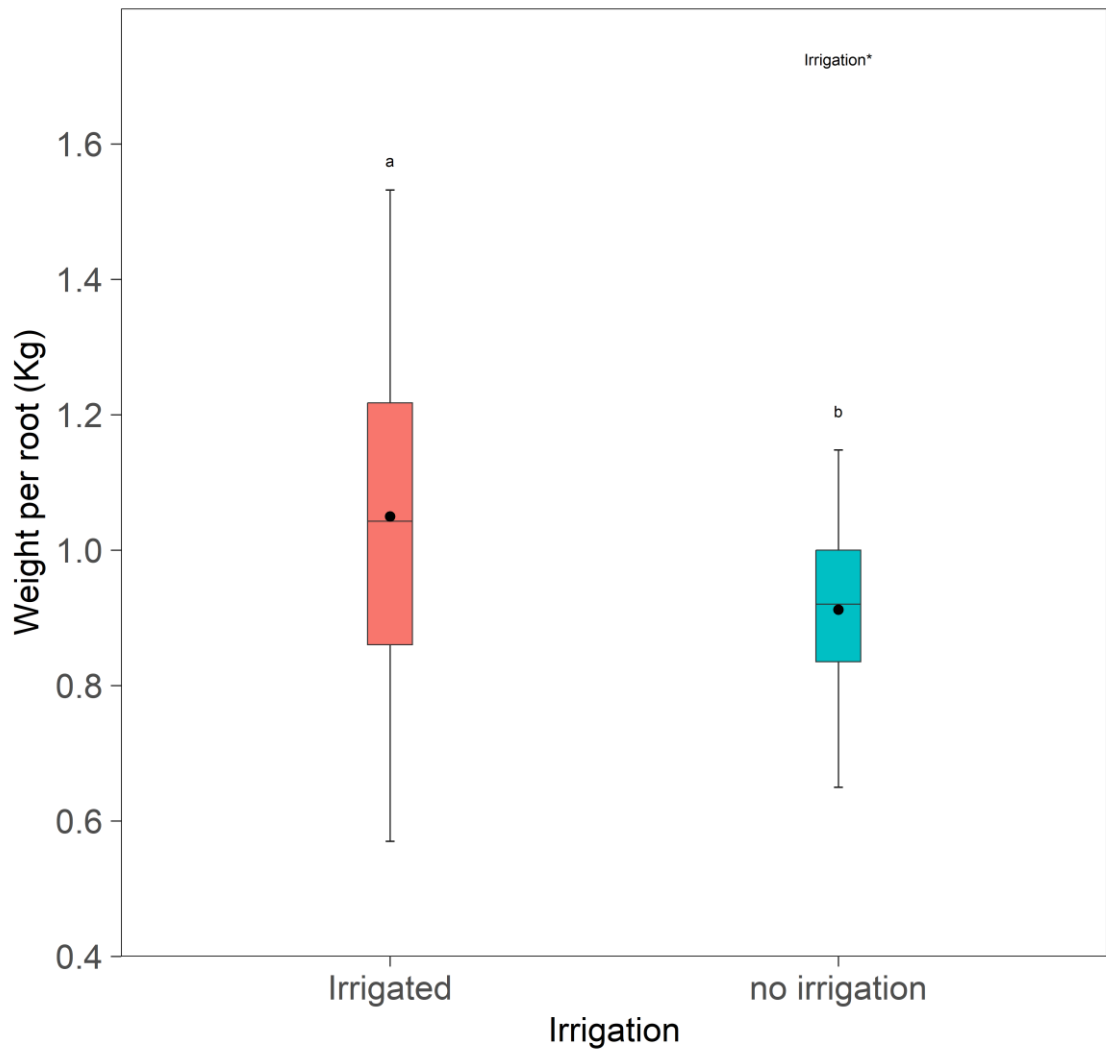


Figure 4-32: Effects of irrigation at harvest on sugar beet root weight. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

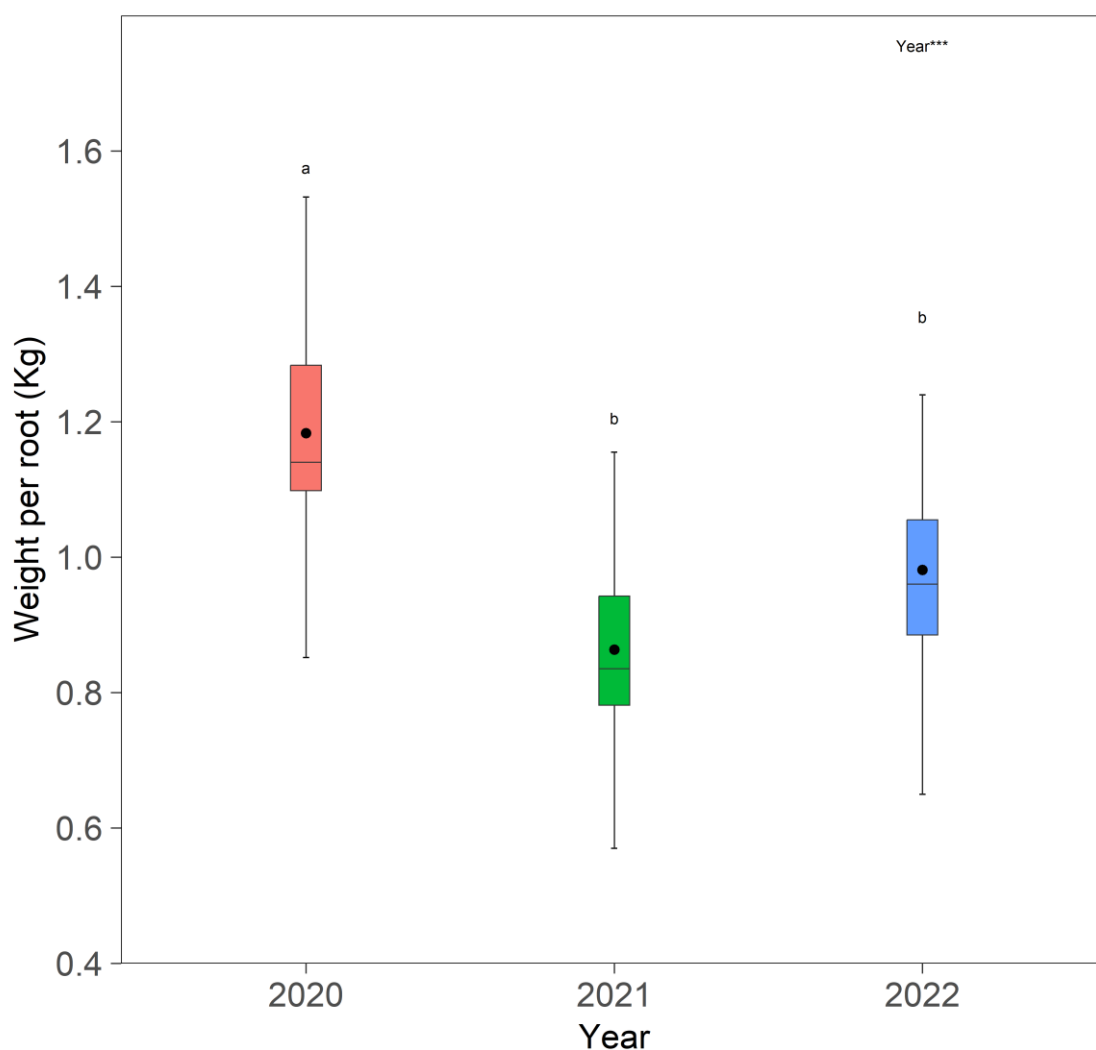


Figure 4-33: Effects of year at harvest on sugar beet root weight. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

4.5.5 Phenotypic traits

Morphological traits included length, width and root tip length before damage (**Table 4-5**). Root tip diameter before damage was not affected by irrigation and year. The average tip diameter before harvest for 2020 was 0.78 cm, for 2021 was 0.99 cm and for 2022 was 1.07 cm. While the average root tip diameter before damage for irrigated roots was 0.99 cm and for the non-irrigated was 0.95 cm. Sugar beet root length was only affected by year ($p < 0.001$) (**Table 4-6**) where roots harvested in 2020 were longer (24 cm) compared to those harvested in 2021 (17 cm) and 2022

(20 cm). The width was affected by year ($p < 0.001$) and irrigation ($p < 0.05$) while their interaction had no influence. On average, 2020 roots were 2 and 1 cm wider than roots harvested in 2021 and 2022, respectively. Irrigated plants had an average root width of 13 cm and were statistically different from non-irrigated plants which were 12 cm wide.

Table 4-5: Effects of irrigation on sugar beet morphology traits.

Irrigation	Width (cm)	Length (cm)	Tip diameter before damage (cm)
Irrigated	12.93 ^a ± 0.72	24.78 ± 0.94	0.81 ± 0.09
No irrigation	12.35 ^b ± 0.74	23.86 ± 1.33	0.74 ± 0.11
P-value	***	NS	NS

Note: Treatments with different superscripts mean they are statistically different. n=136, seasons=3, site=1

Table 4-6: Effects of year on sugar beet root morphology traits.

Year	Width (cm)	Length (cm)	Tip diameter before damage (cm)
2020	14.00 ^a ± 0.28	24.33 ^a ± 0.79	0.78 ± 0.19
2021	12.58 ^b ± 0.21	17.23 ^c ± 0.39	0.99 ± 0.18
2022	11.90 ^b ± 0.22	20.08 ^b ± 0.68	1.07 ± 0.32
P-value	***	***	NS

Note: Treatments with different superscripts mean they are statistically different. n=136, seasons=3, site=1

4.5.6 Relative water content

Root tissue's RWC was significantly affected by irrigation ($p < 0.001$) (**Figure 4-34**). The average RWC for irrigated plants was 93 % while those that were kept dry had a RWC of 89 %. At every irrigation regime, the temperature did not affect RWC (**Figure 4-35**). RWC from the edge to the central part of the root was not statistically different. Average RWC for the edge, middle and central part was 90, 91 and 91 %, respectively (**Figure 4-36**).

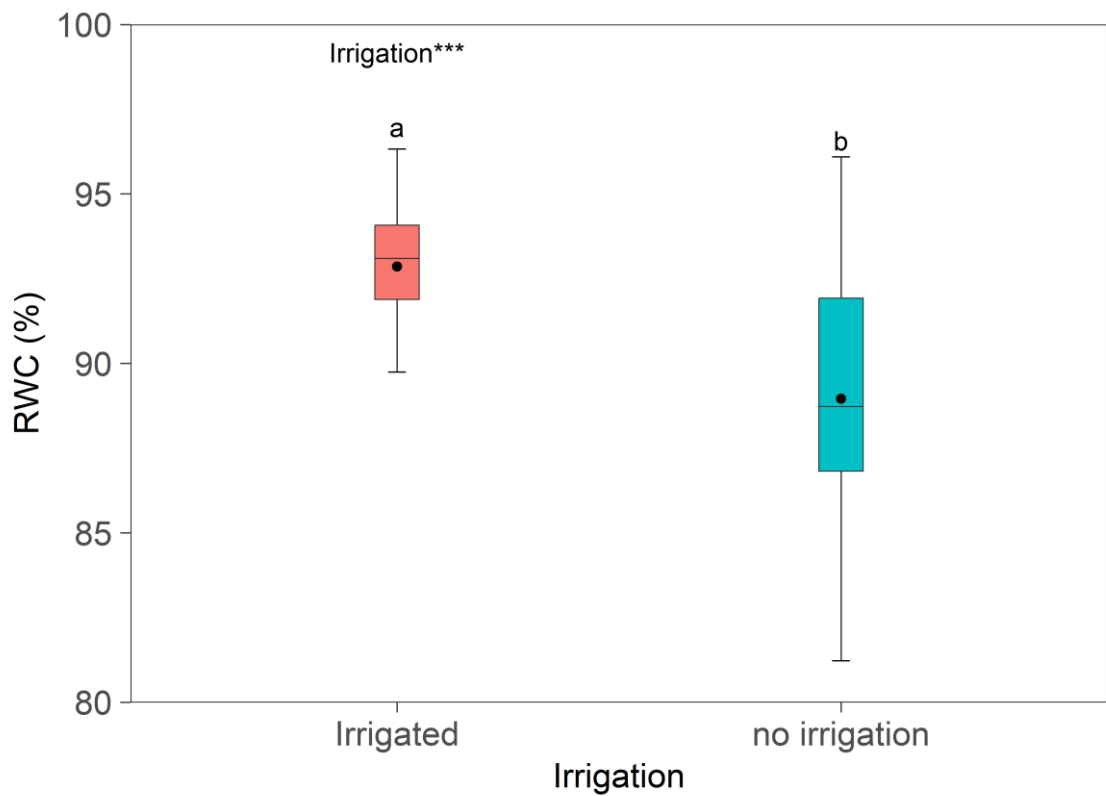


Figure 4-34: Effects of irrigation at harvest on root tissue RWC. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

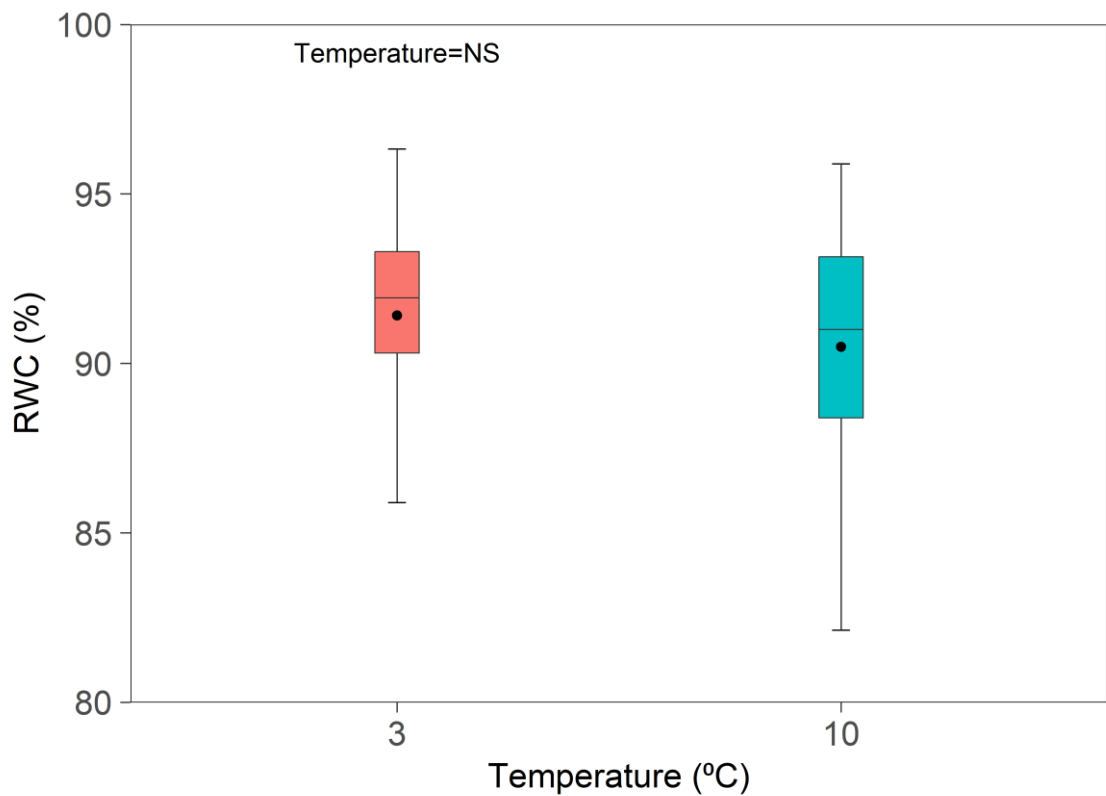


Figure 4-35: Effects of temperature at harvest on root tissue RWC. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

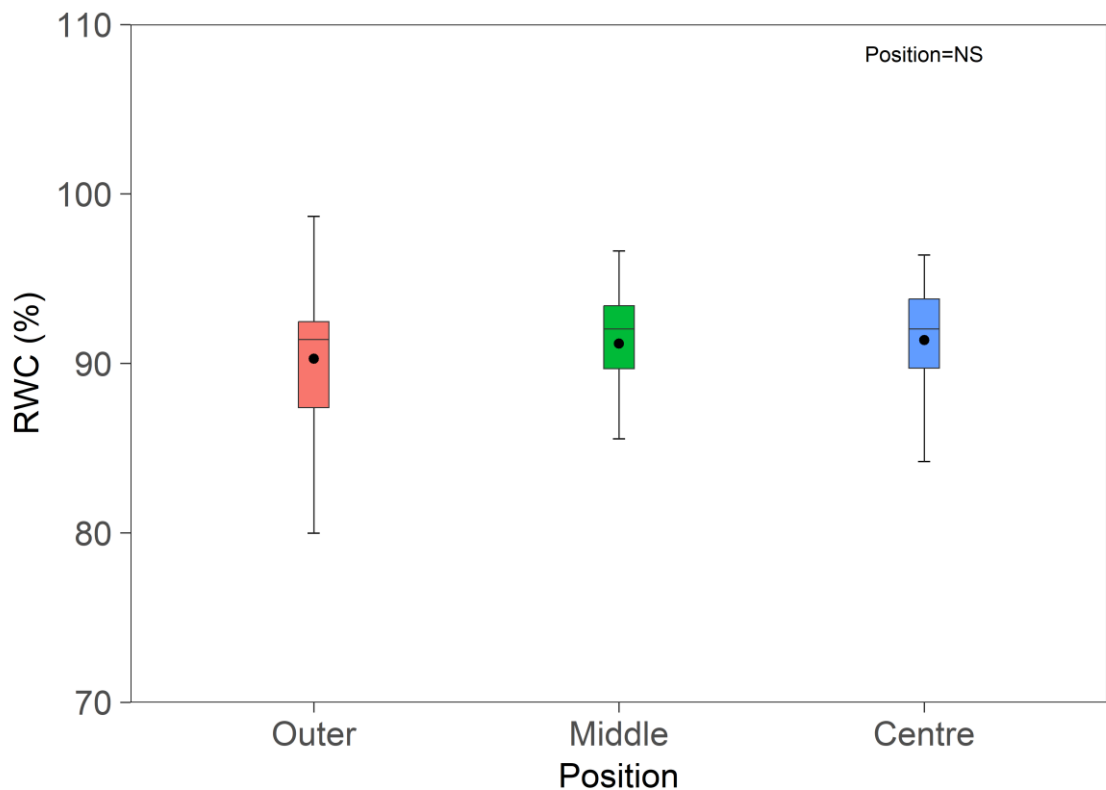


Figure 4-36: Effects of sampling position on root tissue's RWC. n=153, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

4.5.7 Textural properties

Root tissue compression resistance was affected by irrigation (<0.001) and the interaction between temperature and irrigation ($p < 0.05$) (**Figure 4-37**). The average resistance required to compress root tissues was 2.08 MPa. Irrigated and non-irrigated root tissues required 2.00 and 2.17 MPa, respectively. However, a 12 % decrease in compression was observed when irrigated roots were harvested at 3 °C. Root tissue puncture was affected by irrigation ($p < 0.01$), however, the difference is more marked when the roots were harvested at 10 °C. Results show that non-irrigated roots required 9 % more force to puncture compared to irrigated roots (**Figure 4-39**). Both compression and puncture resistance were negatively correlated to RWC with respective R^2 values of 0.41 and 0.46 (**Figure 4-38** and **Figure 4-39**).

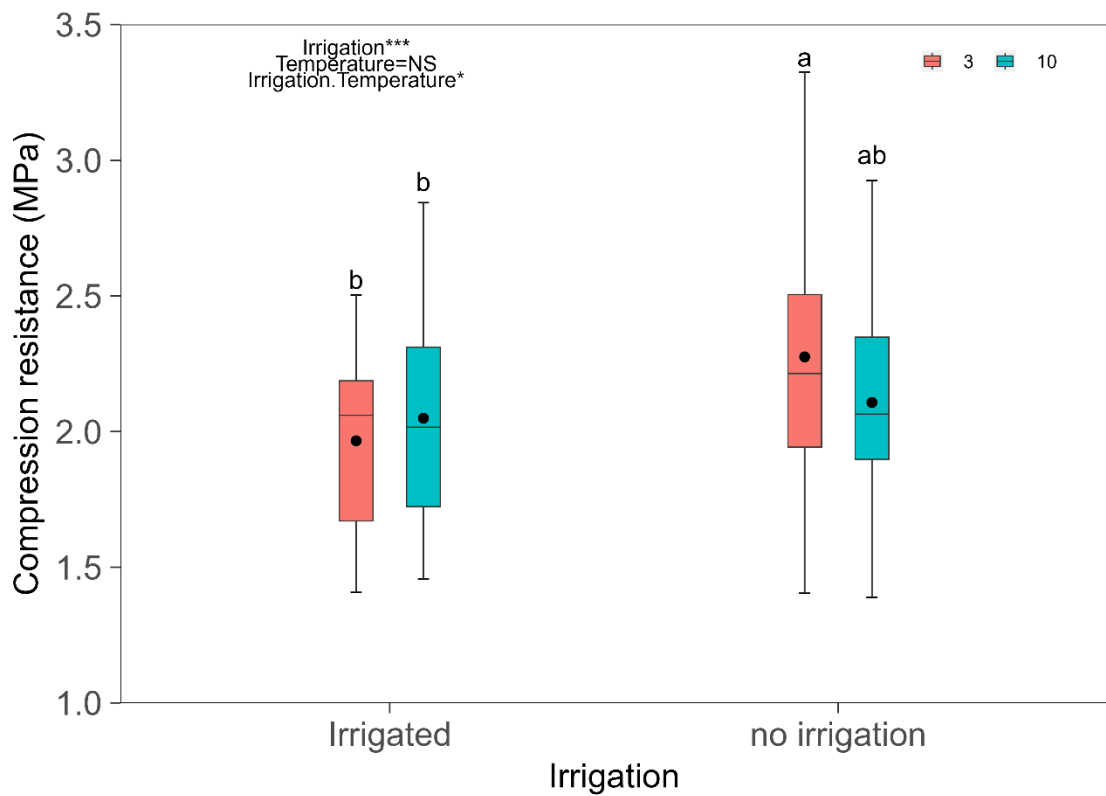


Figure 4-37: Effects of temperature and irrigation on root tissue compression resistance. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

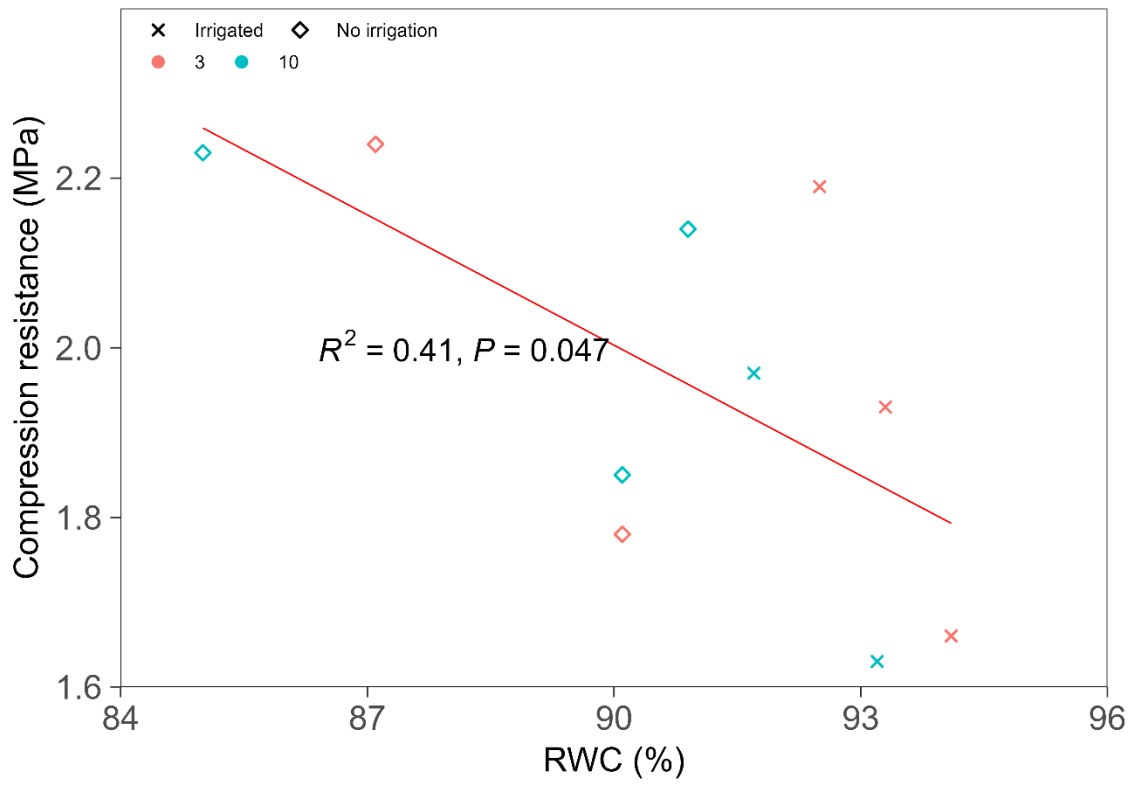


Figure 4-38: Linear regression between RWC and compression resistance

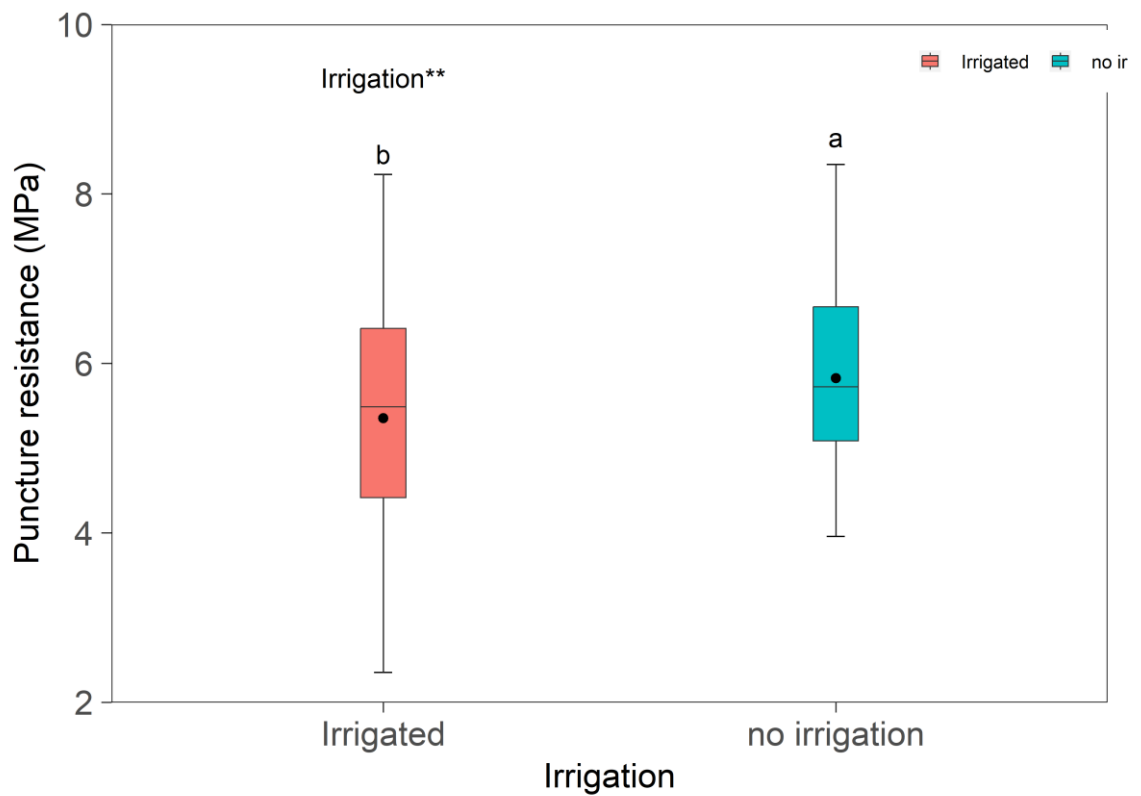


Figure 4-39: Effects of temperature and irrigation on root tissue puncture resistance. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

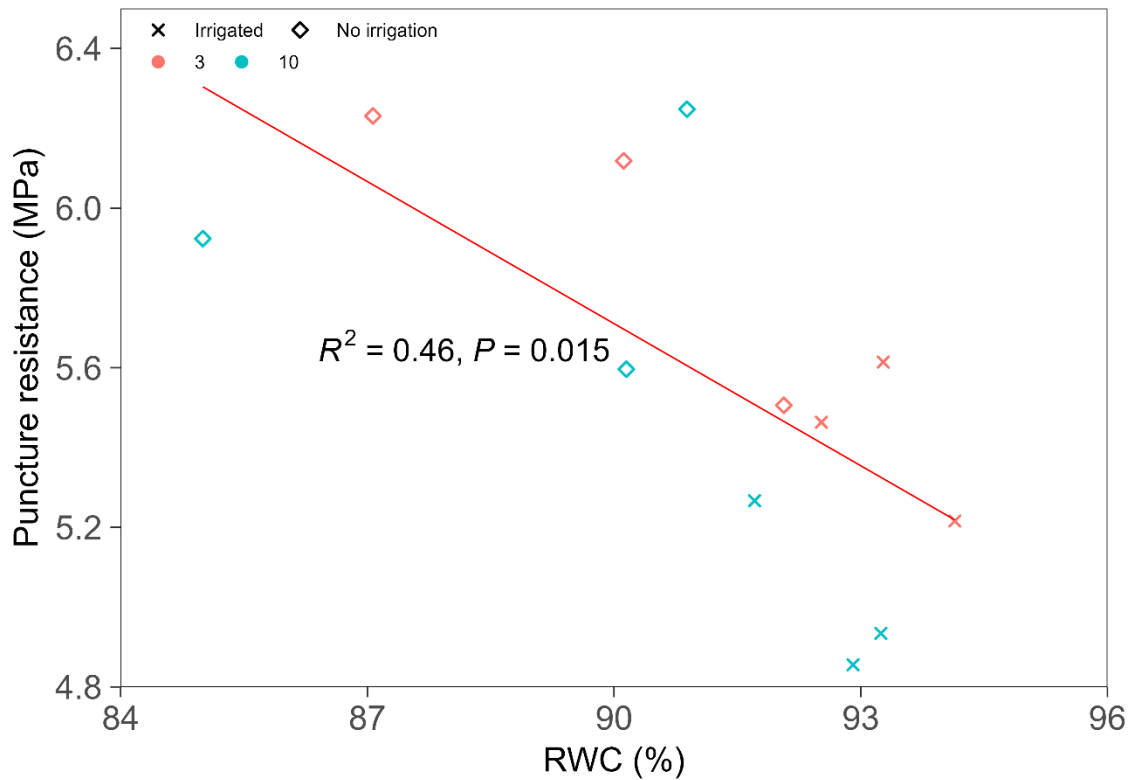


Figure 4-40: Linear regression between RWC and puncture resistance

4.5.8 Root tissue dry matter content

Root tissue dry matter content was statistically affected by irrigation ($p < 0.001$). On average, non-irrigated roots had a higher dry matter content (22 %) compared to the ones that were irrigated (21 %) (**Figure 4-41**). There was a high negative correlation between RWC and dry matter content with an R^2 of 0.69.

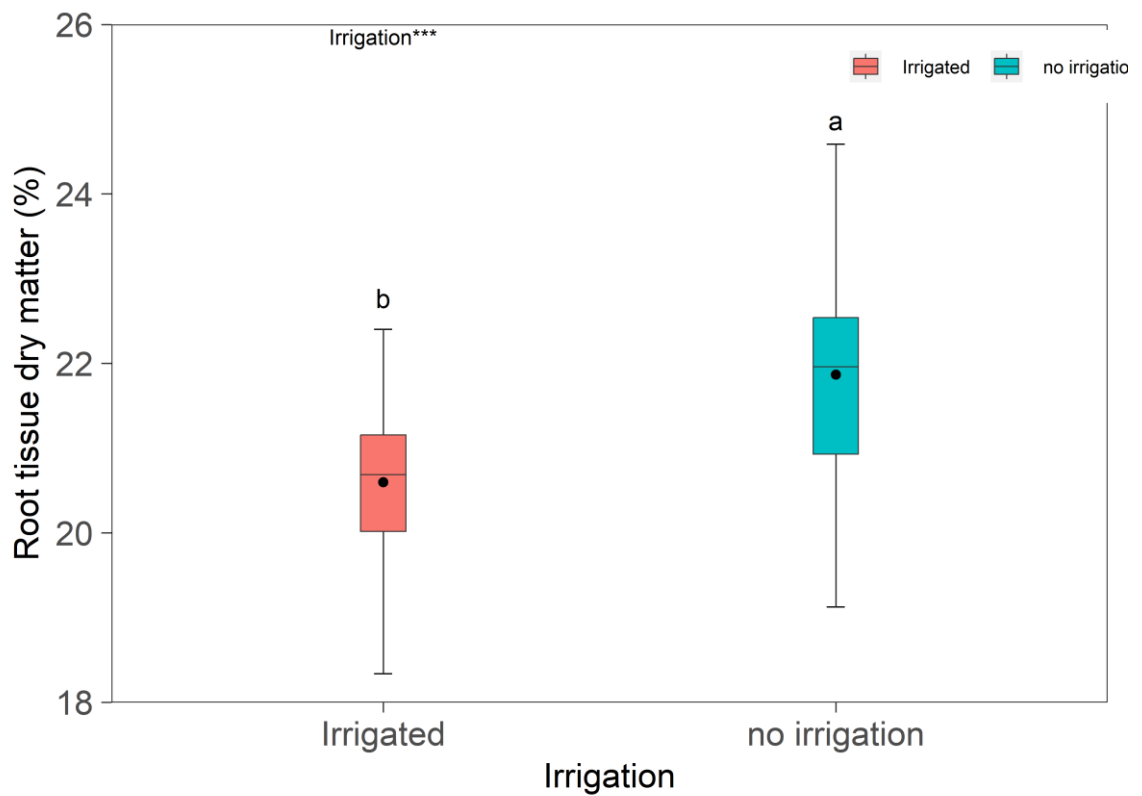


Figure 4-41: Effects of irrigation on root tissue dry matter. n=51, seasons=3. Treatments with different superscripts mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

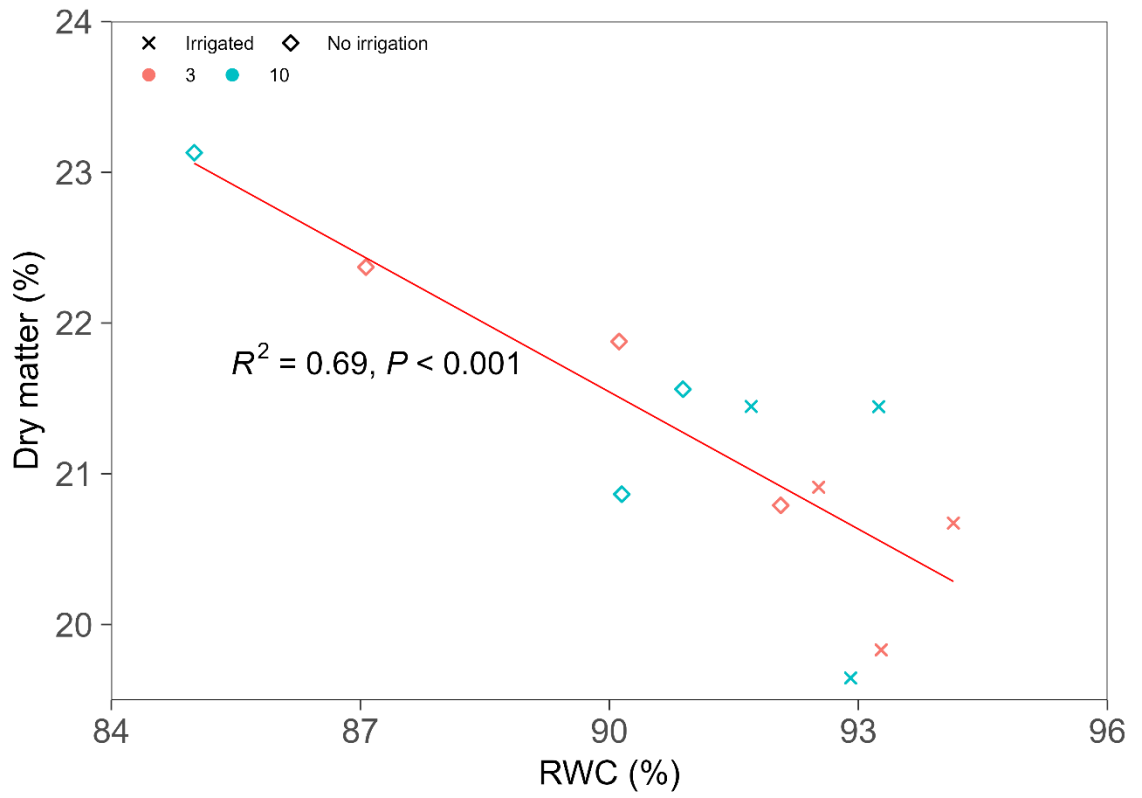


Figure 4-42: Linear regression between RWC and dry matter content.

4.6 Discussion

4.6.1 Water status at harvest and tissue damage susceptibility

A plant's ability to take up water for growth is dependent on water availability in the external environment (Verslues et al., 2006). Increased RWC observed in this study for irrigated roots suggests that even after physiological maturity, sugar beet plants extract more water if the resource is not limiting. It can also be deduced from our study, that subjecting sugar beet to a slowly increasing water deficit induces a decrease in the maximum root tissue water volume (Herppich et al., 2001) and high RWC increases tissue turgidity which has an impact on textural properties (Robbins and Dinneney, 2015). Despite Nause et al., (2020) concluding that sugar beet mechanical properties like puncture resistance remain stable throughout the growing period, a significant reduction in textural properties observed in this study implies that textural properties are dependent on moisture conditions prior to harvesting. A negative correlation between RWC and textural properties supports the observation by Feng et al., (2016) that high water content promotes root tissue frailness through increased exertion of force by cytoplasm on cell walls. Our observation on root tissue failure in resistance to external force under high water content agrees with Lockley et al., (2021) who reported that an increase in radish hypocotyl water content was associated with a decrease in failure force for both compression and puncture resistances.

Increased surface and root tip diameter after damage in irrigated roots suggest that soil water status or rainfall prior to harvesting is an important factor when implementing harvesting plans. As shown in the results, maintaining water status to field capacity for a period of seven weeks decreases tissue strength and dry matter but also increases the width and weight of roots. Both weight (Kenter et al., 2006a; Van Swaaij et al., 2003) and textural strength (Kleuker and Hoffmann, 2022) have been reported to have a direct relationship with surface and root tip damage. Our results also agree with those of Hoffmann (2018) that soil conditions alter the composition of the root's skin and tissue strength, resulting in a higher susceptibility to damage and abrasions. The results suggest that crops which receive considerable rain keeping soils at field capacity for seven consecutive weeks prior to harvesting provide a conducive environment for root tissue damage compared to fields which experience drought for the same duration. These findings, therefore, suggest that apart from utilising varieties that are tolerant to root damage,

scheduling harvesting when the soils have been dry for seven weeks prior to harvesting also offers an opportunity to minimise root damage.

Our study also shows that continuous dry spells start affecting sugar beet's biological processes after three weeks. This was manifested when SPAD values for irrigated roots were statistically not significant throughout the seven weeks while those of no-irrigated roots significantly dropped from the third week. Correlations between SPAD and chlorophyll with an R^2 of greater than 0.80 have been reported in various crops (Jiang et al., 2017; León et al., 2007) justifying the assumption that a water withdrawal at physiological maturity affected chlorophyll content. This finding justifies why the roots from irrigated pots were heavier and wider compared to non-irrigated ones. Our findings are in agreement with those of (Wang et al., 2021) who observed higher SPAD values in irrigated sugar beet than those which just depended on sporadic rains. Practically, these results mean that sugar beet physiological processes that depend on water may be affected if there is a continuous four-week drought.

4.6.2 Root temperature at harvest and tissue damage susceptibility

Temperature alters root characteristics when it is varied throughout the growing season (Kenter et al., 2006b), however, extreme temperatures can damage fragile complexes, mostly proteins and membranes and weaken cell and tissue strength and resistance from deformation (Guihur et al., 2022). The study shows that mild (10 °C) and cold (3 °C) temperatures do not have an impact on root tissue response to damage. This means that harvesting sugar beet in autumn and winter (when temperatures are about 3 °C) has no bearing on both root tip and surface damage. This, therefore, means that in the case of a prolonged harvesting campaign, growers would not experience increased root damage due to the onset of winter unless temperatures go beyond the ones evaluated in this study. Mild temperatures in autumn (10 °C) do not influence root tissue strength variables such as puncture and compression strength. Supporting this finding, Fugate et al., (2016) reported that mild temperatures only affect sugar beet textural strength in storage and this is primarily attributed to increased transpiration rates that reduce cell turgor pressure and in return results in frail cells. In their earlier studies in fresh carrots, Herppich et al., (2002) observed that cutting force only started decreasing after 20 °C with the highest values at 5°C and lowest at 40 °C. However, two years later, Herppich et

al., (2005) observed that a reduction of temperature from 20°C to 10°C increased textural strength in both carrots and radishes. We suggest that our results did not prove harvesting temperature's contribution to tissue strength and damage because temperature ranges were not as wide as those from the literature to induce the required fragility by denaturing cell wall components such as membranes and proteins. According to Met Office (2023), 10 years (2012 to 2022) average temperature data collected at weather stations (Cambridge NIAB, Lowestoft and Waddington) within sugar beet growing areas range from 6-17°C in autumn and 3-10°C in winter. Hence, the study could not be designed for higher temperatures as we wanted to mimic prevailing harvesting temperatures in sugar beet production zones of the UK.

4.6.3 Interaction between temperature and water status and tissue damage susceptibility

Higher temperatures and moisture status at harvest impact plant tissue strength. Herppich et al., (2005) reported that in carrots, cutting force and turgor were higher at a lower tissue temperature (10°C compared to 20°C), meaning that lower temperatures help to maintain high water content in root tissues. However, our findings from this study do not suggest such a relationship between harvesting temperature and water status on the susceptibility of roots to damage and related variables such as puncture and morphological traits. The interaction between the two factors was only influential on root tissue compression where a lower temperature in non-irrigated roots enhanced tissue resistance to compression. RWC of cold stored roots remains more constant than roots stored at a higher temperature suggesting that storage at low temperature largely prevents water loss (Herppich et al., 2001) and water content highly impacts root mechanical properties especially compression (Ansari et al., 2014; Lockley et al., 2021). Despite the interactive effects of temperature and soil moisture not affecting RWC, their minimal effects under low temperature and low soil moisture play a part in compression resistance. This implies that under dry conditions, root tissues for sugar beet will be stronger when exposed to low temperatures.

4.7 Conclusion

Harvesting conditions are an issue of concern when mitigating factors that accelerate root tissue damage in sugar beet post-harvest handling. Findings from this study suggest that harvesting sugar beet when the field has been receiving rain that maintain the soils at field capacity for 7 consecutive weeks prior to harvesting, increases root tissue RWC which renders the tissues frail leading to increased surface and root tip damage. The study also revealed that mild and cold harvesting temperatures in autumn and winter respectively, do not affect textural properties as well as root tissue vulnerability to damage. Interactive effects of soil moisture and harvesting temperature only influence root tissue's ability to resist compression.

Chapter 5: Effects of calcium and boron foliar application on root damage, quality and tissue integrity

5.1 Abstract

Nutrition plays an important role in sugar beet cell structure and impurities. Calcium (Ca) is responsible for the binding of cell wall pectin and boron (B) is essential in promoting cell wall formation, carbohydrate metabolism and sugar translocation. Despite B and Ca's proven direct link with tissue strength in other crops like carrots, most sugar beet studies focused on their contribution to yield and yield components. There is no or little information on how their deficiency or toxicity affects root tissue strength and quality in sugar beet. The study hypothesised that B and Ca affects sugar beet root textural properties, tissue damage susceptibility and root quality. The trials were planted at The Morley Agricultural Foundation farm (tMAF) in Norfolk during the 2021 and 2022 seasons. The two trials (B and Ca trials) were planted separately in randomised complete block designs with three replicates each. For the B trial, YaraVita BORTRAC 150 (Yara fertiliser, Pocklington, UK) was applied at the six-leaf stage (SLS), 4 weeks after SLS and 8 weeks after SLS at a rate 0, 1 and 2 L ha⁻¹ per application making a total of 0, 3 and 6 L ha⁻¹ treatments. While for Ca trial, Omex CalMax® Ultra (East Riding Horticulture Ltd, Sutton Upon Derwent, UK) was applied at SLS and 4, 8, 12 and 16 weeks after SLS at a rate 0, 1 and 2 L ha⁻¹ per application making a total of 0, 5 and 10 L ha⁻¹ treatments. In both trials, Sabatina and Daphna were used as test varieties. Results indicate that a 5 L ha⁻¹ foliar Ca application reduced surface damage but did not affect root tip diameter after damage. Ca application reinforced root tissues' ability to resist puncture when applied during a season characterised by well-distributed rainfall. However, root quality was not affected by the Ca application. B did not affect root tissue damage, however a 6 L ha⁻¹ application of B enhanced root tissue's resistance to compression. These results suggest that at tMAF, a 5 L ha⁻¹ Ca foliar application is agronomically ideal when minimising tissue damage.

5.2 Introduction

Plant cell wall structure integrity and associated tissue mechanical properties are key determinants for root tissue damage. Together with the cell's internal pressure and intercellular adhesion, the properties of plant cell walls influence how plant tissues undergo mechanical deformation and subsequently break up during post-harvest handling (Holland et al., 2020). The morphological structure and molecular architecture of plant cell walls have an important bearing on their mechanical properties. A plant cell wall is a heterogeneous and dynamic structure composed of a three-dimensional interwoven network of cellulose microfibrils embedded in a complex matrix of pectin, hemicelluloses, and structural proteins (Singh et al., 2010). The absence of side chains allows cellulose molecules to associate closely and form microfibril structures, providing a mechanical framework for the cell wall and its rigidity and resistance to osmotic pressures (Marry et al., 2006). Hemi-celluloses are thought to form links between cellulose microfibrils, modulating cell wall strength and influencing extensibility.

Mineral nutrients (macro elements P, K, Mg, S, Na, Ca, and N and microelements Cl, B, Fe, Mn, Zn, Cu, Ni, and Mo) play a crucial role in the growth, survival, and reproductive success of plants. Among these essential elements for higher plants, Ca and B have been shown to play important roles in maintaining the integrity of plant cell walls through their localisation in cell walls and their ability to bind pectic polysaccharides (Cosgrove, 2005; Vincken et al., 2003).

B deficiency is reported to cause brittleness in sugar beet leaves and petioles due to impaired cell wall development and the risk of crop losses due to brittle petioles are minimised by foliar applications (Shorrocks, 1997). There is limited information as to whether a deficiency in B leads to the brittleness of sugar beet root tissues, but in other root crops like carrots, glasshouse studies have revealed that increased levels of B in root tissues enhance plant cell wall structural integrity and its resistance to tissue damage (Singh et al., 2010). Since sugar beet root damage is benchmarked on tissue strength, further field studies are required, especially in areas where plants show B deficiency, to quantify the impact of foliar applications of B and Ca on root tissue properties. Ca deficiency results in small leaf blades with a black tip at the apex of the petiole. Ca's role in strengthening root tissues has not

been widely reported in the literature but a few studies have focused on the contribution to leaves and stem rigidity. For example, Li et al., (2012) reported increased pectin, lignin and breaking forces in peony stems after Ca application. Over-application of some nutrients such as nitrogen has been reported to affect root quality by increasing α -amino N impurities. At the same time, a deficiency in some nutrients like sulphur has also been proven to increase α -amino N impurities (Thomas et al., 2003). However, there is no information on whether a deficiency or toxicity of B or Ca reduces or increases root quality.

Despite Ca and B playing a very important role in plant stability and tissue robustness, and their deficiencies being widely reported in various crops, deficiency of the two nutrients has not been observed widely in most soils (Rashid and Ryan, 2004) implying that deficiencies are related to their availability for plant uptake. This has made a foliar application the most effective way of correcting B and Ca deficiencies. Thus, aside from soil analyses to quantify B and Ca deficiencies, in this study, we applied foliar treatments of B and Ca to assess their contribution towards sugar beet root tissue strength and damage susceptibility. The study hypothesised that B and Ca affects sugar beet root textural properties, tissue damage susceptibility and root quality.

5.3 Materials and methods

5.3.1 Site

The two experiments were conducted at the tMAF in Norfolk (52°33'18.8"N 1°02'24.2"E) during the 2021 and 2022 seasons. The site has predominantly sandy loam soils (**Table 5-1**) and received a total of 195 mm of rain in 2021 and 141 mm in 2022 from planting to harvesting (**Figure 5-1** and **Figure 5-2**). In 2021, the area was only dry during the early growth stage until day 30, but later, rainfall distribution was normal until the 150th day. In 2022 the area received little rain with a very sporadic distribution from planting to 35 days after planting and between 80 and 160 days after planting. Soil analysis was done as detailed on section 3.3.1 and shows that except for Na, all nutrients were within the optimum range for sugar beet production (AHDB, 2019). Specifically, B and Ca were not deficient in both years.

Table 5-1: Soil characteristics for tMAF farm.

Measurement	2021	2022
pH	7.5	7.2
P mg L ⁻¹ (Available)	20	18
K mg L ⁻¹ (Available)	139	160
Mg mg L ⁻¹ (Available)	52	40
Cu (EDTA Extractable) mg L ⁻¹	3.6	3.4
B (Hot Water Soluble) mg L ⁻¹	1.5	1.2
Na (Ammonium Nitrate Extractable) mg L ⁻¹	7.1	9.0
Zn (EDTA Extractable) mg L ⁻¹	3.1	2.6
Ca (Ammonium Nitrate Extractable) mg L ⁻¹	2181	1600
Fe (DPTA Extractable) mg L ⁻¹	59	52
Organic matter (LOI) %	3	4
S (Phosphate Buffer Extractable) mg L ⁻¹	19	16
Mn (DPTA Extractable) mg L ⁻¹	10	7
Sand (2.00 - 0.063mm) %	76	60
Silt (0.063 - 0.002mm) %	13	23
Clay (< 0.002mm) %	11	17
Textural Classification	Sandy loam	Sandy loam
Estimated CEC meq 100 ⁻¹	15	12

Morley 2021

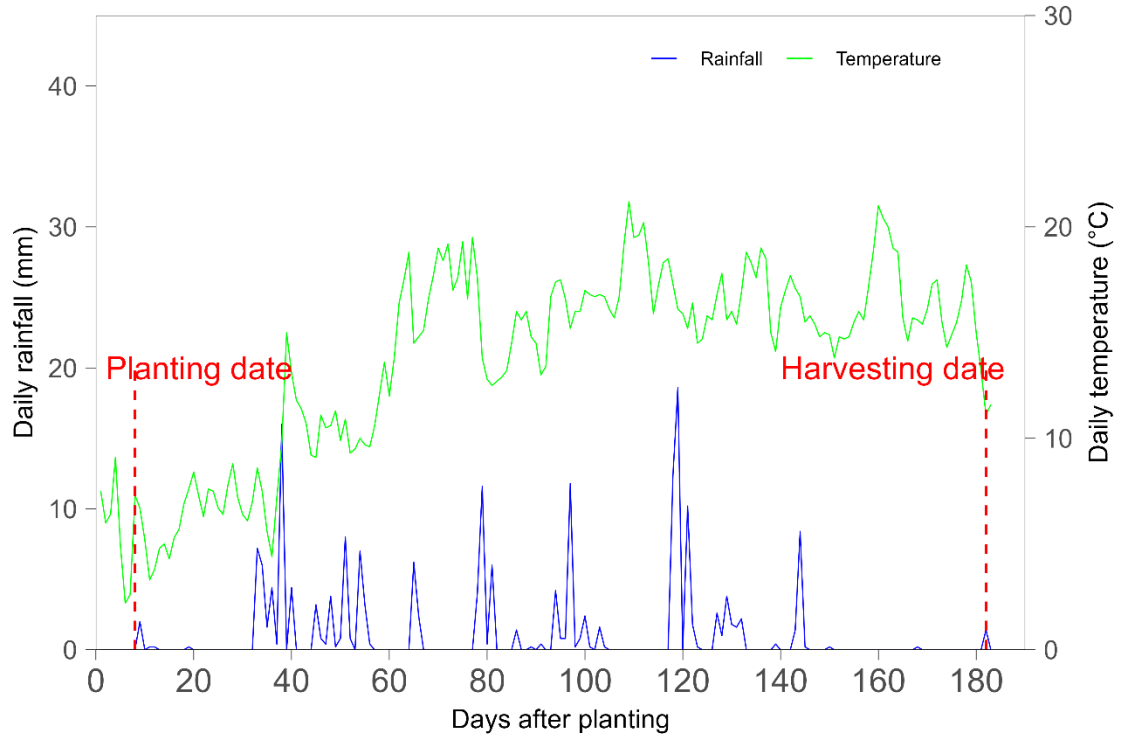


Figure 5-1: Daily rainfall and temperature distribution at tMAF in 2021

Morley 2022

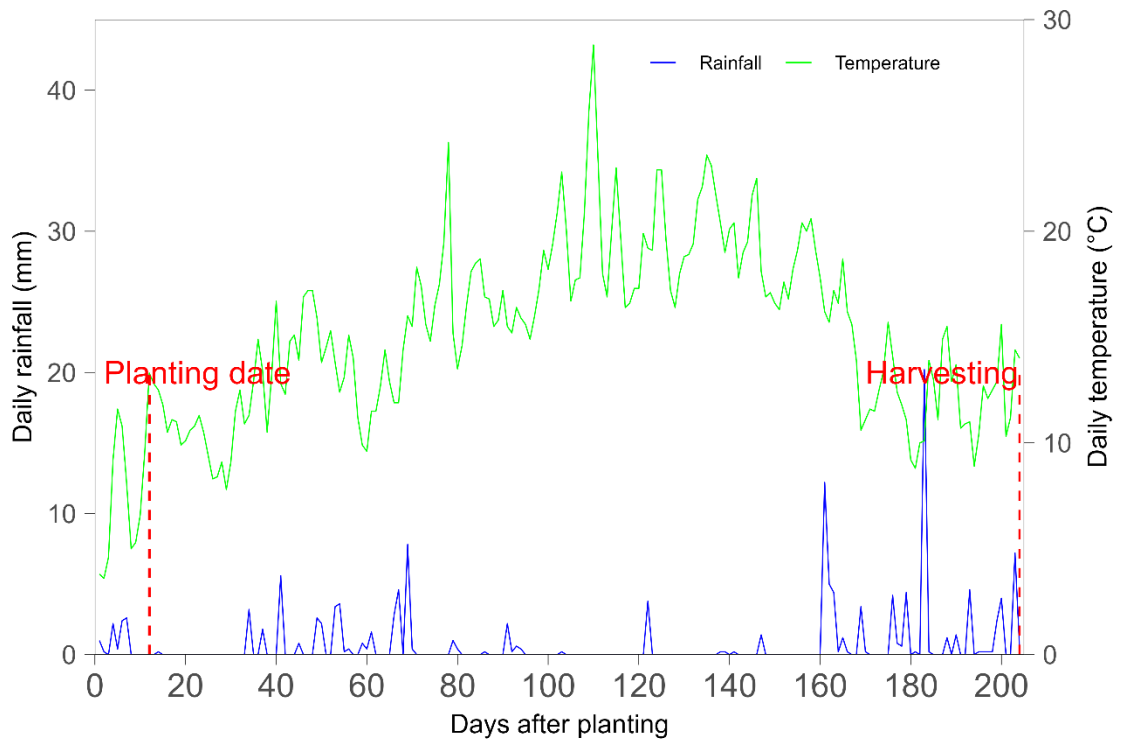


Figure 5-2: Daily rainfall and temperature distribution at tMAF in 2022

5.3.2 Field management

Plots contained 6 rows each 7.5 m long and spaced at 50 cm forming a total area of 22.5 m². Seed was drilled at a space of 16 cm giving a total plant population of 100 000 ha⁻¹. In 2021, the trials were drilled on the 8th of April while in 2022 the trials were drilled on the 12th of April. In both trials, a handheld plot sprayer (Norman Smith Equipment Ltd, UK) was used when applying C and B foliar fertilisers. Apart from the nutrients under test, all agronomic practices and other nutrients (N = 120 kg ha⁻¹, Phosphate (P₂O₅) = 50 kg ha⁻¹, Potash (K₂O₅) = 100 kg ha⁻¹) required were applied prior to planting were done following standard commercial practice (2022).

5.3.3 Experimental design

5.3.3.1 Calcium experiment

The experiment was a 2 x 3 factorial (6 treatments) with variety and fertiliser rate as treatment factors. It was laid in a randomised block design with three replications. To assess the effects of Ca, varieties (Sabatina and Daphna) characterised as most susceptible to damage were selected from the field trials in **chapter 3**. A foliar fertiliser Omex CalMax® Ultra with nutrient contents as detailed in **Table 5-2** was used as a source of Ca. The fertiliser was applied at three levels (0, 5 and 10 L ha⁻¹) which technically provided the crop with 0, 0.5 and 1 L ha⁻¹ of Ca, respectively. However, the application was split into five dozes starting at the six-leaf growth stage as detailed in. When applying Omex CalMax®, the fertiliser was dissolved in water at a rate of 1 to 200 litres. Since the plot size was 15 square metres, the 1 L ha⁻¹ application was achieved by mixing 3 ml of Omex CalMax® and 450 ml of water which were applied per plot and per application. When applying 2 L ha⁻¹, the amount of fertiliser was doubled to 6 ml while water was maintained at 450 ml.

Table 5-2: Nutrient contents for Omex CalMax® Ultra

Nutrient	Concentration (%)
N	9.60
CaO	14.50
MgO	1.90
Mn	0.10
Fe	0.05
B	0.05
Cu	0.05
Zn	0.02
Mo	0.001

Table 5-3: Calcium fertilizer application in experimental plots

Application rate and time					
At six leaf stage	4 weeks after six leaf stage	8 weeks after six leaf stage	12 weeks after six leaf stage	16 weeks after six leaf stage	Total
0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹
1 L ha ⁻¹	1 L ha ⁻¹	1 L ha ⁻¹	1 L ha ⁻¹	1 L ha ⁻¹	5 L ha ⁻¹
2 L ha ⁻¹	2 L ha ⁻¹	2 L ha ⁻¹	2 L ha ⁻¹	2 L ha ⁻¹	10 L ha ⁻¹

5.3.3.2 Boron experiment

The experiment was a 2 x 3 factorial (6 treatments) with variety and B fertiliser rate as factors. It was laid in a randomised block design with three replications. Sabatina and Daphna constituted the two varieties used. Just like in the Ca trial, the two varieties were chosen as they proved to have weak textural properties and vulnerable to damage (**Chapter 3**). A straight foliar fertiliser (YaraVita BORTRAC 150) was used as a source of B. The fertiliser contains 150g of B per litre. The fertiliser application was split into 3 starting at the six-leaf growth stage. At every application time, 0, 1 and 2 litres were applied for the 0, 3 and 6 L ha⁻¹ treatments respectively (**Table 5-4**). The 0, 3 and 6 L ha⁻¹ applications of YaraVita BORTRAC 150 technically provided the crop with 0, 0.45 and 0.9 L ha⁻¹ of B, respectively. During application, YaraVita BORTRAC 150 was dissolved in water at a rate of 1 to 200 litres. When applying YaraVita BORTRAC 150, the fertiliser was dissolved in water at a rate of 1 to 200 litres. Since the plot size was 22.25 m², the 1 L ha⁻¹ application was achieved by mixing 3 ml of YaraVita BORTRAC 150 and 450 ml of water which were applied per plot and per application. When applying 2 L ha⁻¹, the amount of fertiliser was doubled to 6 ml while water was maintained at 450 ml.

Table 5-4: Boron fertiliser application in experimental units

Application rate and time			
At six leaf stage	4 weeks after six leaf stage	8 weeks after six leaf stage	Total
0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹	0 L ha ⁻¹
1 L ha ⁻¹	1 L ha ⁻¹	1 L ha ⁻¹	3 L ha ⁻¹
2 L ha ⁻¹	2 L ha ⁻¹	2 L ha ⁻¹	6 L ha ⁻¹

5.3.4 Plant sampling

Fifteen sugar beet roots were randomly uprooted by hand from the middle rows. Ten uniform roots were then selected and put in storage sacks and taken to Harper

Adams University (HAU) where they were stored in a cold room at 6 °C and relative humidity of 85 %. After one day, two roots were randomly picked from the sacks, washed and taken to the laboratory for textural analysis. Five roots from the remaining eight were used for the damage test.

5.3.5 Texture and analysis

Textural properties conducted in this study include puncture, shear, and compression resistance which were done as described in **3.3.5.1** and **3.3.5.2**.

5.3.6 Damage test

Damage test was done as described in **3.3.6**.

5.3.7 Sugar and impurities analysis

Potassium, α -amino N, sodium impurities and sugar content were analysed to assess root quality. The analysis of beet brei samples was carried out with an automated analyser (Anton Paar OptoTec GmbH) following (Kenter and Hoffmann, 2009) where beets were washed, weighed and cut to prepare brei, which was clarified with 0.3% Al-sulphate solution (Ebmeyer and Hoffmann, 2022). Sucrose was measured polarimetrically and potassium and sodium by flame photometry. Amino N was analysed by the fluorometric method (Felix and Terkelsen, 1973).

5.3.8 Data analysis

An analysis of variance (ANOVA) was performed on the collected variables in R version 4.2.2 within *lmerTest* package (Kuznetsova et al., 2017) using a linear mixed model. Before the analysis, each variable was tested for normality using Shapiro-Wilk test and showed normal distribution of the population. Data for each season was analysed separately where fertiliser application and variety were fitted as fixed factors while replication was considered a random factor. A post hoc analysis was performed in *emmeans* (Russell et al., 2023) package using a Tukey HSD test to compare treatment means at $p \leq 0.05$ significance level. Principal component analysis was done on B experiment data using seven variables. Variables that were highly correlated to one of the loaded variables but also explained a small percentage of variation were dropped to avoid multicollinearity. Graphs were prepared using *ggplot2* (Wickham, 2009) R package and where box plots have been used, black dots represent the mean value.

5.4 Results

5.4.1 Calcium experiment

5.4.1.1 Textural properties

Results show that an interaction between Ca and variety did not have an influence on textural properties. Resistance to puncture was affected by calcium application ($p < 0.05$) in 2021 (**Figure 5-3**) while variety influenced puncture resistance ($p < 0.05$) in both seasons. The average resistance to puncture for 2021 was 6 MPa while for 2022 was 5 MPa. Sabatina had a higher resistance to puncture in both seasons (6.04 and 5.93 MPa in 2021 and 2022, respectively) compared to Daphna (5.42 and 5.12 MPa in 2021 and 2022, respectively) (**Figure 5-4**). In 2021, applying 10 L ha⁻¹ of Ca significantly increased resistance to puncture. Resistance to shear was affected by Ca application in 2021 ($p < 0.05$) (**Figure 5-5**) and variety in both seasons ($p < 0.05$ in 2021 and $p < 0.001$ in 2022) (**Figure 5-6**). Resistance to compression was affected by calcium application in 2021 while in 2022, there were no significant differences. However, varieties response to compression resistance only differed in 2022 where Daphna required less force (2.51 MPa) to compress its tissues compared to Sabatina (2.89 MPa).

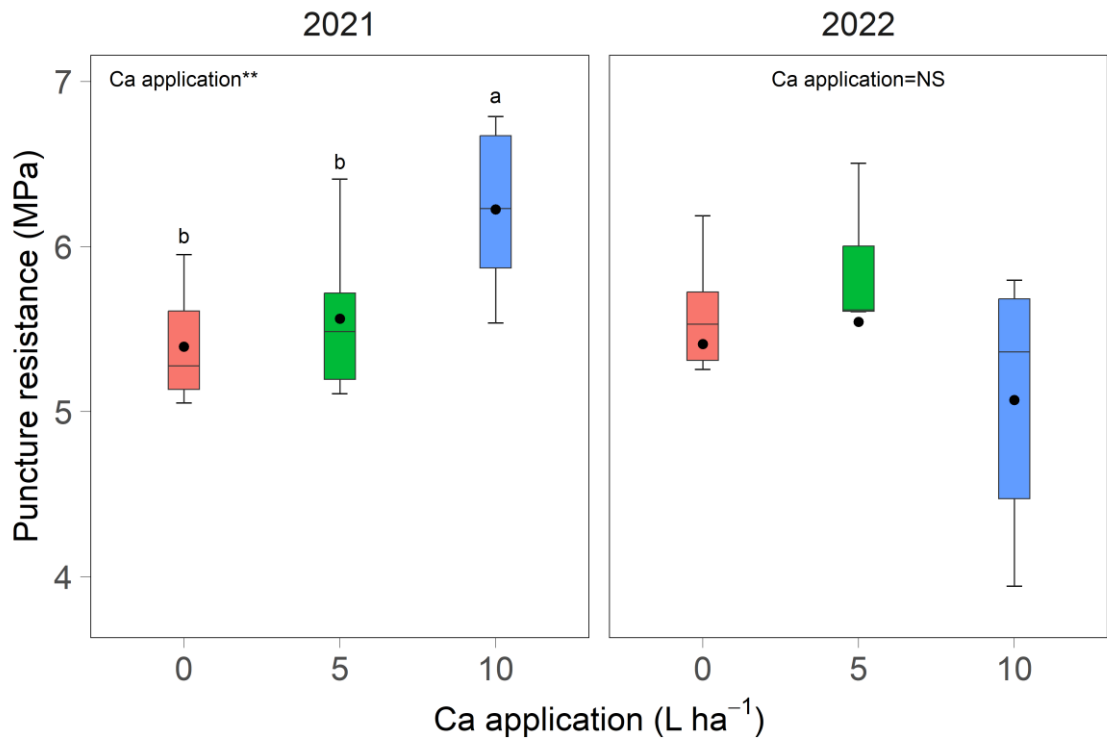


Figure 5-3: Effects of Ca application on sugar beet puncture resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

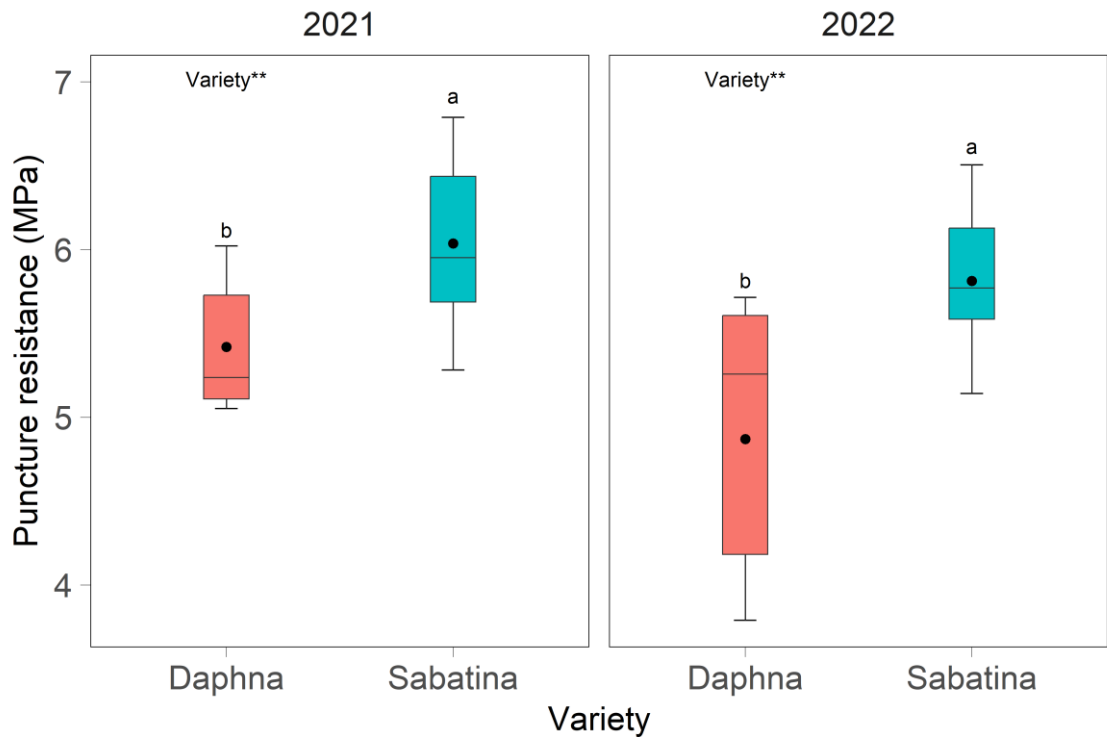


Figure 5-4: Effects of sugar beet variety on puncture resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

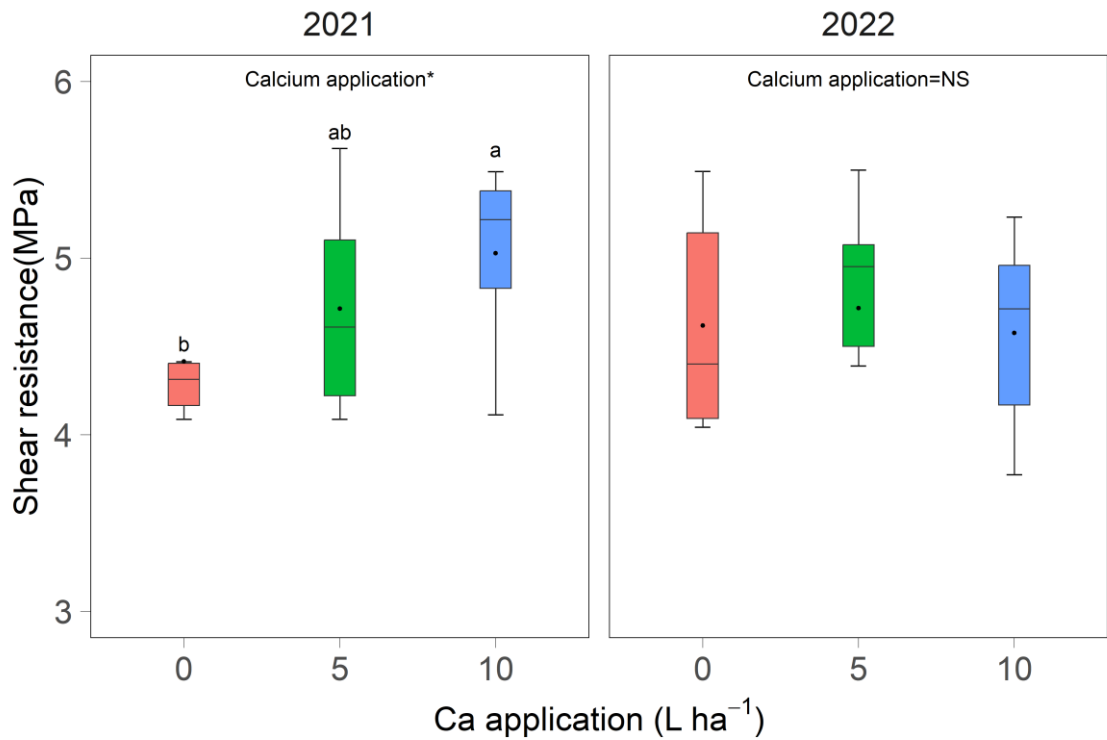


Figure 5-5: Effects of Ca application on sugar beet shear resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

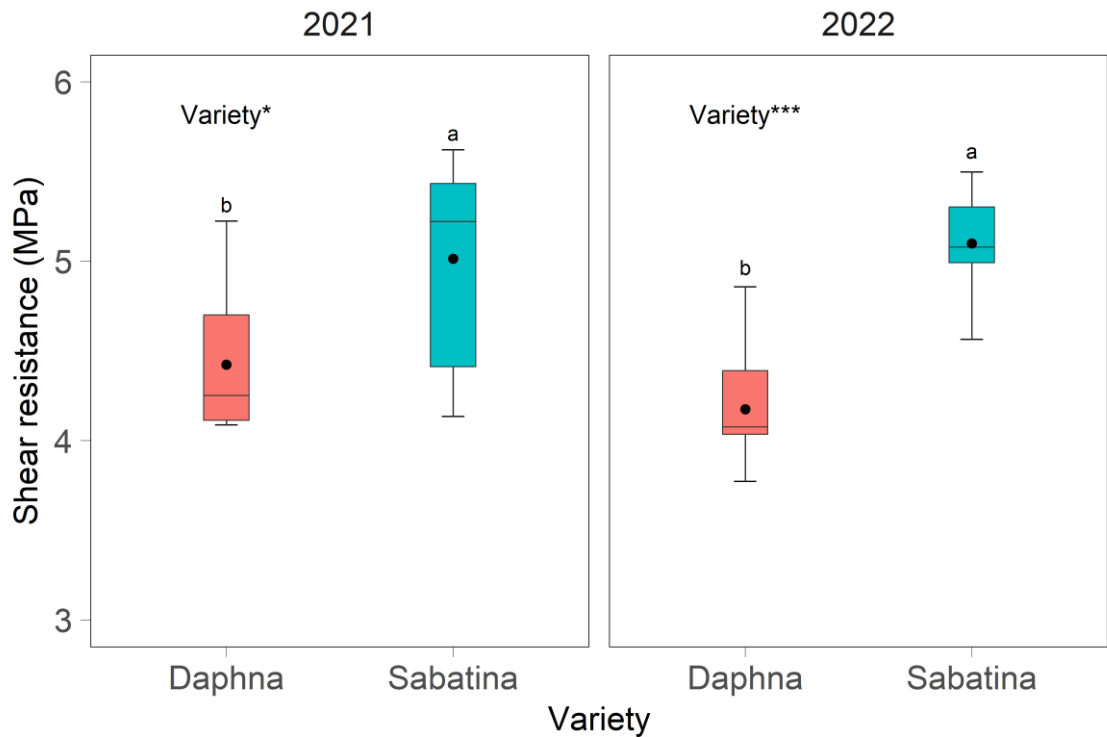


Figure 5-6: Effects of sugar beet variety on shear resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

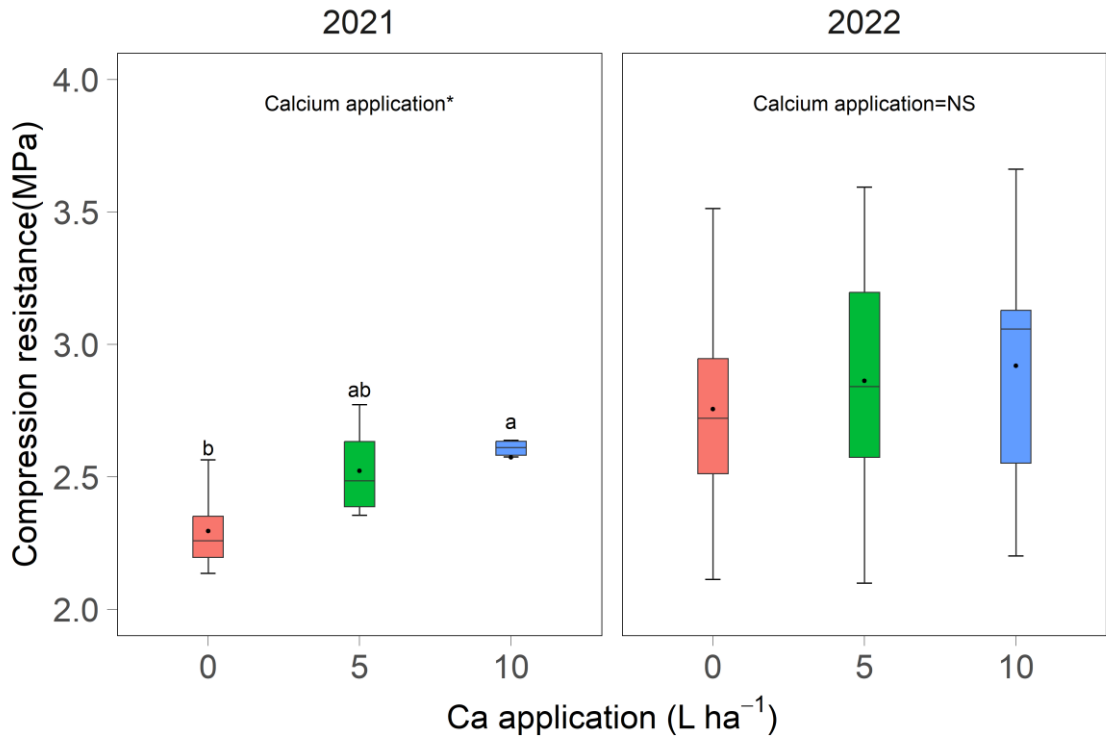


Figure 5-7: Effects of Ca application on compression resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

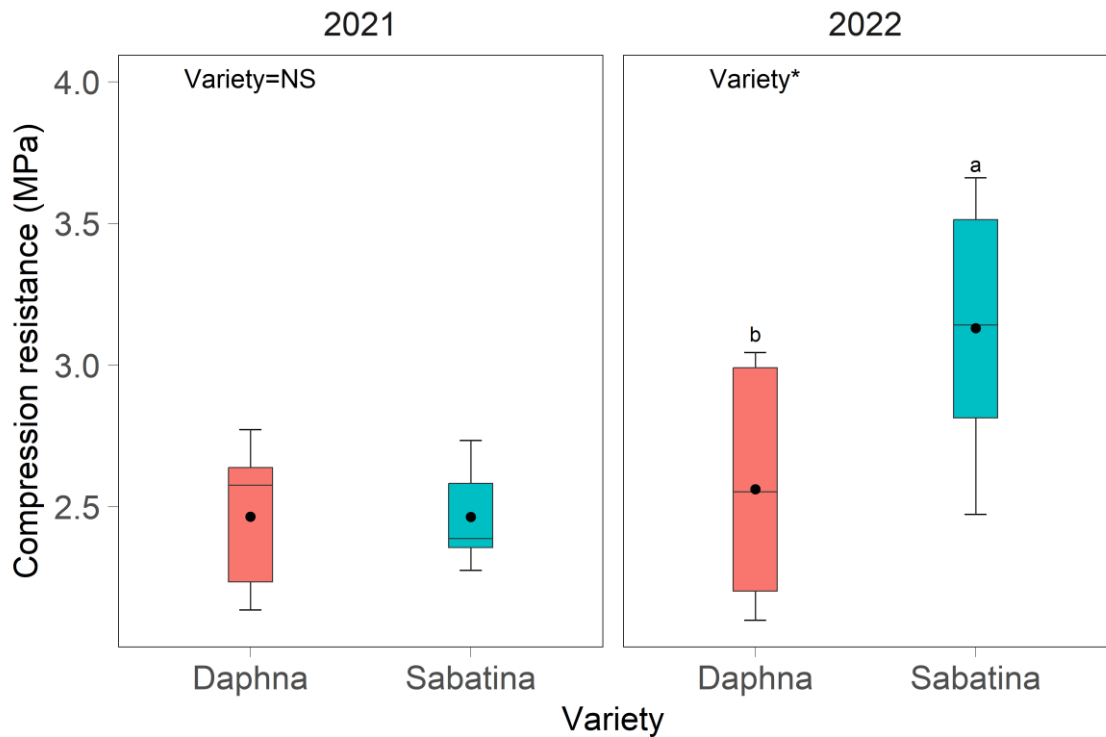


Figure 5-8: Effects of sugar beet variety on compression resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.1.2 Weight

Ca application did not influence sugar beet root weight (**Figure 5-9**). However, root weight was observed to be higher in 2021 than in 2022. The average weight for 2021 was 87 T ha⁻¹ while for 2022 was 51 T ha⁻¹ representing a 41 % decrease.

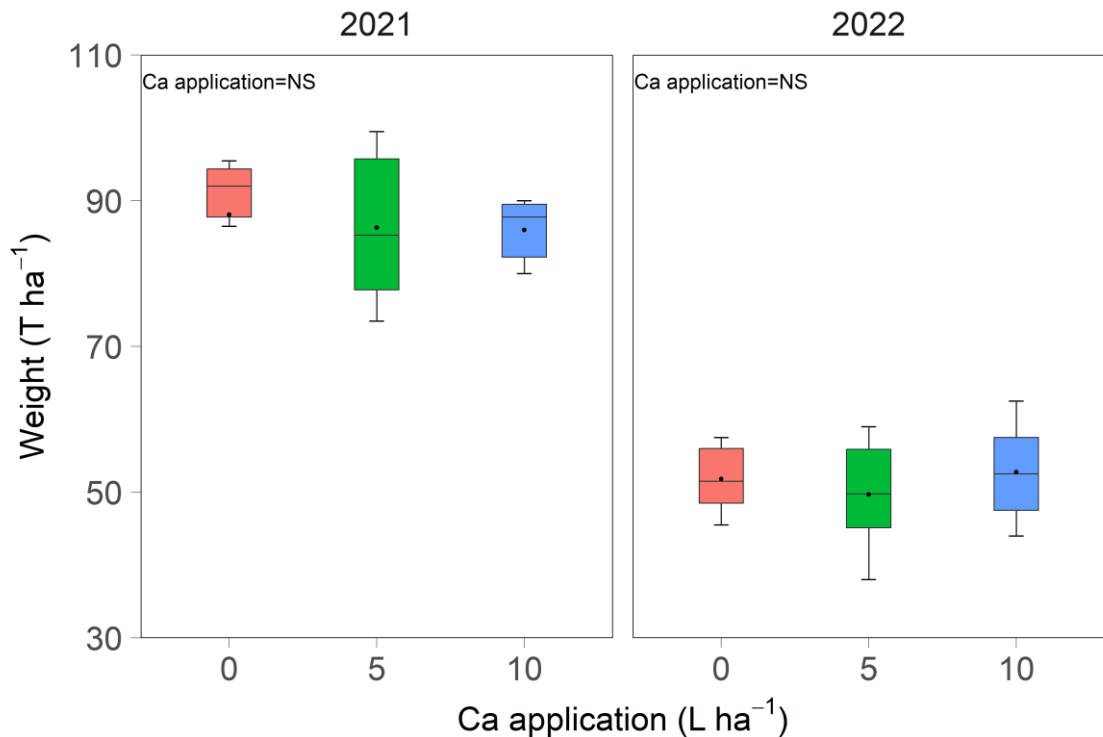


Figure 5-9: Effects of Ca application on root weight during 2021 and 2022 at Morley farm. For each season, $n=18$. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.1.3 Root tissue damage

Tissue damage was measured in terms of root tip diameter after damage and surface damage. Surface damage was affected by Ca application ($p < 0.05$) (**Figure 5-10**) in 2021 where Ca rates of 0,5 and 10 $L ha^{-1}$ resulted in respective surface damage percentages of 11, 9 and 8. However, in 2022, there were no significant differences in surface damage when the three Ca rates were applied. In both seasons, the interaction between variety and Ca application was not significant. Root tip diameter after damage was not influenced by Ca application (**Figure 5-11**). However, there was a significant difference between Sabatina and Daphna in both seasons with regard to root tip diameter after damage. In 2021, average root tip diameter after damage for Sabatina was 5 cm while Daphna was 4 cm while in 2022, Sabatina registered an average of 4.4 cm and Daphna registered an average of 3.7 cm.

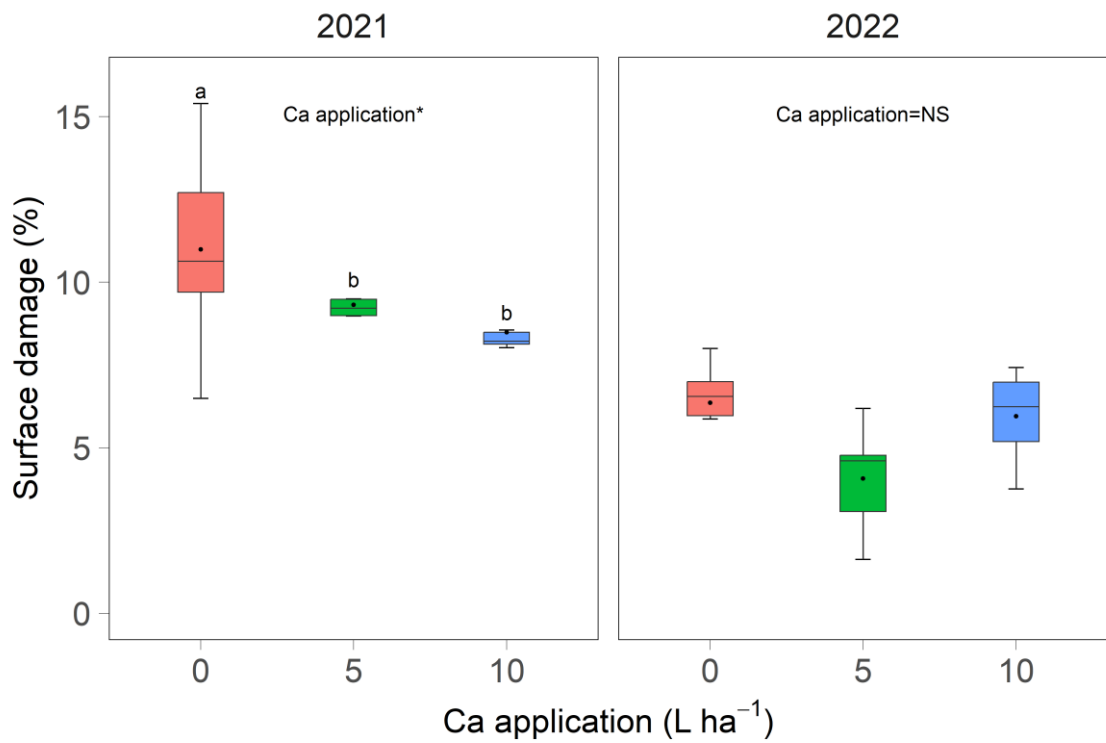


Figure 5-10: Effects of Ca application on surface damage during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

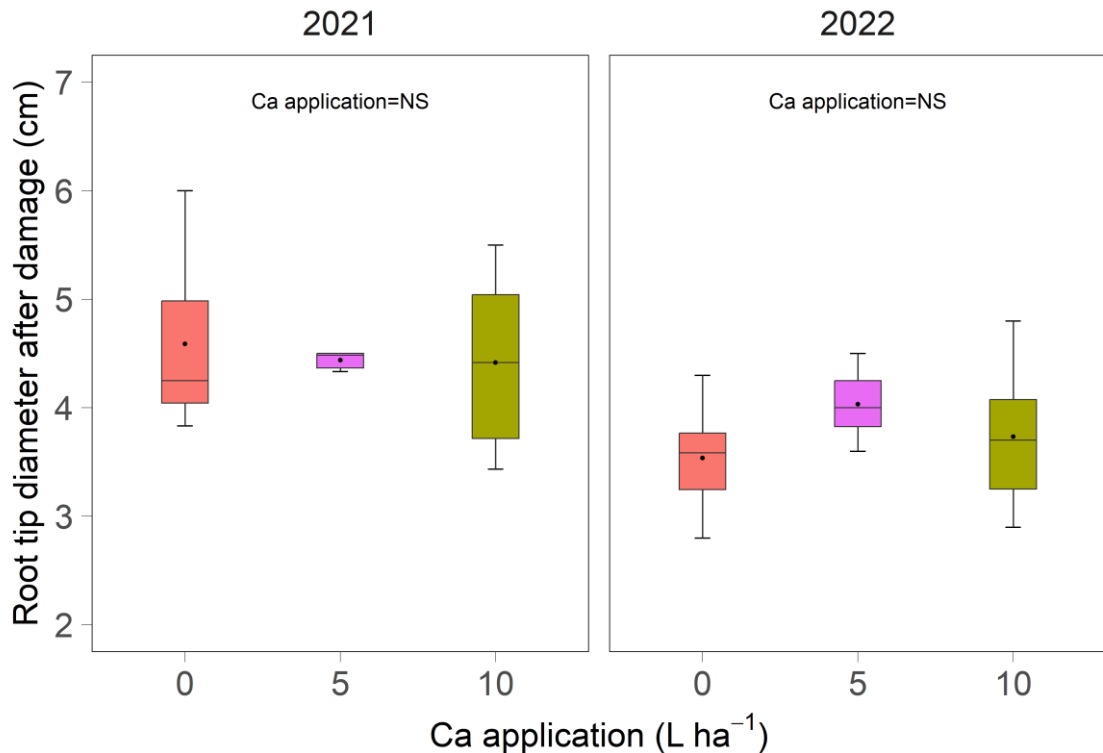


Figure 5-11: Effects of Ca application on root tip diameter damage during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.1.4 Root quality

Root quality was assessed through quantification of sugar content and impurities (Na, K and α -amino N). Sugar content was affected by Ca application in 2022 season only (**Figure 5-12**). On average, there was a relatively higher sugar content in the roots that were harvested in 2022 (17 %) compared to those harvested in 2021 (16 %). α -amino N impurities were not affected by Ca, varieties, and interaction between Ca and variety. However, higher average α -amino N impurities (21 %) were observed in 2022 than 2021 (8 %). This trend was consistent for both varieties and at every Ca application rate (**Figure 5-13**). Sodium was not significantly affected by Ca application (**Figure 5-14**) but variety (**Figure 5-15**). Sabatina had a significantly higher sodium content during both seasons (25 and 21 meg/100g in 2021 and 2022, respectively) compared to Daphna (15 and 16 meg/100g in 2021 and 2022,

respectively) Ca and variety did not influence potassium concentration in the roots (Figure 5-16).

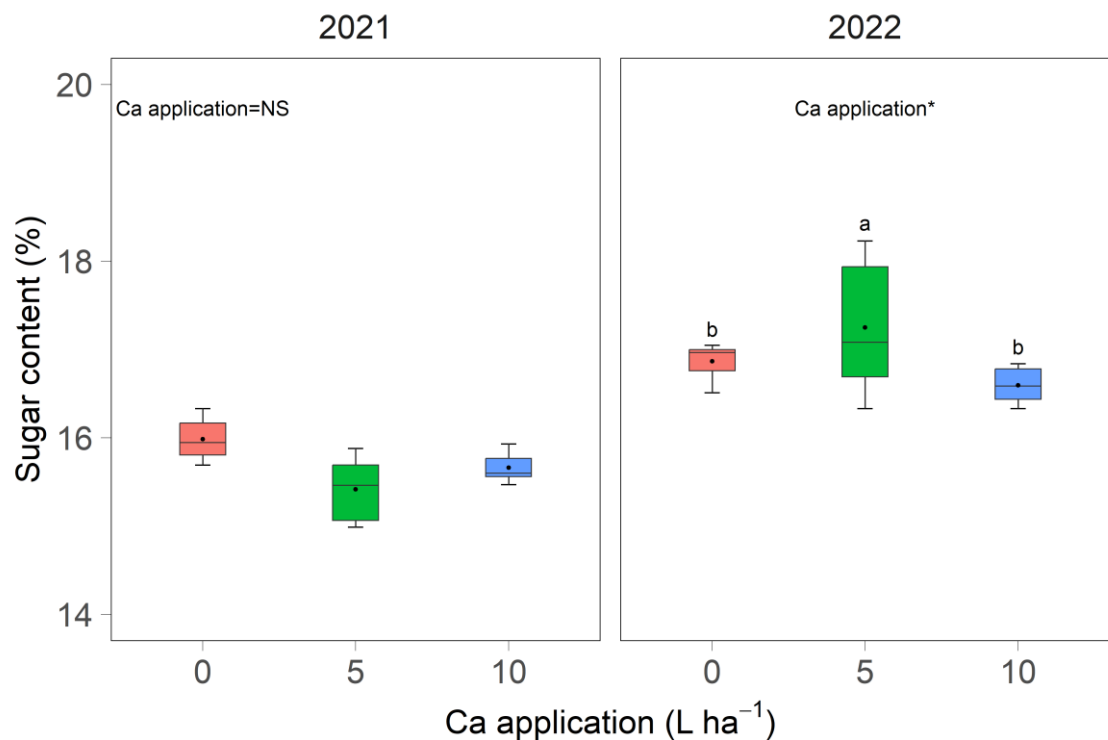


Figure 5-12: Effects of Ca application on sugar content during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point

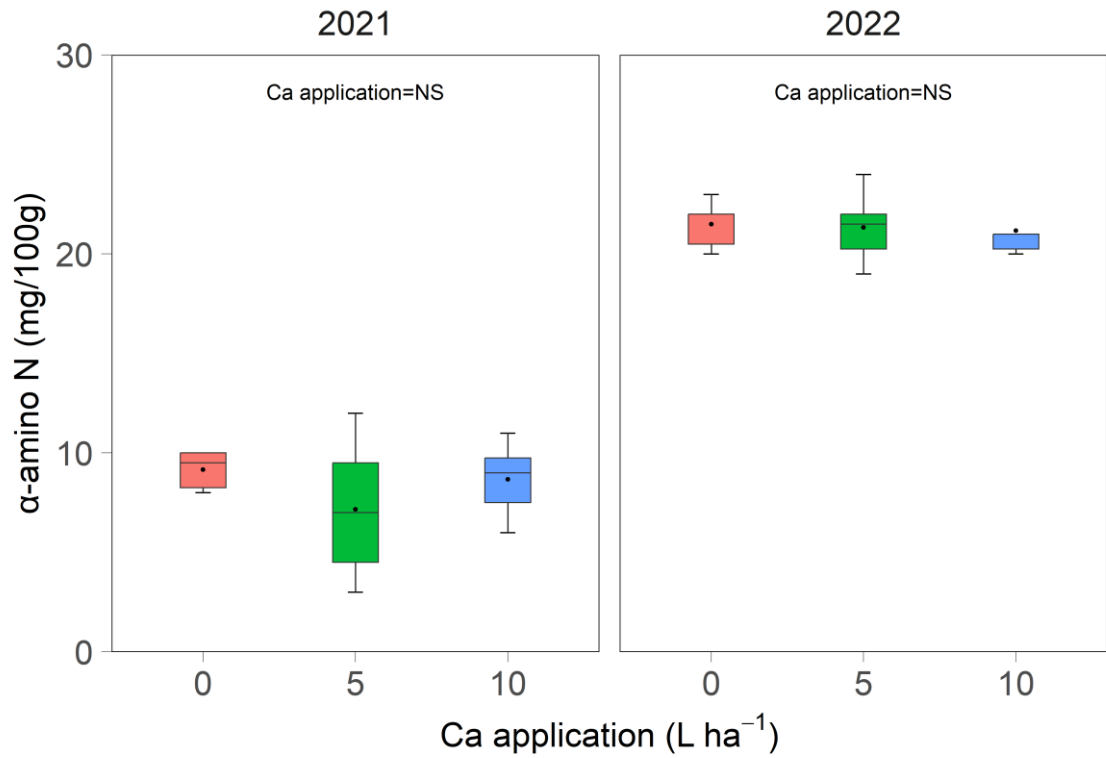


Figure 5-13: Effects of Ca application on α -amino N during 2021 and 2022 at Morley farm. For each season, $n=18$. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point

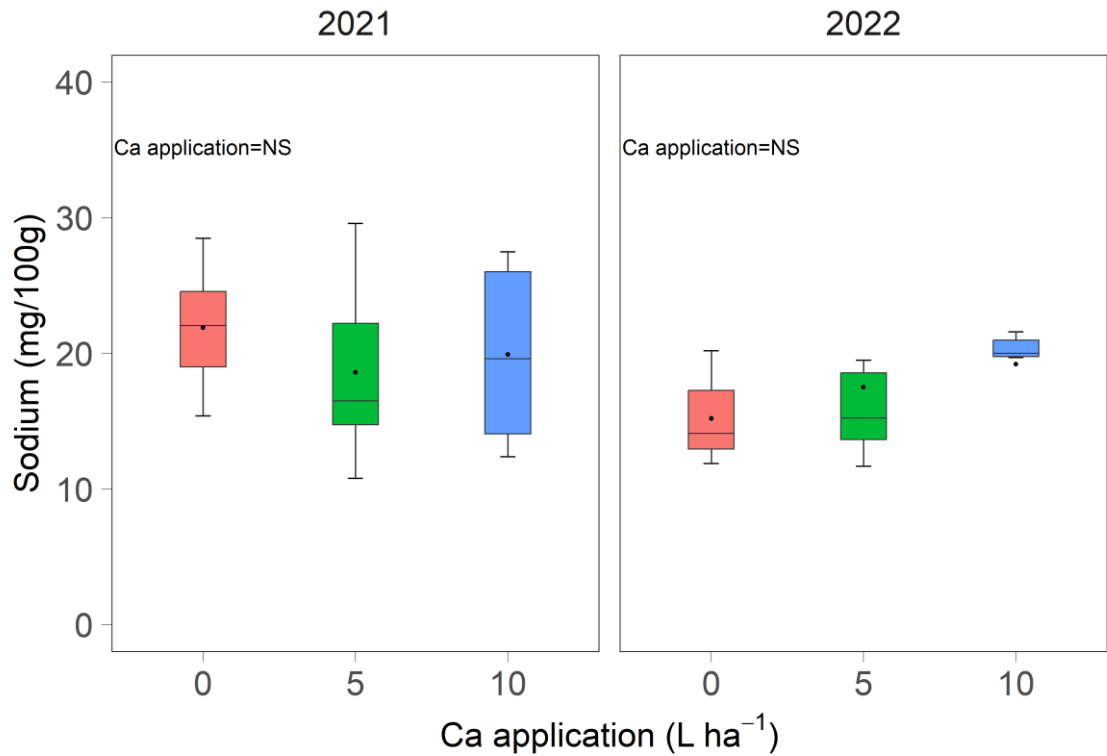


Figure 5-14: Effects of Ca application on sodium content during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

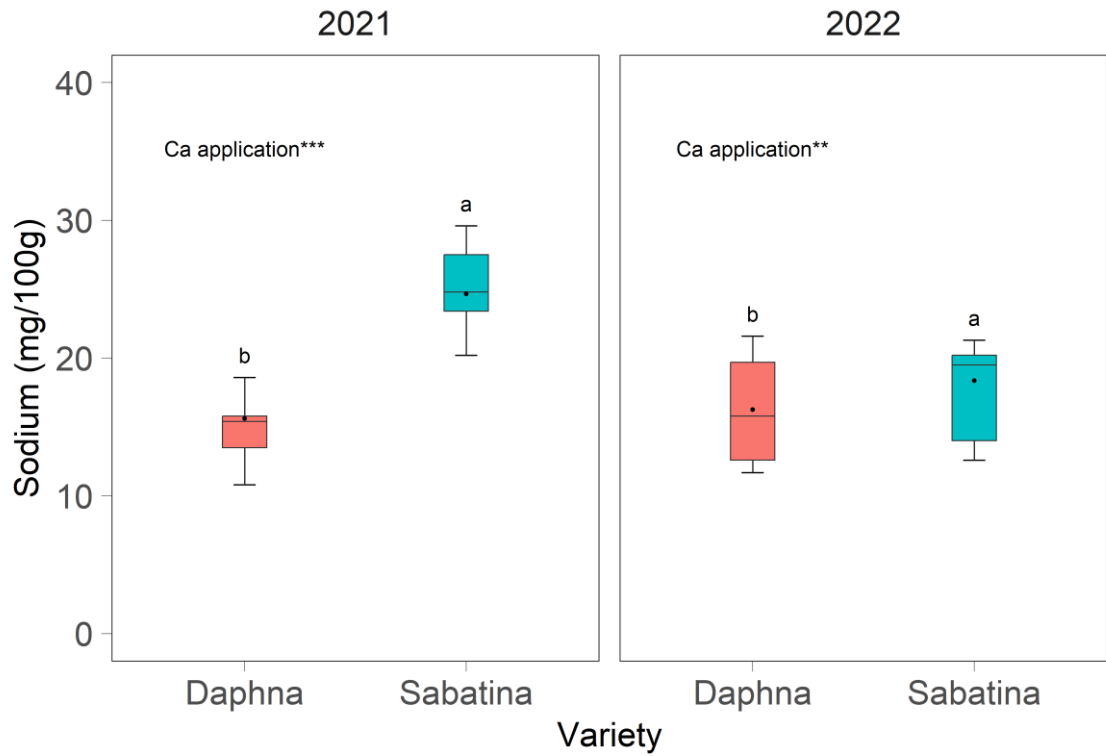


Figure 5-15: Effects of sugar beet variety on sodium content during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

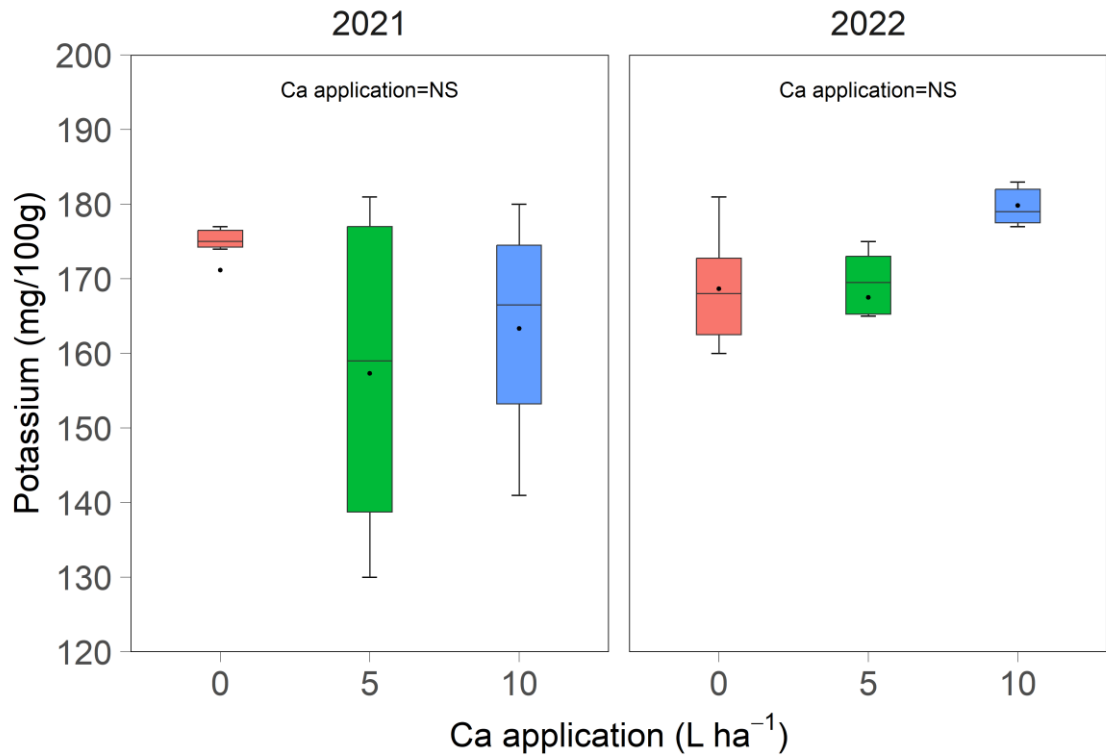


Figure 5-16: Effects of Ca application on potassium content during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.2 Boron experiment

5.4.2.1 Textural properties

Textural properties under consideration for the B experiment were puncture and compression resistance. Compression was affected by B ($p < 0.05$) and variety ($p < 0.01$) but not their interaction. Roots from plots that did not receive B recorded an average of 2.24 MPa while an application of 3 and 6 L ha⁻¹ B registered compression values of 2.57 and 2.63, respectively. Despite each variety showing an increase in compressive strength when B is applied, the within-variety increase was not statistically significant across B application rates (**Figure 5-17**). Variety 5 was on average stronger in compression compared to variety 3. Despite variety 3 showing an increase of puncture resistance when B was applied, results also show that

variety, B and their interaction did not affect root tissues' response to puncture (Figure 5-18).

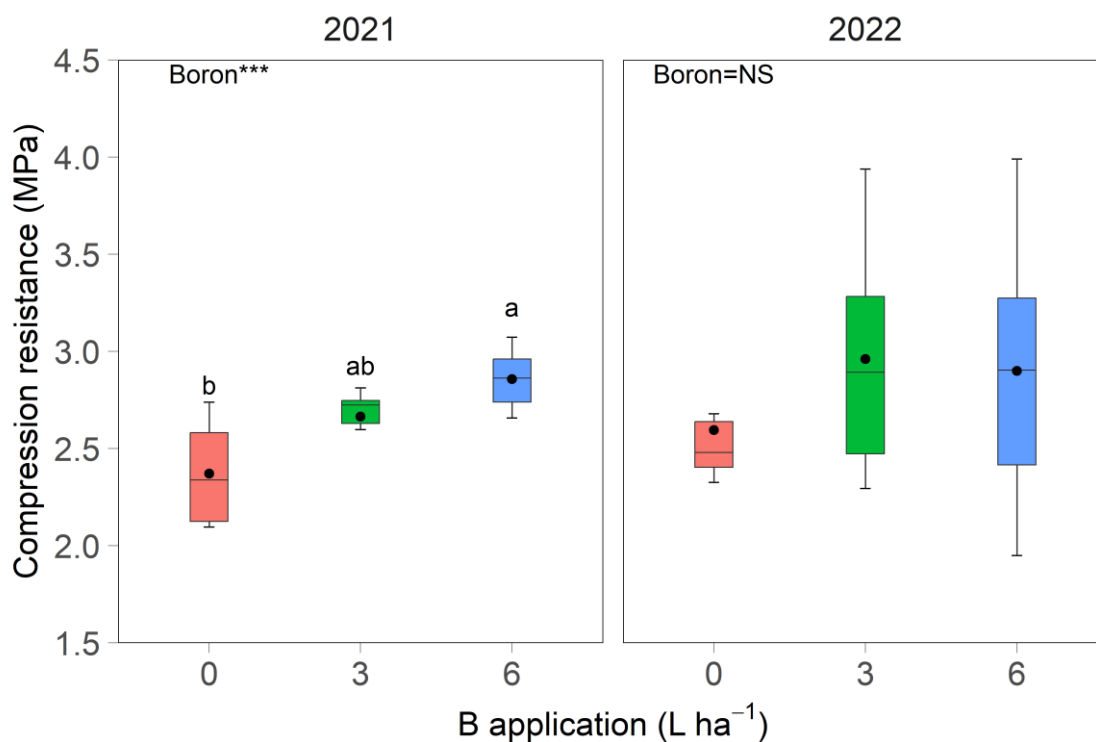


Figure 5-17: Effects of B application on compression resistance during 2021 and 2022 at Morley farm. For each season, n=18. Treatments with different superscripts within a season mean they are statistically different. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

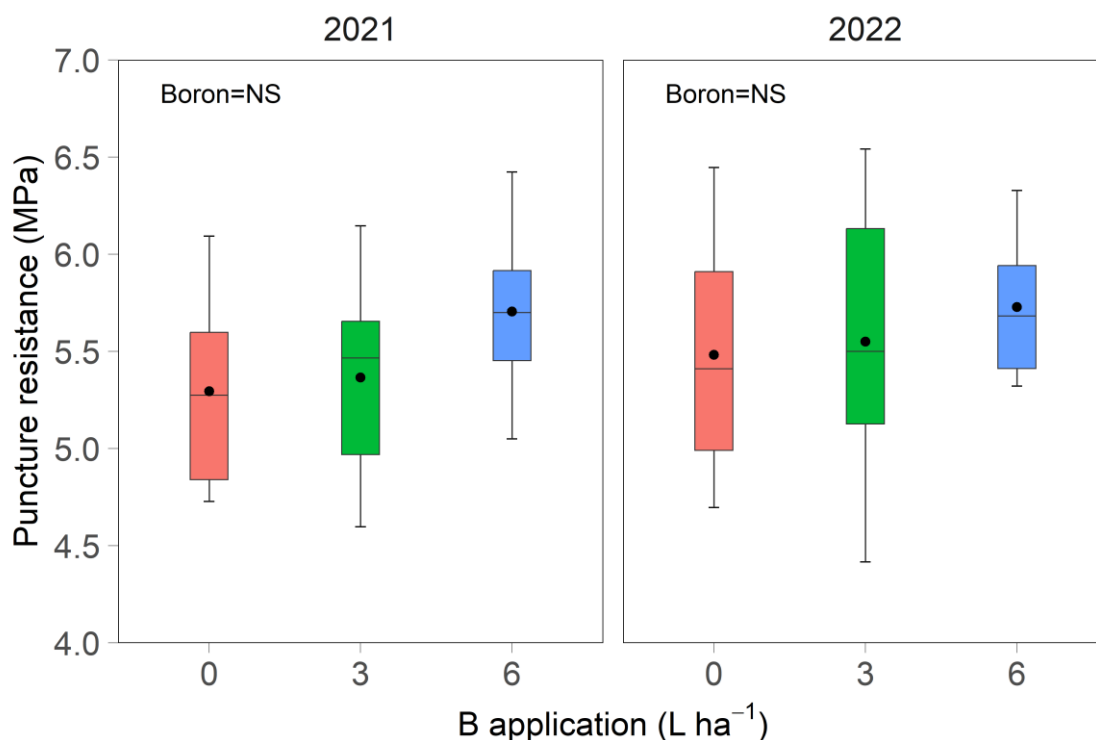


Figure 5-18: Effects of B application on puncture resistance during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.2.2 Weight

B application did not influence sugar beet root weight (**Figure 5-19**). However, the weight of the roots was influenced by the season ($p < 0.001$) with 2021 registering a higher weight (87 tonnes ha⁻¹) compared to the 2022 season (50 tonnes ha⁻¹). There was an average weight reduction of 43 % in 2022 which was statistically uniform across the B application rates.

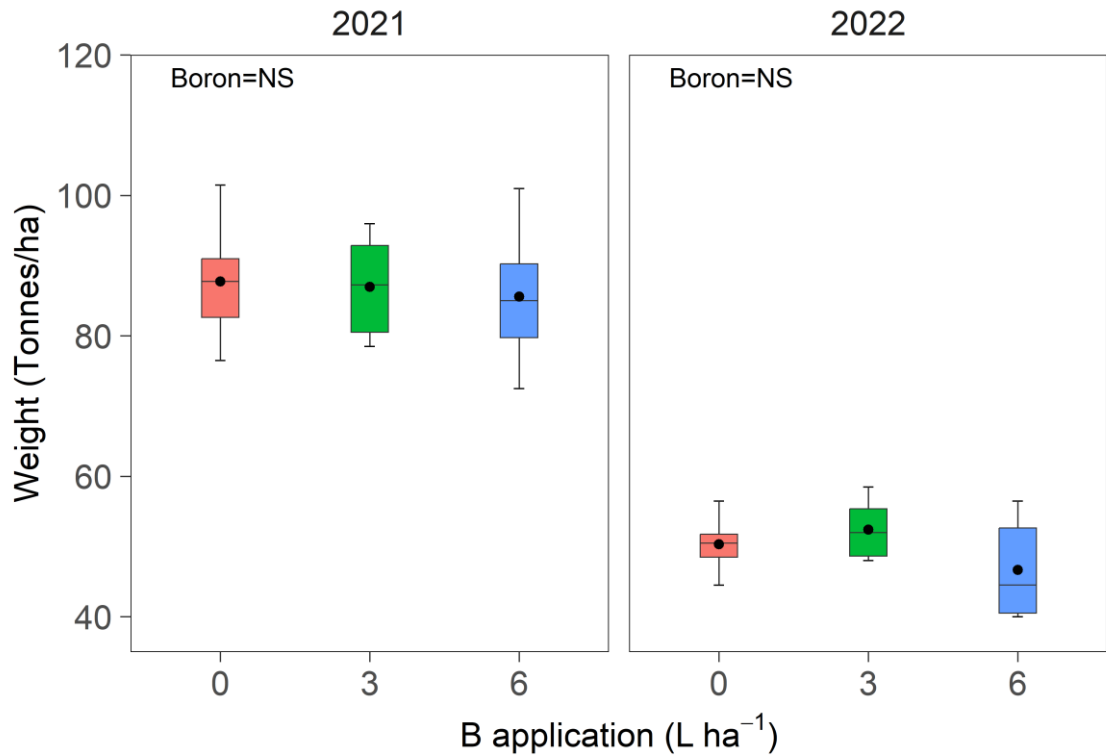


Figure 5-19: Effects of B application on weight during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.2.3 Root tissue damage

Root tissue damage was measured as root tip diameter after damage and surface damage. B and the interaction between season and B did not statistically reduce or increase both root tip diameter after damage and surface damage (**Figure 5-20** and **Figure 5-21**). However, season affected root tip diameter after damage ($p < 0.001$) with low diameters observed in 2022. The average root tip diameter after damage in 2021 was 5.16 and in 2022 was 3.95 cm.

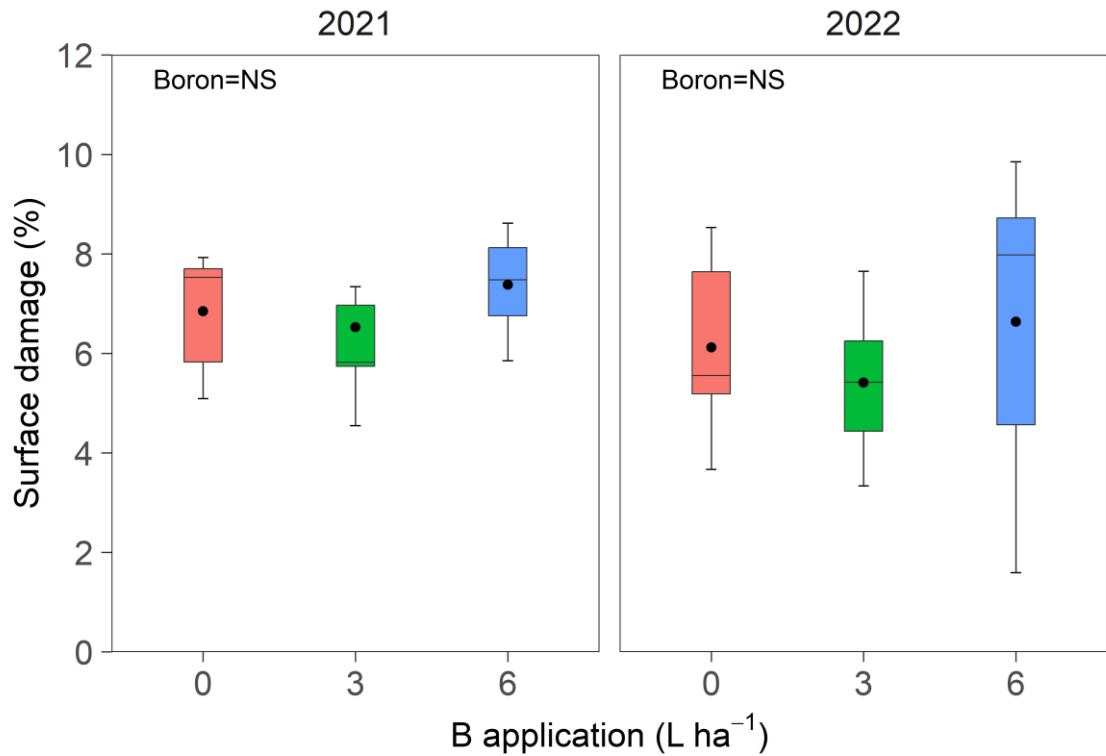


Figure 5-20: Effects of B application on sugar beet root surface damage during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

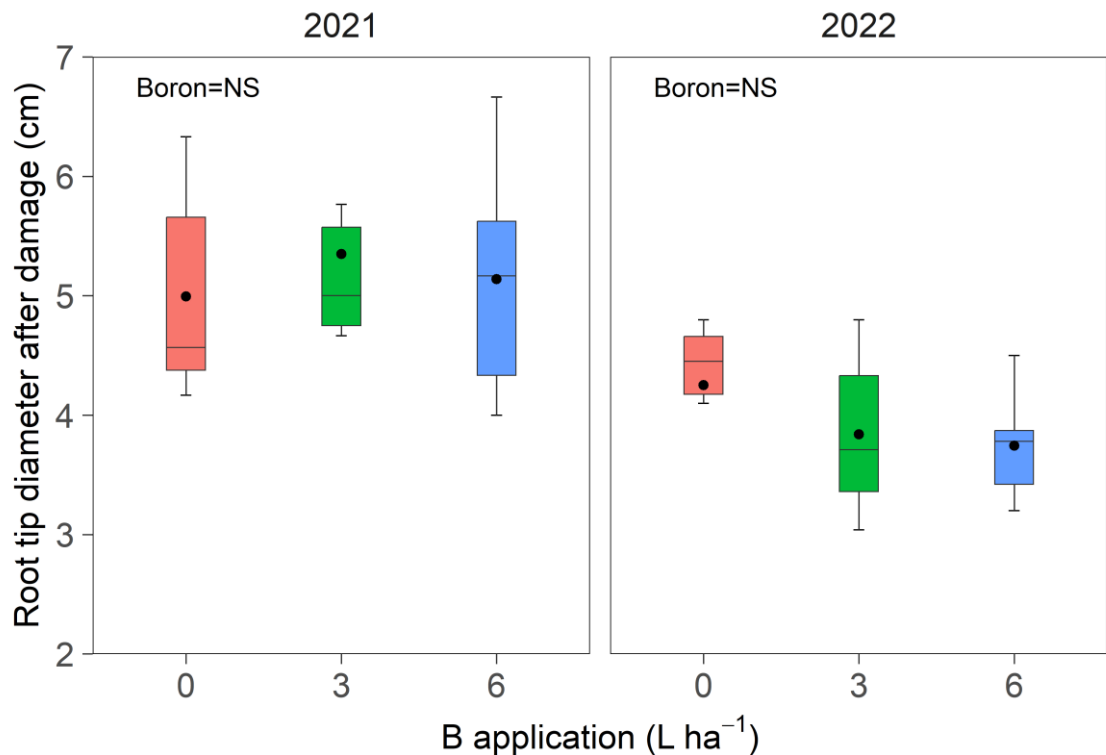


Figure 5-21: Effects of B application on sugar beet root tip diameter after damage during 2021 and 2022 at Morley farm. For each season, n=18. Black dots represent the mean value, the horizontal line in the box represent the median. The upper whisker represents the maximum value, the lower whisker represents the minimum value, the box represents the interquartile range where 50 % of the values fall, the lower part of the box represents the lower quartile where 25 % of the values fall below this point and the upper part of the box represent the upper quartiles where 25 % of the values fall above this point.

5.4.2.1 Root quality

Root quality was assessed by measuring sugar content and impurities. Impurities under study included sodium, potassium and α -amino N. Results show that B application and interaction between B and variety did not affect impurities and sugar content in the roots (**Table 5-5**). However, there were seasonal differences for α -amino N where 2022 had statistically higher α -amino N across all B application rates than 2021. A similar trend was observed for sugar content and potassium. However, potassium concentration in root tissues for 2021 was different from that of 2022 in non-treated plants and the ones that received 6 L ha⁻¹ B. Sodium impurities were not affected by both season and B application despite 0 L ha⁻¹ application rate showing a relatively higher concentration in 2021. Our results also show that the two varieties were not statistically different for all impurities. However,

the varieties were statistically different in terms of sugar content. Variety 5 had a higher sugar content (17 %) compared to variety 3 (16 %).

Table 5-5: Effects of B application on sugar beet impurities and sugar content in 2021

B (L ha⁻¹)	Sugar (%)	Potassium (mg/100g)	Sodium (mg/100g)	α-amino N (mg/100g)
0	15.64±0.12	163±10	22±3	8±2
3	15.62±0.15	164±9	18±2	9±2
6	15.92±0.10	152±8	18±2	5±2
P-value	NS	NS	NS	NS

Note: For each variable, n=18.

Table 5-6: Effects of B application on sugar beet impurities and sugar content in 2022

B (L ha⁻¹)	Sugar (%)	Potassium (mg/100g)	Sodium (mg/100g)	α-amino N (mg/100g)
0	16.91±0.07	174±3	16±1	21±1
3	16.74±0.13	168±5	18±1	21±1
6	16.82±0.09	173±5	15±1	22±1
P-value	NS	NS	NS	NS

Note: For each variable, n=18.

Table 5-7: Effects of sugar beet variety on impurities and sugar content in 2021

B (L ha ⁻¹)	Sugar (%)	Potassium (mg/100g)	Sodium	α-amino N
			(mg/100g)	(mg/100g)
Daphna	15.66±0.11	150±8	17±2	6±1
Sabatina	15.80±0.11	170±5	21±2	9±1
P-value	NS	NS	NS	NS

Note: For each variable, n=18.

Table 5-8: Effects of sugar beet variety on impurities and sugar content in 2022

B (L ha ⁻¹)	Sugar (%)	Potassium (mg/100g)	Sodium	α-amino N
			(mg/100g)	(mg/100g)
Daphna	16 ^b ±0.06	175 ^a ±3	17±1	21±2
Sabatina	17 ^a ±0.05	168 ^b ±3	16±1	22±2
P-value	*	*	NS	NS

Note: For each variable, n=18.

5.4.2.2 Principal component analysis

A principal component analysis (PCA) was performed for impurities (sodium, potassium, and α-amino N), weight, textural properties (puncture and compression) and sugar content (**Figure 5-22**). Other variables were dropped because they were highly correlated with one of the loaded variables. Since there were only three B application rates, the PCA had only three components. PC1 and 2 explained 69.12 and 30.88 % of the variation, respectively, totalling 100 %, hence PC 3 was dropped since it was almost negligible. PC1 was affected by puncture, weight sugar, α-amino N, and potassium while sodium and compression are the only traits that contributed to PC2. The 0 L ha⁻¹ B application is associated with high potassium and sodium impurities, 3 L ha⁻¹ application rate is associated with high weight and α-amino N while the 6 L ha⁻¹ application rate is associated with high puncture and sugar content.

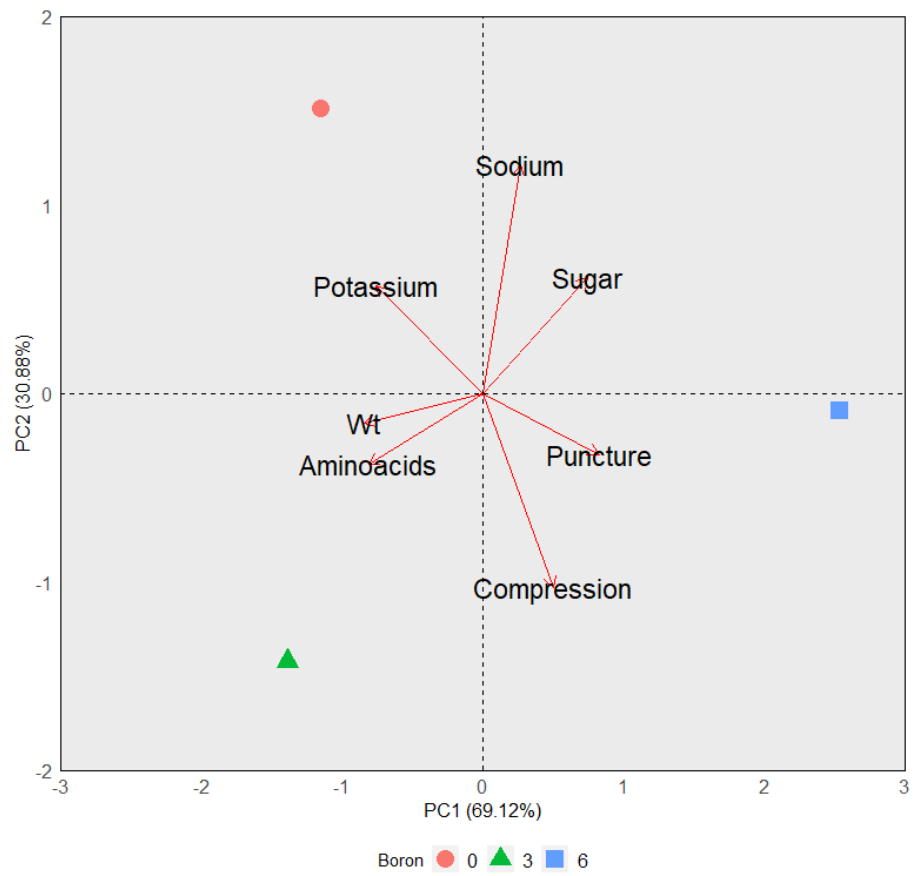


Figure 5-22: Principal component analysis for impurities (sodium, potassium, and α -amino N), weight, textural properties (puncture and compression) and sugar content.

5.5 Discussion

5.5.1 Calcium experiment

5.5.1.1 Soil calcium

The sugar beet threshold value for Ca content in the soil is about 700 mg L⁻¹ (Draycott and Christenson, 2003). Soil analysis for tMAF show that Ca content for 2021 and 2022 were 2181 and 1600 mg L⁻¹, respectively. According to AHDB, (2019), such Ca concentrations are excessive for sugar beet production. This suggests that apart from supplementation of Ca through foliar application, the plants also had a chance to absorb residual Ca from the soil. However, it must be noted that the primary cause of Ca deficiency in sugar beet is not usually related to supply from the soil, but rather to uptake, translocation and utilisation in the plant. Barber (1995) showed that Ca moves to the root surface by mass flow which is influenced by the rate of transpiration which is generally affected by seasonal factors like rainfall and temperature.

5.5.1.2 Root tissue damage susceptibility

Our study suggests that an application of foliar Ca reduces root tissue surface damage. This was more marked in 2021 when the site experienced well-distributed rainfall. Ulrich and Mostafa (1976) reported that Ca be present in optimum quantity for sugar beet to maintain plasma membrane integrity and when deficient leads to root vascular tissues browning and weak tissues. Our findings indicate that 5 L ha⁻¹ Ca reduced surface damage in 2021 suggesting Ca sufficiency in the roots. Increasing Ca from 5 to 10 L ha⁻¹ during the 2021 season did not have a significant decrease on surface damage suggesting that the nutrient has a point of diminishing return. This indicates that depending on the season, an application of 5 L ha⁻¹ Ca at Morley Farm suffices to a significant reduction in surface damage and increasing the rate to 10 L ha⁻¹ is a wastage. Ca's contribution to the reduction in root tissue surface damage was not robust in 2022 when the experimental site experienced severe drought. As earlier discussed, drought is associated with low transpiration for beet roots which decreases mass flow to the root surface of soil water containing soluble nutrients hence reducing Ca uptake and translocation (Hosseini et al., 2019). One explanation for the reduced response to Ca in 2022 could be low uptake from the available soil Ca. However, further Ca glasshouse studies on drought may be required to affirm this assertion. In terms of seasons, the study suggests that

surface damage was reduced in a season that was associated with drought (2022). This could be explained by lighter roots which were experiencing lower kinetic impact when cleaning in the rotating drum.

Root tip diameter after damage did not respond to Ca application regardless of seasonal differences. Roots' resilience to tip damage is a function of cross-sectional forces offered by cambium rings and tissues and is mainly controlled by compressive strength (Kleuker and Hoffmann, 2022, 2021; Nause et al., 2020). This suggests that the failure of Ca to influence root tip diameter after damage was due to its non-significant contribution to compression resistance. These results support the findings by Hoffmann and Schnepel, (2016) who reported that root tip tissue damage is mainly a factor of the growing environment and genotypic differences. The difference in the growing environment created by sporadic rainfall distribution in 2022 manipulated morphological traits for the roots hence the variations in the root's response to root tip diameter after damage. In 2022, a season associated with drought, the storage roots were generally lighter with small bases hence resulting in low root tip diameter after damage.

5.5.1.3 Textural properties

The study shows that Ca supplementation at tMAF affected the ability of root tissues to resist puncture especially when drought was not severe. This was manifested by a significant increase in root resistance to puncture when a higher Ca rate (10 L ha⁻¹) was applied in 2021. The results also suggest that variety and season are the main driving forces for differences in textural properties where both varieties gain resistance to compression and lose puncture resistance in a drought season. Textural properties are highly influenced by the cell wall content (Kleuker and Hoffmann, 2022) but our results are contrary to Li et al., (2012) who discovered that breaking forces of peony plant tissues are positively correlated with the ratio of cell wall content after Ca application. On the contrary, (2010) reported that an additional supply of Ca does not affect the mechanical properties of mature carrot tissues. One explanation is that foliar supplementation did not influence the root's response to textural properties in the dry season because the nutrient might not have reached sufficiency levels due to low uptake from soil available Ca and reduced translocation

within the plant as a consequence of reduced water fluxes associated with drought conditions.

5.5.1.4 Root quality

Ca application in a dry season relatively increased sugar concentration in the roots. The results indicate that in a drought season, applying 5 L ha⁻¹ Ca is an appropriate measure for the enhancement of sugar content. On the contrary, Artyszak et al., (2016) found no differences in sugar levels when foliar Ca was applied to sugar beet. Unlike their study which was done under optimum rainfall distribution, our study was implemented in two distinct seasons where one (2022) was characterised by severe drought. Hosseini et al., (2019) attributed sugar increase in roots to Ca's ability in inducing sucrose transporters (BvSUC3 and BvTST1) which support the loading of more sucrose into roots. The effect of Ca on sugar concentration in 2022 can be explained by an interaction between season and Ca whereby in a dry season, low Ca uptake in the plants that did not receive Ca affected the loading of sugar in the phloem cells. Ca's failure to influence impurities when varying rates were applied implies that soils at tMAF do not require foliar supplementation to influence the accumulation of sodium, potassium and α -amino N in sugar beet roots. These results agree with those of Artyszak et al., (2016) who reported that foliar application of Ca and silicon had no significant effect on sugar beet root quality parameters including α -amino N, potassium and sodium.

Hoffmann (2010) reported increased impurities in drought-stressed sugar beet. Our results also indicate that sugar beet impurities respond differently to seasons with α -amino N increasing in a drought season and potassium increasing in a non-drought season. This suggests that an increase in either of the two impurities due to seasonal differences results in a decrease in the other. Farley and Draycott (1975) found similar patterns where an increase of sodium and potassium root impurities due to fertilisation proportionately decreased α -amino N impurities. Increased concentration of impurities under drought stress is due to the accumulation of non-utilised metabolites that result from limited growth (Bloch et al., 2006). The effects of interaction between variety and season on sodium accumulation in root tissues suggest that varieties respond differently to this impurity, especially in a drought season.

5.5.2 Boron experiment

Studies have reported no loss in top, root yield or sugar when soil B exceeds 0.50 mg L⁻¹ (Smilde, 1970). Furstenfeld and Burcky (2000) recommended that B should not be applied in soils with greater than 0.95 mg L⁻¹. Soils at tMAF had greater than 0.95 mg L⁻¹ indicating sufficiency. However, as Draycott and Christenson, (2003) indicated that drought exacerbates B deficiency in sugar beet due to decreased flow of soil solution to the root. This necessitated a B foliar application since the experimental site was characterised by drought in 2022.

A significant increase in tissue compression especially at 6 L ha⁻¹ confirms that B is of agronomic importance in root tissue strength. However, the micronutrient did not influence most variables including puncture resistance, yield, shear resistance, sodium, potassium, sugar content and α -amino N. These findings are contrary to the literature. For example, in carrots, cross-linking of pectin has been reported to have a greater influence on mature tissue's mechanical properties when B was supplied during plant growth (Singh et al., 2010). B application has been shown to not only substantially increase sugar levels (Bonilla et al., 1980) and their quantitative distribution patterns (Pommerrenig et al., 2019) but also increase both α -amino N and K impurities (Zewail et al., 2020) in sugar beet. However, unlike our study which was done in the field where the soil also acted as the source of B, the above studies were done in glasshouses where feeding solutions were the only source. Therefore, our findings suggest that B levels (**Table 5-1**) and uptake by the plants were optimum at the experimental site and hence could not influence variables collected in this study. Despite not being statistically significant, PCA analysis results suggest that when B was not applied, there was a relative increase in sodium and potassium impurities and a 6 L ha⁻¹ relatively increased sugar content and resistance to puncture.

5.6 Conclusion

The study revealed that under field conditions, a 5 L ha⁻¹ Ca application enhances root tissue textural properties which in turn reduces tissue damage on the surface of the roots, especially in a season with adequate rainfall. In a drought season, our study also confirms findings by other researchers that Ca application helps to increase sugar levels. However, Ca does not influence the accumulation of impurities and damage of root tip tissues. Ca's efficacy in sugar beet production is dependent on the season. Under field conditions, a 6 L ha⁻¹ B application only enhances the root's ability to resist compression. However, it does not improve on other traits implying that B absorbed from the soil was enough for plant growth and production. In conclusion, our study suggests that Ca is more critical when optimising sugar beet tissue strength than B most especially in seasons with adequate rainfall.

Chapter 6: General discussion and conclusions

6.1 Variety and root tissue damage susceptibility and resilience

Our findings in **Chapter 3** support the observation that sugar beet root's ability to resist tip damage is dependent on the variety (Hoffmann and Schnepel, 2016; Kenter et al., 2006a). Sabatina and Daphna were identified as the most vulnerable and resilient to root tip damage, respectively. We also observed that while root tip diameter after damage was statistically different among varieties used in this study, surface damage was not statistically different suggesting that the underlying physiological basis of these two traits are different. This was contrary to the literature (Hoffmann et al., 2018a, 2018b) which reported significant differences among varieties for both traits. However, apart from reporting significant differences in surface damage, the cited literature also reported significant differences in weight. Therefore, one explanation is that variety's failure to influence surface damage was due to relatively uniform weight observed across varieties used in this study.

Our results indicate that Fotheringhay produced roots with relatively longer root tip diameter after damage and surface damage, especially in seasons with adequate rainfall. We attribute this to the higher average weight per root observed at this site which may have increased the kinetic impact (Akeson and Stout, 1978) when cleaning in the rotating drum. Seasonal effects on variety's response to tissue damage were also observed with lighter roots produced in drought seasons registering less tissue damage. This supports earlier observation by Pidgeon et al., (2001) that roots produced in a drought season are associated with less damage when exposed to an external force and that yield can be reduced by over 40 %.

Our results also suggest that vulnerable varieties damage their tissues consistently, especially at wet sites. This implies that growing vulnerable varieties in sites with heavy soils that hold water for a long time should be accompanied by good storage practices or direct loading to the factory. In general, our results mean that there is a possibility to reduce root tip and surface damage during post-harvest handling through utilisation of resilient varieties in sites with lighter soils that allow water to drain freely.

6.2 Root morphology and variety resilience to damage

Hoffmann (2017) highlighted the importance of root morphology and concluded that morphologically, root yield is correlated to width. However, there was no explanation of how morphological variables influence varieties resilience to damage. Thus, our study is the first to explore root morphology's impact on tissue damage. Our PCA results and cluster analysis in **chapter 3** morphologically characterised varieties in this study into two categories. The first category usually has varieties that are small and long with either weak or strong textural properties and the other one comprises of varieties that are wide and short with weak textural properties. Our results contradict Hoffmann et al., (2018a) and Hoffmann and Schnepel, (2016) who linked damage to weak tissues only without considering morphological traits. Our findings show that while surface damage has no relationship with morphological traits, root tip diameter after damage does not only depend on tissue strength but is also correlated with morphological traits like root tip diameter before damage, root weight per beet, length and width. However, we observed that the impact of morphology on root tip diameter after damage is more pronounced in varieties with weak textural properties. For example, root tip diameter after damage for Sabatina and Daphna was statistically different despite both having weak textural properties. The difference could be explained by morphology whereby Daphna was significantly smaller and longer than Sabatina.

6.3 Textural properties and root resilience to tissue damage

Kleuker and Hoffmann, (2019) defined threshold values for strong puncture, shear and compression resistance as 5.98, 3.62 and 2.1 MPa, respectively. Based on these thresholds, our results mean that varieties used in this study possess strong textural properties apart from Daphna and Sabatina. Tissue strength is a factor of water-insoluble cell wall components (Kleuker and Hoffmann, 2022, 2021; Nause et al., 2020). We, therefore, suggest that Daphna and Sabatina have lower dry matter and future studies could consider measuring this trait. Our findings on puncture and compression confirm an earlier observation by Kleuker and Hoffmann (2019) that the root's ability to resist compression and puncture increases as you move from the central to peripheral zone and from the base to the crown, respectively. This explains why the tips are prone to damage but also suggests that the root's ability to resist surface damage or cracking is mainly dependent on the peripheral tissues.

High positive correlations between compression and puncture resistance but also puncture and shear resistance suggest that either of the three is enough when predicting variety's tissue strength. English et al., (2022) measured sugar beet root's resistance to puncture and found a high correlation between puncture values measures in the field using a handheld penetrometer and those from a texture analyser in the laboratory implying that puncture resistance can practically be used as a proxy to predict tissue strength.

Our results also show that at Fotheringhay which was a wet site compared to Bracebridge, surface damage was negatively correlated to resistance to puncture and root tip damage was negatively correlated with compression resistance. Correlation between resistance to compression and root tip damage would be expected since root tip damage involves inner tissues including parenchyma cells whose strength is measured through compression. Surface damage mainly involve root periderm tissues and the puncture test was measured within 5 mm of the periderm, hence a negative relationship between surface damage and resistance to puncture can be explained by periderm tissues contribution to puncture resistance. These findings agree with Kleuker and Hoffmann, (2022) who reported high negative correlations between root tip damage and compression resistance in wet ($R^2=0.41$) rather than dry ($R^2=0.20$) environments. Our results therefore suggest that root tip and surface damage are influenced differently whereby root tip diameter after damage is more correlated to compression resistance and surface damage is more correlated to puncture resistance.

6.4 Harvesting time influences textural properties and root resilience to tissue damage.

As observed by Van Swaaij et al., (2003) our study also suggests that proper scheduling of harvesting may help to reduce both root and surface damage. However, the two are influenced differently where root tip damage is dependent on the actual harvesting time while surface damage is positively correlated to moisture conditions prior to harvesting. The effect of soil moisture prior to harvesting on surface damage was studied later in **Chapter 4** as the correlation used rainfall as a proxy. We attribute the increase in root tip damage when harvested one month later

to continued secondary growth which led to an increase in width and root tip diameter before damage. Our findings also agree with Cakmakci and Oral, (2002) who reported a 13 % increase in root weight when a one-month harvesting delay was imposed. Delayed harvesting did not affect the root tissue's ability to resist compression, but it significantly reduced their ability to resist puncture. We observed that harvesting delay did not influence compression resistance which is in agreement with Nause et al., (2020) and Bentini et al., (2005) who reported that textural properties remained stable after a three months delay in harvest. However, our results on resistance to puncture do not agree with this literature because they suggest that compared to inner tissues from the parenchyma zone, tissues forming the skin of sugar beet roots become less resistant to puncture when harvest was delayed, especially in wet soils.

6.5 Water status at harvest and sugar beet's tissue damage susceptibility

SPAD values in **chapter 4** indicate that a continuous dry spell affects sugar beet biological processes when extended for four consecutive weeks. This was confirmed as SPAD values for irrigated and non-irrigated plants were constant up to the fourth week when those of non-irrigated plants significantly dropped. SPAD is an indicative value for chlorophyll content and higher correlations have been reported between the two variables (Jiang et al., 2017; León et al., 2007). Our findings also support an observation by Wang et al., (2021) that irrigated beet had higher SPAD values than non-irrigated. This means irrigated roots were actively growing and explains why the roots from irrigated pots were heavier and wider compared to non-irrigated roots.

Our study also reveals that RWC increases with an increase in soil water content (Herppich et al., 2001) prior to harvesting, hence affecting textural properties (Robbins and Dinneney, 2015). However, this was contrary to Nause et al., (2020) who reported that textural properties remain stable throughout the growing period. However, our treatments involved moisture manipulation while Nause et al., (2020) just sampled fields at different times without quantifying their moisture conditions. Negative correlations between RWC and textural properties support the observation by Feng et al., (2016) that high water content promotes root tissue frailness through increased exertion of force by cytoplasm on cell walls. We also find our results to

agree with Lockley et al., (2021) who reported that an increase in radish hypocotyl water content is negatively correlated with compression and puncture resistance.

Maintaining water status at field capacity for seven weeks not only weakens root tissues but also reduces dry matter. High water status prior to harvesting also increases the width and weight of the roots. Weight and textural properties are positively correlated with both root tip and surface damage (Kenter et al., 2006a; Kleuker and Hoffmann, 2022; Van Swaij et al., 2003) and our results also agree with Hoffmann (2018) who highlighted that soil conditions alter the composition of the root's skin rigidity and tissue strength, hence promote damage. Therefore, our results suggest that prolonged high-water status prior to harvesting is a precursor for root tissue damage. We, therefore, suggest that minimisation of damage requires a holistic approach where tolerant varieties must be accompanied by proper agronomic practices such as harvesting when the soil is relatively dry.

6.6 Root temperature at harvest affects sugar beet tissue damage susceptibility

In **Chapter 4** our study demonstrates that autumn and winter temperatures at harvest do not influence the root's resilience to damage. Despite evidence on temperature's effects on tissue's resilience to damage (Guihur et al., 2022; Herppich et al., 2005, 2002), our findings reveal that temperatures used in this study do not contribute to tissue's puncture and compression resistance. This practically means that if sugar beet is harvested when temperatures are within 3 -10 °C, growers will not experience more damage due to a change in temperature. In other crops like carrots, root tissues have been reported to start losing resistance to damage when temperatures exceed 20 °C (Herppich et al., 2002). Hence, the temperature's failure to contribute to tissue damage can be explained by the low-temperature range (3 - 10 °C) used in this study. However, this study did not go beyond this range to represent harvesting campaign temperatures in the UK.

6.7 Interaction between temperature and water status affects sugar beet's tissue damage susceptibility

Our findings from this study do not show a significant interaction between harvesting temperature and water status on the susceptibility of roots to damage, puncture and morphological traits. This is contrary to Herppich et al., (2002) who reported that at a lower temperature and high turgor, cutting forces for carrots increased. Interaction

between water status and temperature only influenced root tissue resistance to compression whereas a lower temperature in non-irrigated roots enhanced the tissue resistance to compression. However, when temperatures were increased to 10°C, resistance to compression was not statistically significant in both irrigated and non-irrigated plants.

Low RWC strengthens roots resistance to compression by reducing the amount of force exerted on the cell wall by the cytoplasm (Feng et al., 2016). Hence, one of the explanations for an increase in resistance to compression for root tissues that were kept dry prior to harvesting and at a higher temperature could be reduced RWC content observed in tissues extracted from such roots. Despite interaction affecting compression, other variables were not significantly affected implying that temperatures used in this study were not high enough to dry the tissues or low enough to maintain water or freeze the tissues.

6.8 Foliar application of Ca affects sugar beet response to root damage, quality and textural properties

Soils at tMAF had Ca content of above 1600 mg L⁻¹ in both seasons. According to AHDB (2019), such Ca concentrations are excessive for sugar beet production because the crop performs well when soil Ca content is about 700 mg L⁻¹. However, Ca deficiency in sugar beet is related to uptake, translocation and utilisation in the plant. Our findings in **Chapter 5** indicate that Ca application reduced surface damage and increased puncture. While surface damage was reduced when 5 L ha⁻¹ Ca was applied, it was relatively increased when a 10 L ha⁻¹ was applied. This means that 5 L ha⁻¹ Ca was enough to enhance tissue's resistance to surface damage and increasing the rate to 10 L ha⁻¹ is of no benefit.

Surface damage mainly occurs in periderm tissues and the puncture test is performed on the periderm as well. Plant periderm tissue (phellem, phellogen and phelloderm) strength is determined by an apparent increase in pectin which is held together by Ca (Sabba and Lulai, 2002). Hence an enhancement in resistance to puncture and a reduction in surface damage when Ca is sufficient is expected.

The reduction in surface damage when Ca was applied was more marked in 2021 than in 2022. As explained by Hosseini et al., (2019) that inadequate rains affect

residual Ca absorption through reduced transpiration rate, one explanation for Ca's effectiveness in 2021 would be adequate distribution of rain during the growing season which may have improved uptake of residual Ca from the soil which when combined with the foliar supplement improved Ca content in the tissues. However, we recommend further Ca glasshouse studies on drought to confirm this observation. Our study also demonstrates that Ca does not influence root tip damage. Ca did not affect the root's resistance to compression which was in early observed (**in chapter 3**) to be linked to root tip damage. We therefore attribute Ca's failure to influence root tip damage to its inability to increase resistance to compression.

Ca application in a dry season increased sugar concentration in the roots. The results indicate that in a drought season, applying 5 L ha⁻¹ Ca is an appropriate measure for the enhancement of sugar content. Hosseini et al., (2019) attributed sugar increase in roots to Ca's ability in inducing sucrose loading into roots. Ca's failure to influence impurities when varying rates were applied implies that plants grown at tMAF did not require foliar supplementation to influence the accumulation of Na, K and α -amino N in sugar beet roots. These results agree with those of Artyszak et al., (2016) who reported that foliar application of Ca and Si had no significant effect on sugar beet root quality parameters including α -amino N, K and Na.

6.9 A foliar application of B affects sugar beet response to root damage, quality and textural properties

Soils at tMAF had respective B concentrations of 1.5 and 1.2 mg L⁻¹ during the 2021 and 2022 seasons which are both above the minimum requirement (0.95 mg L⁻¹) (Fürstenfeld and Bürcky, 2000). However, foliar B supplementation have been necessitated due to deficiencies which emanate from uptake and transportation challenges by the plants. Our findings in **chapter 5** indicate that a 6 L ha⁻¹ B application enhanced root tissue resilience to compression. However, B did not influence puncture, yield, shear, Na, K, sugar content and α -amino N. These findings are contrary to the literature where greater influence on mature tissues' mechanical properties when supplied during plant growth have been reported (Singh et al., 2010). B application has been shown to increase sugar levels (Bonilla et al., 1980), distribution patterns (Pommerrrenig et al., 2019) and impurities (Zewail et al., 2020)

in sugar beet. Hence, our findings suggest that residual B uptake by the plants was optimum at the experimental site and hence foliar supplementation could not influence variables collected in this study.

6.10 General conclusions

This study addressed the research hypotheses listed on section 2.9 as follows.

- 1) Variety affects sugar beet root tissue damage susceptibility and resilience
- 2) Root morphology influences the variety's resilience to damage
- 3) Textural properties affect sugar beet root resilience to tissue damage
- 4) Harvesting time influences textural properties and the root's resilience to tissue damage.
- 5) Water status at harvest affects sugar beet's tissue damage susceptibility
- 6) Root temperature at harvest affects sugar beet's tissue damage susceptibility
- 7) Interaction between temp and water status affects sugar beet's tissue damage susceptibility
- 8) Foliar B application affects sugar beet response to root damage, textural properties and impurities
- 9) Foliar application of Ca affects sugar beet response to root damage, textural properties and impurities

After a thorough test of these hypotheses, we finally draw the following conclusions:

- 1) Variety affects sugar beet root tip damage with Sabatina and BTS3325 being the most vulnerable and resilient, respectively.
- 2) Surface damage is affected by site and not variety with the roots being more resilient in dry sites
- 3) Resistance to puncture and compression is influenced by variety
- 4) Root morphology influences resilience to root tip damage in weak varieties
- 5) The correlation between root tip damage and morphological factors is stronger than that of root tip damage and textural properties
- 6) Depending on site, compression and puncture negatively correlate to root tip and surface damage, respectively.
- 7) Late harvesting increases root tip damage but also reduces root resistance to puncture.
- 8) High water status prior to harvesting increases tissue frailness, hence increasing both root tip and surface damage
- 9) Temperature at harvest does not affect tissue strength and tissue damage
- 10) 5 L ha⁻¹ foliar application of Ca reduces surface damage especially when rainfall is adequately distributed

- 11) Foliar Ca application does not influence root responses to root tip damage
- 12) Foliar Ca application improves root tissue resistance to puncture especially in a non-drought season
- 13) Foliar Ca application does not improve impurities and root tissue resistance to compression
- 14) Foliar B application only improves root tissue resistance to compression

6.11 Limitations and future studies

This research managed to identify various traits and agronomic practices that would be optimised to minimise tissue damage in sugar beet production processes. However, there is a need for continuous assessment of varieties since the UK's Recommended List of varieties changes every year. In this research, we only evaluated eight varieties out of 22 at two sites. All varieties need to be evaluated in every sugar beet producing zones in the UK. Future studies must also incorporate molecular work to find out the genetics of vulnerable varieties. On Ca and B experiment, our study was only conducted in the fields where residue soil Ca and B might have countered some of the results. Future studies must consider a glasshouse experiment where the crops grown in Ca and B free media can be compared to treated ones so that results can be only ascribed to nutritional differences.

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