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### Keywords:

root tip damage; surface damage; tissue puncture; tissue compression

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# Water status but not mild and cold temperatures affect harvest damage susceptibility and tissue integrity of sugar beet (*Beta vulgaris*) roots

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## Abstract

Sugar beet root damage at harvest promotes sucrose losses of circa 0.1 – 0.4 % day<sup>-1</sup> in storage. However, root response to environmental stresses at harvest and their consequential rates of damage are not known. We investigated the effects of temperature and water stress at harvest on root resilience to damage and tissue strength. Water (irrigated to field capacity and non-irrigated) and temperature (cold and mild) treatments were imposed on physiologically mature sugar beet plants for seven weeks prior to and for three days after harvesting, respectively. Water status at harvest significantly affected relative water content (RWC) ( $p < 0.001$ ), root weight ( $p < 0.001$ ) and root width ( $p < 0.001$ ). RWC was positively correlated to surface damage ( $R^2 = 0.43$ ,  $p = 0.02$ ), root tip damage ( $R^2 = 0.42$ ,  $p = 0.03$ ), tissue compression ( $R^2 = 0.41$ ,  $p = 0.05$ ) and tissue puncture ( $R^2 = 0.46$ ,  $p = 0.01$ ). Tissue damage was not affected by root tissue temperature of 4 °C compared to 12 °C. We conclude that sugar beet damage at harvest is not influenced by root temperatures over the range commonly observed in the UK and temperate production areas. However, higher water status at harvest, such as would be observed in a wet season, increases root tip and surface damage. These findings will help to inform optimum harvesting conditions to minimize sugar loss from the sugar beet crop.

## Introduction

Sugar beet root tissue strength is influenced by several factors including biochemical constituents, water content, cellular components including cell wall composition and temperature (Nause *et al.* 2020). Weaker root tissues are more prone to harvest damage which promotes sugar losses when in storage (Kleuker and Hoffmann, 2022). Cellular water content influences tissue strength via the regulation of turgidity which is fundamental in maintaining structure and rigidity (Ali *et al.* 2023). Poor water status maintenance can compromise tissue structure and cell membrane integrity (Gorzeleny and Puchalski, 2000; Lewicki and Jakubczyk, 2004). Root crops with a high proportion of parenchyma cells like sugar beet are more brittle and at high turgidity, high pressure is exerted on cell walls reducing the amount of force required to induce fracture (McGarry, 1995; Smittle *et al.* 1974). Studies in carrots (*Daucus carota* subsp. *sativus*) 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 (*Raphanus sativus*) 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. Schäfer *et al.* (Schäfer *et al.*, 2020) observed less compressive strength in sugar beet samples that had a higher water content.

High tissue strength has been linked with low temperatures mostly in vegetable crops. Herppich *et al.* (2002) reported that in temperature ranges of 0 – 45 °C, forces required to cut a cross-section of carrot roots are dependent on root tissue 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 increased 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.

Water content and therefore tissue integrity in sugar beet could be influenced by irrigation and temperature. However, the impact of high soil moisture content and tissue temperature on tissue water content and strength of commercial sugar beet prior to harvesting have not been thoroughly investigated. Despite evidence of water and temperature's ability to manipulate root tissue mechanical properties and the resilience to damage in other crops other than sugar beet, growers harvest sugar beet under varying moisture and temperature conditions. This is due to differences in weather patterns, soil types and delayed harvesting which extends into winter

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**Table 1.** Climatic data prior to moving into the polytunnel at Harper Adams University (HAU)

Experiment	Duration	Rainfall (mm)	Rain days	Temperature range (°C)	Average temperature (°C)
1	Planting to moving into polytunnel - HAU	438	101	4 to 24	13
2	Planting to transplanting - Bracebridge	127	41	2 to 21	12
2	Transplanting to moving into polytunnel - HAU	150	23	5 to 19	14
3	Planting to transplanting - Bracebridge	55	30	3 to 24	13
3	Transplanting to moving into polytunnel - HAU	132	21	2 to 27	16

(Gabarron-Galeote *et al.* 2019; Okom *et al.* 2017). Growers in the UK 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 existing in sugar beet production zones within the UK at harvest could be a precursor for root tissue frailness and damage. This is likely to be exacerbated by increased weather variability as a consequence of climate change (Beacham *et al.* 2018). However, most research in sugar beet has focused on the contribution of water availability and temperature to economic yield during the crop growing period and post-harvest storage (Fitters *et al.* 2017, 2018, 2022; Fugate *et al.* 2016; Gummerson, 1986; Haagenson *et al.* 2008; Klotz and Finger, 2004). There is little information on whether water and temperature status of sugar beet root tissues is correlated with susceptibility to damage during the harvesting process.

This current study hypothesised that 1) water status at harvest affects sugar beet's tissue damage susceptibility, 2) temperature at harvest affects sugar beet's tissue damage susceptibility and 3) interaction between temperature and water status affects sugar beet's tissue damage susceptibility.

## Materials and methods

### Experimental design

Three experiments were conducted, one per year, from 2020–2022. Experiment 1 (2020) was grown from seed in large boxes at Harper Adams University (HAU), Shropshire (52°78' N, 2°43' W); Experiments 2 (2021) and 3 (2022) utilized plants that were initially field-grown at the British Beet Research Organisation (BBRO), Bracebridge, Lincolnshire (53°09'N 0°28'W) before transplanting into plastic buckets, transporting to HAU and growing on. The alternative research design for Experiments 2 and 3 was adopted owing to the COVID-19 lock down where the university was closed and work resumed after the sugar beet planting season had already begun. Hence transplanted plants were opted and the use of plastic buckets which were easier to transport than large box. Climatic data for HAU and Bracebridge were obtained from the university's weather station (Table 1) and BBRO's weather station, respectively.

### Experiment 1

Experiment 1 was planted on 15/04/2020 in wooden potato boxes of 1 m wide, 1.2 m long and 1 m high, after Fitters *et al.* (2018). Before filling, the boxes were lined with woven plastic sheeting (Permatex Premium, Tildenet, Bristol, UK) to reduce loss of water from the sides. The boxes were filled with sandy clay loam, (Landscape20, Topsoil, Cambridgeshire, UK). The bottom of the boxes had some spaces between the woods to allow drainage of

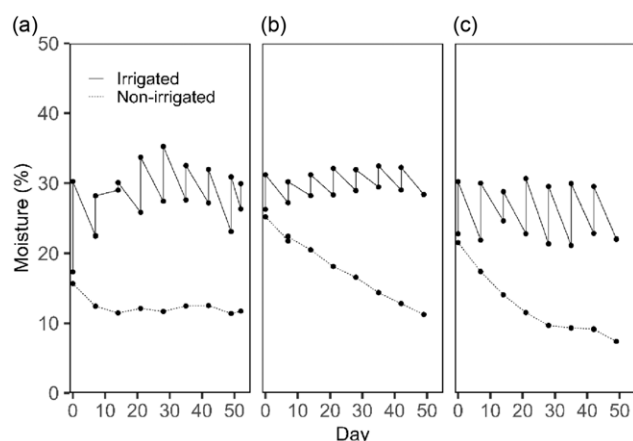
water. Soils used in 2020 were the washed-away particles from the sugar beet delivered by growers at the Wissington sugar factory. The soil was a sandy loam and was analysed following Ministry of Agriculture, Fisheries and Food (1986). Chemical and physical properties analysis for the soil are as described on Table S1. The sugar beet cultivar Sabatina KWS was studied. Our preliminary work categorized this variety as relatively weak in textural properties, vulnerable to damage and large in size (data not presented). Seed was planted 10 cm from the edges, one seed was planted per station in three rows per box using an intra and inter-row spacing of 16 and 50 cm, respectively. This resulted in 7 plants per row giving a total of 21 plants per box.

Experiment 1 was laid out in a completely randomized design with three replications in a 2 × 2 factorial design consisting of irrigation (irrigated to field capacity and non-irrigated) and temperature (3 and 10°C which were imposed after harvesting) as factors. The boxes were initially laid out in a field at HAU farm until moving into a large polytunnel for irrigation treatments to be applied with the same experimental layout maintained in each environment.

The plants received standard recommended agronomic inputs (Table S2) and were hand weeded. Prior to entering the polytunnel, soil moisture readings were taken every two weeks using a Time domain reflectometry (TDR) 100 soil moisture metre (Spectrum Technologies, Inc, Aurora, United States) to a depth of 20 cm and, when necessary, the plots were irrigated to avoid wilting.

### Experiments 2 & 3

Plants (cv. Sabatina KWS) were drilled at two BBRO field trials in Bracebridge, Lincolnshire (2021 - 52°78' N, 2°43' W; 2022 - 52°78' N, 2°43' W). The field used in 2021 was characterized by sandy loam soils while in 2022 the field was dominated by clay loam soils (Table S1). The seeds were drilled on three, 100 m long rows at Bracebridge on 01/04/2021 and 02/04/2022 for experiment 2 and 3, respectively. Each row was spaced 50 cm apart and the seeds were drilled at an intra-row spacing of 16 cm with the aim of an established plant density of no less than 100 000 plants ha<sup>-1</sup>. Approximately four months after drilling (26/07/2021 and 08/07/2022), 20 plastic buckets of 40 and 50 cm in height and diameter respectively, were filled with soils from the same plot. Two plants were gently uprooted from the middle row and transplanted into each bucket at 10 cm from each other. Upon arrival at HAU, the buckets were located outside the polytunnel for 90 days, to grow on to physiological maturity and these are part of the days that the crop was grown outside the polytunnel. The 90 days timing was after (Scott and Bremner, 1966) who observed a reduced growth rate in sugar beet 90 days after transplanting and concluded that the roots had reached physiological maturity. Prior to entering the polytunnel, moisture readings were taken every two weeks using a TDR 100 soil moisture metre to a depth of 20 cm and when



**Figure 1.** Soil moisture content for irrigated and non-irrigated sugar beet crop while in the polytunnel in 2020 (a), 2021 (b) and 2022 (c). For 2020, day 0 = 29/10/2020, for 2021, day 0 = 26/10/2021 and for 2022 day 0 = 08/10/2022.

necessary, the plots were irrigated to avoid wilting. The experiment was laid out in a completely randomized design with five replications. Treatments were arranged in a  $2 \times 2$  factorial consisting of irrigation and harvesting temperature as factors.

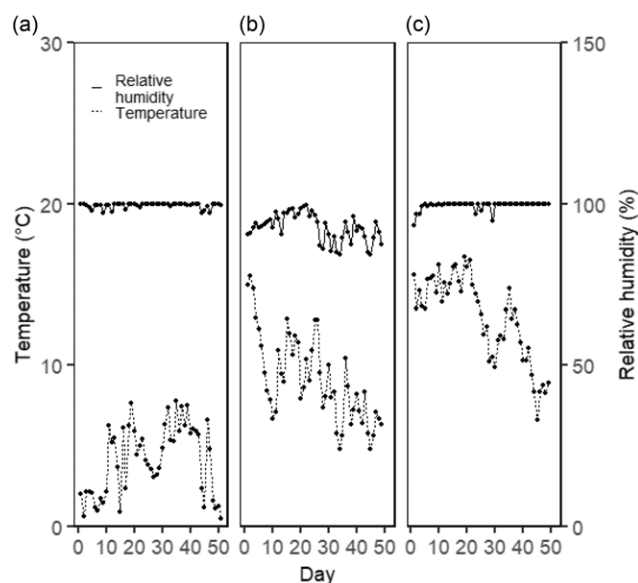
### Water treatments

#### Experiment 1

The plants were moved into a polytunnel on the 29/10/2020 for the imposition of water treatments. Irrigation was applied at two levels: maintaining the soil moisture at field capacity (30%) and without irrigation for 52 days. The final soil moisture content for non-irrigated plants in experiment 1 was 12 %. The roots were harvested on 20/12/2020. Soil moisture in the irrigated treatment was returned to field capacity on a weekly basis in the well-watered boxes. Before each irrigation, soil moisture content (Figure 1a) was measured in every box to a depth of 20 cm using a TDR 100 soil moisture metre. 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 deficit volume was then measured using measuring cylinders and the water was manually irrigated into the boxes. The temperature and relative humidity of the polytunnel were measured using data loggers (Tinytag plus 2 GP-4505, Gemini Data Loggers, UK) (Figure 2a).

#### Experiments 2 & 3

The buckets were moved into a polytunnel on 26/10/2021 and 08/10/2022 for experiment 2 and 3, respectively. Irrigation treatments were applied as described for experiment 1 for 49 days. The final soil moisture content for non-irrigated plants was 12 and 7 % for experiment 2 and 3, respectively. The roots were harvested on 14/12/2021 and on 26/11/2022 for experiments 2 and 3, respectively. Maintenance of soil moisture content (Figure 1b & c) for well-watered and measurement of temperature and relative humidity (Figure 2b & c) of the polytunnel were performed as described in experiment 1. Compared to experiment 1 and 3, experiment 2 had a higher rate of drying and this is attributed to the sandy soils used in experiment 2.



**Figure 2.** Daily temperature and relative humidity in the polytunnel in 2020 (a), 2021 (b) and 2022 (c). For 2020, day 0 = 29/10/2020, for 2021 day 0 = 26/10/2021 and for 2022 day 0 = 08/10/2022.

### Temperature treatments

In both experiments, the roots were immediately washed in the polytunnel using tap water (temperature not recorded) before being transported to a cold room for imposition of temperature treatments. At the time of washing the roots, respective polytunnel temperatures for experiment 1, 2 and 3 were 0.5, 6 and 9 °C. The same treatments were applied for all three experiments where roots were held at either 3 °C or 10 °C for three days. The three days between harvesting and testing were important as we wanted the roots to have ample time for tissue temperature manipulation.

### Harvesting

The non-irrigated plants had most of its leaves wilting at harvest compared to the irrigated plants. Harvesting was done by gently uprooting the roots from the soil using hands. Full details on harvesting, transplanting and moving into the polytunnel were as detailed in Table S3.

### Root assessments

Roots assessments were, in order: morphology, damage assay, textural analysis (Kleuker and Hoffmann, 2019) and relative water content (RWC). When conducting textural tests, RWC and damage tests, only tissues from the true root which excludes the crown were considered. In experiment 1, five roots from the middle row were used for the damage assay and two were used for texture analysis and relative water content. In experiments 2 and 3, two roots were used for all the tests. This approach was based on Kleuker and Hoffmann (2019), who after conducting textural analysis on sugar beet roots concluded that a reduction on the number of roots from thirty to two per plot provided stable results.

### Morphological assessment

Root length was measured from the top to the tip of the root while root width was measured on the widest axis. Root tip diameter was measured at the widest circumference of the tips. Root weight was





**Figure 3.** The rotating drum used to damage the sugar beet roots (a), and sugar beet roots after damage (b).

also recorded using a digital scale. Root length and diameter were both measured using a ruler.

### Damage assay

A root damage assay (after Van Swaaij *et al.* 2003; Kenter *et al.* 2006a and Hoffmann and Schnepel, 2016) was utilized to impose controlled damage in a rotating drum (Figure 3a). The rotating drum had a diameter of 0.6 m and length of 1.0 m and was set at a speed of 45 rpm. The drum was made of 30 mm × 30 mm metal bars spaced at 6 cm apart and the sides were covered by a 2 mm metal plate (Van Swaaij *et al.* 2003). These settings and specifications were chosen after Van Swaaij *et al.* (2003) who reported an  $R^2$  of 0.94 with damage caused by machine harvesting in the field when such a rotating drum was used. The roots were then exposed for 15 seconds to dynamic stresses akin to those experienced during the cleaning step of the commercial harvest process using the rotating drum. After this simulated cleaning, weight and root tip diameter (Figure 3b) were measured. When measuring root tip diameter, the longest diameter was considered. Surface damage which resulted due to tissues being scraped from the surface of the root was scored visually as a percentage. Each sample was returned to the cold room immediately after the damage test.

### Texture analysis

Texture analysis was performed 24 hours after the damage test. Before each textural test, root tissue temperature was measured using a Thermo 20 thermometer (Electronic Temperature Instruments Ltd, UK). Resistance to tissue puncture was assessed according to Kleuker and Hoffmann (2019) where a TA.HD.plus texture analyser (Stable Micro Systems, Godalming, UK) fitted with a 500 kg load cell and a P/2 cylindrical probe at a crosshead speed of 60 mm minute<sup>-1</sup> was used to determine the force required to puncture the periderm of each root to a depth of 5 mm. Puncture resistance values were determined after Kleuker and Hoffmann (2019) who defined puncture resistance as the maximum force recorded between 0 to 0.5 mm. One puncture test was performed on the top, middle and tip of the roots and the average value was used as a representative for the whole root. The top, middle and tip puncturing positions are defined after Kleuker and Hoffmann (2019) as the root surface below the crown, between top and the tips and at the tip, respectively.

Resistance to compression was tested according to Kleuker and Hoffmann (2019). A 25 mm high cylindrical section was cut using a knife at the largest diameter and samples were taken using an 18 mm cork borer from the middle, edge and central part of the cross-section. The edge, the middle and the central parts are defined as the root tissues after the periderm, between the roots centre and the edge and the centre of the root, respectively. Samples from these three positions were further reduced in height consistently, to 20 mm using a knife. 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.

### Relative water content

RWC for root tissue was measured immediately after texture analysis using two roots per plot and following Fitters *et al.* (2018). Three cylindrical 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 length of 18 mm using a knife and weighed on a digital scale. The samples were then put in plastic containers, which were filled with sterile distilled water. The containers were covered and stored in a cold room at 5 °C for three days. The resulting turgid samples were weighed and then dried at 75 °C to a constant weight (dry weight). The constant weight was determined when the sample was registering the same weight for the last three consecutive days. RWC was calculated using the formula below:

$$RWC = \frac{(WW - DW)}{(TW - DW)} * 100 \quad (1)$$

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

### Data analysis

An analysis of variance (ANOVA) was performed using a linear mixed model in R version 4.2.2 within the *lmerTest* package (R Core Team, 2022). Before the analysis, each variable was tested for normality using the Shapiro-Wilk test and showed normal distribution of each dataset. Irrigation, year and temperature were fitted in the model as fixed factors while replication was fitted as a random factor. When analysing morphological traits and weight, temperature was dropped as a fixed factor in the model since a

**Table 2.** Effect of year on morphological characteristics and weight of sugar beet cv Sabatina KWS roots

Year	Width (cm)	Length (cm)	Tip diameter before damage (cm)	Weight (kg/root)
2020	14.0 <sup>a</sup> ± 0.28	24.3 <sup>a</sup> ± 0.79	0.78 ± 0.19	1.18 <sup>a</sup> ± 0.05
2021	12.6 <sup>b</sup> ± 0.21	17.2 <sup>c</sup> ± 0.39	0.99 ± 0.18	0.86 <sup>b</sup> ± 0.04
2022	11.9 <sup>b</sup> ± 0.22	20.1 <sup>b</sup> ± 0.68	1.07 ± 0.32	0.98 <sup>b</sup> ± 0.04
<i>p</i> -value	***	***	NS	***

\*\*\* denotes significant at  $p < 0.001$ , NS denotes not significant. The means ( $\pm$  SE) are drawn from three replicates in 2020 and five replicates in 2021 and 2022. Values with different superscripts within a variable are significantly different ( $p < 0.05$ ).

**Table 3.** Effects of irrigation on root phenotypic traits and weight of sugar beet cv Sabatina KWS roots

Irrigation	Width (cm)	Length (cm)	Tip diameter before damage (cm)	Weight (kg/root)
Irrigated	12.9 <sup>a</sup> ± 0.72	24.8 ± 0.94	0.81 ± 0.09	1.05 <sup>a</sup> ± 0.05
Non-irrigated	12.4 <sup>b</sup> ± 0.74	23.9 ± 1.33	0.74 ± 0.11	0.91 <sup>b</sup> ± 0.03
<i>p</i> -value	***	NS	NS	***

\*\*\* denotes significant at  $p < 0.001$ , NS denotes not significant. The means ( $\pm$  SE) are drawn from three replicates in 2020 and five replicates in 2021 and 2022. Values with different superscripts within a variable are significantly different ( $p < 0.05$ ).

three-day imposition of temperature treatment did not affect morphological features. A post hoc analysis was performed in *emmeans* package (Lenth *et al.* 2022) using the HSD Tukey test to compare treatment means at  $p \leq 0.05$  significance level. A Pearson's correlation was performed to check the relationship between some variables at a significance of 0.05. Graphing was done using the *ggplot2* (Wickham, 2009) R package. Treatments with different superscripts are statistically different from each other at  $p \leq 0.05$ .

## Results

### Root morphological traits and weight

There was a significant effect of year on root width ( $p < 0.001$ ), length ( $p < 0.001$ ) and weight ( $p < 0.001$ ) (Table 2) while irrigation significantly affected root width ( $p < 0.001$ ) and weight ( $p < 0.001$ ) (Table 3). In 2021, average root weight was similar to 2022. However, roots harvested in 2020 were heavier compared to those harvested in 2021 and 2022. Average root weight for 2020, 2021 and 2022 were 1.18, 0.86 and 0.98 kg, respectively. Non-irrigated roots were lighter and weighed an average of 0.91 kg/root while irrigated roots weighed 1.05 kg/root. Roots harvested in 2020 were significantly longer (24 cm) compared to those harvested in 2021 (17 cm) and 2022 (20 cm).

On average, roots harvested in 2020 were significantly 1 and 2 cm wider than roots harvested in 2021 and 2022, respectively. Root tip diameter before damage was not significantly affected by irrigation or year. The average tip diameter before damage for 2020 was 0.78 cm, for 2021 was 0.99 cm and for 2022 was 1.07 cm. The

**Table 4.** Effects of storage temperature, irrigation and year on sugar beet cv Sabatina KWS root tissue temperature ( $^{\circ}\text{C}$ ) at time of assessment

Storage temperature ( $^{\circ}\text{C}$ )			
Irrigation	Year	3	10
Irrigated	2020	4.06 ± 0.37	12.7 ± 0.37
	2021	4.78 ± 0.29	12.5 ± 0.29
	2022	4.44 ± 0.29	11.7 ± 0.29
Non-irrigated	2020	3.86 ± 0.37	12.2 ± 0.37
	2021	4.18 ± 0.29	12.2 ± 0.29
	2022	4.36 ± 0.32	12.2 ± 0.29
Mean		4.28 <sup>b</sup> ± 0.15	12.3 <sup>a</sup> ± 0.14
<i>p</i> -value			
Temperature***			
Year = NS			
Irrigation = NS			
Temperature × Year = NS			
Temperature × Irrigation = NS			
Year × Irrigation = NS			
Temperature × Year × Irrigation = NS			

\*\*\* Denotes significant at  $< 0.001$ , NS denotes not significant. The means ( $\pm$  SE) are drawn from three replicates in 2020 and five replicates in 2021 and 2022. Values with different superscripts within a variable are significantly different ( $p < 0.05$ ).

overall average root tip diameter before damage for irrigated roots was 0.99 cm and for the non-irrigated was 0.95 cm. The interaction between year and irrigation did not have an influence on any of the morphological traits as well as weight.

### Root tissue temperature and relative water content

Root tissue temperature (internal temperature) differed significantly following imposition of storage temperature treatment (Table 4). The average temperature for roots that were stored for three days at 3  $^{\circ}\text{C}$  and 10  $^{\circ}\text{C}$  was 4 and 12  $^{\circ}\text{C}$ , respectively. This difference between the storage temperature and the actual root temperature was expected due to sample handling when the roots were taken out of the cold rooms. Despite storage temperature affecting internal root tissue temperature, temperature on its own did not affect any of the measured variables (data not presented). However, temperature's interaction with irrigation affected roots response to compression resistance (Table 5) where roots that were irrigated had lower compression resistance than the non-irrigated when stored at 3  $^{\circ}\text{C}$ .

Root tissue RWC was significantly affected by irrigation ( $p < 0.001$ ), year ( $p < 0.001$ ) and the interaction between irrigation and year ( $p < 0.05$ ) (Table 6). RWC for non-irrigated and irrigated roots ranged 81–96 and 88–96 %, respectively. Mean RWC for irrigated and non-irrigated were of 89 % and 93 %, respectively. There was a slight but significantly lower RWC for root tissues in 2021 (90%) compared to 2020 (92 %) and 2022 (92%). There was also an interaction between year and irrigation whereby in 2020 and 2021 irrigated roots had a significantly higher RWC (4 and 7 % higher, respectively) while in 2022, there was no statistical significance.

**Table 5.** Effects of irrigation at harvest and temperature on sugar beet cv Sabatina KWS root tissue's compression resistance (MPa)

Storage temperature	Irrigation		Mean
	Irrigated	Non-irrigated	
3	1.9 <sup>a</sup> ± 0.07	2.24 <sup>b</sup> ± 0.11	2.09 ± 0.07
10	2.05 <sup>ab</sup> ± 0.09	2.11 <sup>ab</sup> ± 0.07	2.08 ± 0.06
Mean	2.01 <sup>A</sup> ± 0.06	2.17 <sup>B</sup> ± 0.06	2.09 ± 0.04
<i>p</i> -value			
Temperature = NS			
Irrigation**			
Temperature × Irrigation*			

\*\*Denotes significance at <0.01 and \*denotes significance at <0.05 while NS denotes not significant. The means (± SE) are drawn from three replicates in 2020 and five replicates in 2021 and 2022. Values with different lower-case superscripts are significantly different ( $p < 0.05$ ). Mean values for irrigation with different upper-case superscripts are significantly different ( $p < 0.05$ ).

**Table 6.** Effects of irrigation at harvest and year on sugar beet cv Sabatina KWS root tissue RWC

Year	Irrigation		Mean
	Irrigated	Non-irrigated	
2020	94 ± 1.11	90 ± 1.12	92 <sup>a</sup> ± 1.11
2021	93 ± 0.84	86 ± 0.84	90 <sup>b</sup> ± 0.84
2022	93 ± 0.84	92 ± 0.90	92 <sup>a</sup> ± 0.84
Mean	93 <sup>A</sup> ± 0.57	89 <sup>B</sup> ± 0.58	91 ± 0.50
<i>p</i> -value			
Year**			
Irrigation***			
Year × Irrigation*			

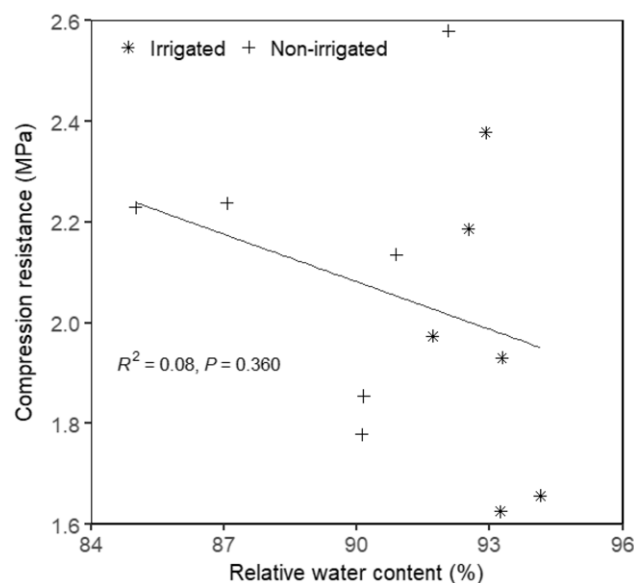
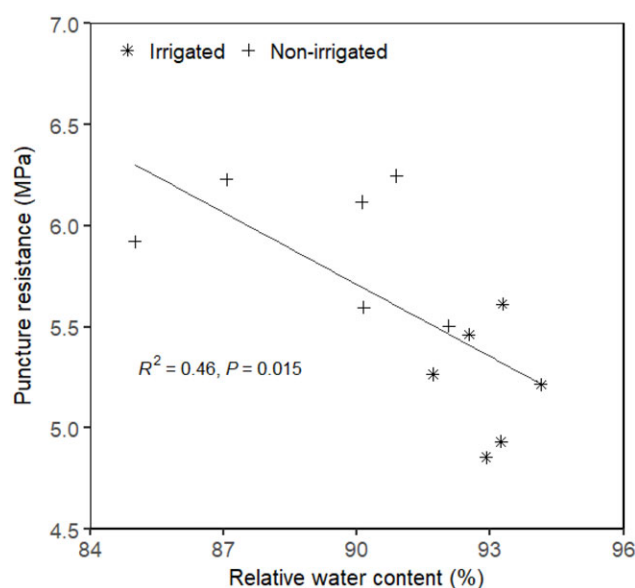
\*\*\*Denotes significant at <0.001, \*\*denotes significance at <0.01 and \*denotes significance at <0.05 while NS denotes not significant. The means (± SE) are drawn from three replicates in 2020 and five replicates in 2021 and 2022. Mean treatment values for year with different lower-case superscripts are significantly different ( $p < 0.05$ ). Mean values for irrigation with different upper-case superscripts are significantly different ( $p < 0.05$ ).

### Textural properties

There was no relationship between compression resistance and RWC while puncture resistance had a negative relationship ( $R^2 = 0.46$ ,  $p < 0.05$ ) with RWC (Figure 4 and 5). Irrigated and non-irrigated root tissues resisted deformation until compression forces of 2.00 and 2.17 MPa were applied, respectively. Non-irrigated roots required 9 % more force to puncture the periderm compared to irrigated roots.

### Surface and root tip damage

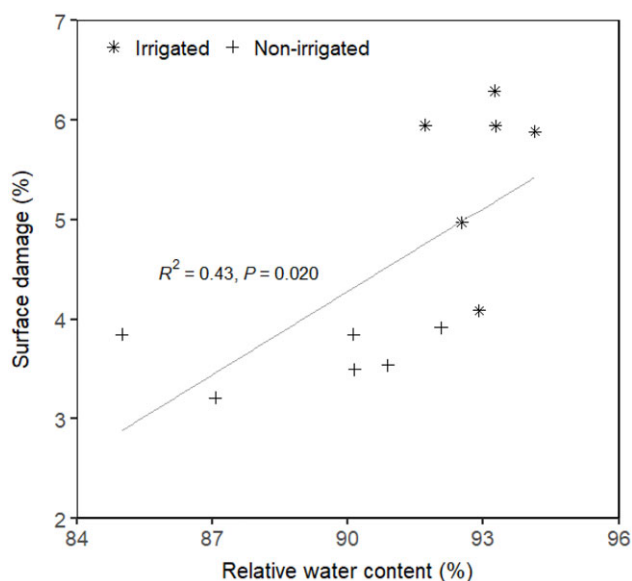
Surface damage had a positive relationship with RWC ( $R^2 = 0.43$ ,  $p < 0.05$ ). Root tip after damage was positively related to RWC ( $R^2 = 0.41$ ,  $p < 0.05$ ) (Figure 6 and 7) and negatively related to compression resistance ( $R^2 = 0.69$ ,  $p < 0.05$ ) (Figure 9). Resistance to puncture was also significant and negatively proportional to

**Figure 4.** Linear regression between RWC and compression resistance across years. The data points are an average of 12 roots of a particular year, irrigation and temperature (= 2 × 2 × 3).**Figure 5.** Linear regression between RWC and puncture resistance. Each data point is an average of 12 roots of a particular year, irrigation and temperature (= 2 × 2 × 3).

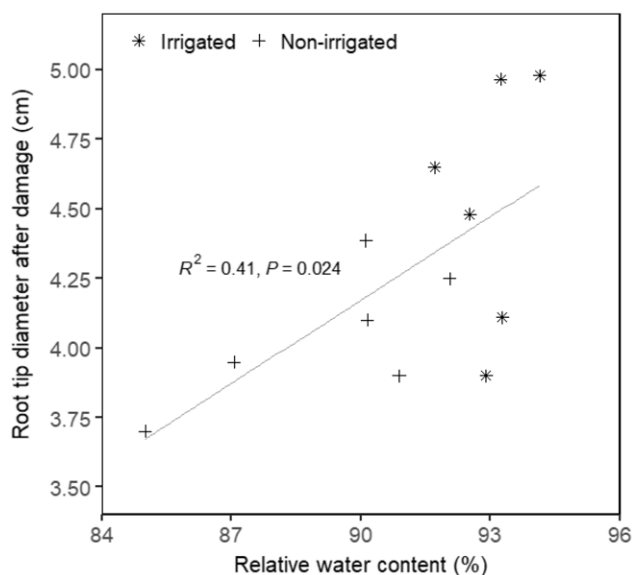
surface damage ( $R^2 = 0.42$ ,  $p < 0.05$ ) (Figure 8). On average, irrigated roots had a root tip diameter after damage of 4.47 cm while the non-irrigated roots had an average of 3.91 cm. There was no direct relationship between surface damage and root width ( $R^2 = 0.05$ ,  $p < 0.577$ ) while root tip damage was directly proportional to root width ( $R^2 = 0.61$ ,  $p < 0.003$ )

### Discussion

Root damage at harvest contributes to post-harvest sugar loss through lost tissue, damage-induced respiration and microbial growth (Hoffmann and Schnepel, 2016; Kenter *et al.* 2006b). These losses will be more pronounced in longer-term storage and at



**Figure 6.** Linear regression between sugar beet surface damage and relative water content. Each data point is an average of 12 roots of a particular year, irrigation and temperature ( $= 2 \times 2 \times 3$ ).

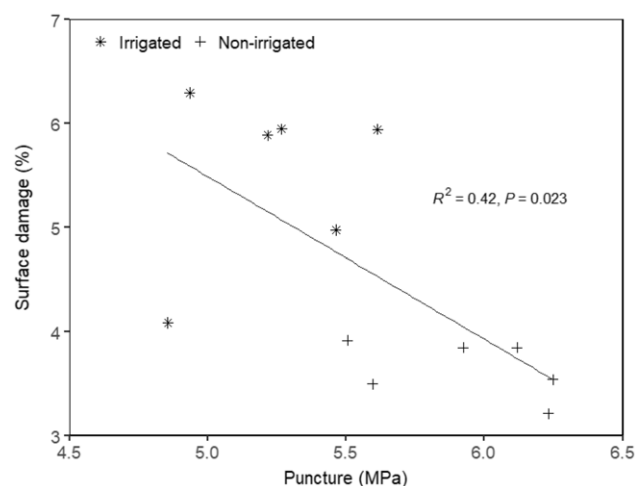


**Figure 7.** Linear regression between sugar beet root tip diameter after damage and relative water content. Each data point is an average of 12 roots of a particular year, irrigation and temperature ( $= 2 \times 2 \times 3$ ).

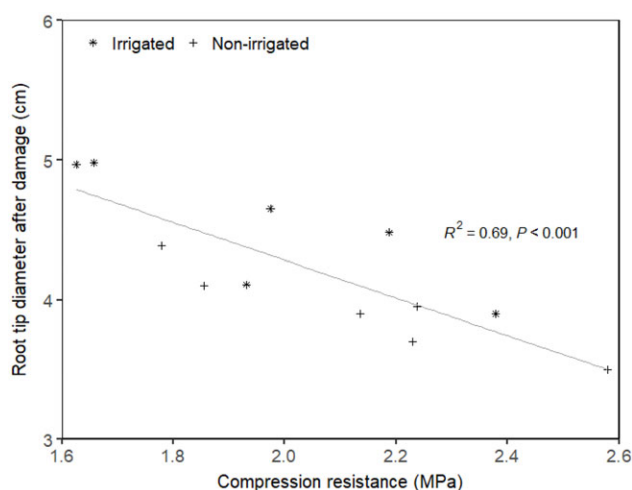
higher temperatures (Hoffman, 2018). The underlying physiology of resilience to root damage and associated sugar loss has been linked to the influence of variety, growing environment and nutrition on cellular factors that contribute towards sugar beet root tissue strength (Nause *et al.* 2020). In this current study we examined the impact of water content and root temperature on resilience to mechanical damage.

### Root morphology

Morphologically, our results suggest that transplanted plants produce small roots that are lighter in weight. This was manifested



**Figure 8.** Linear regression between sugar beet root tip diameter after damage and root tissue compression resistance. Each data point is an average of 12 roots of a particular year, irrigation and temperature ( $= 2 \times 2 \times 3$ ).



**Figure 9.** Linear regression between sugar beet surface damage and root tissue puncture resistance. Each data point is an average of 12 roots of a particular year, irrigation and temperature ( $= 2 \times 2 \times 3$ ).

as roots harvested in 2020 when the plants were sown directly registered heavier, longer and wider roots compared to when they were transplanted in 2021 and 2022. However, these results are contrary to Khozaei *et al.* (2020) who reported higher root yield in transplanted sugar beet compared with direct sowing. We attribute the reduced size and weight found in the current study to physiological stress on the plants as they were transplanted four months after planting while Khozaei *et al.* (2020) transplanted them after one month.

The results also suggest that wider and heavier roots are more prone to root tip but not surface damage as the current study shows no link between size or weight of the root with surface damage. This clearly suggest that surface and root tip damage are influenced by different physiological factors. These results are also in line with those of Van Swaaij *et al.* (2003) who observed that sugar beet with significantly heavier roots usually record high root tip damage compared to light roots due to increased kinetic energy on impact



during cleaning. Gemtos (1999) attributed higher root tip damage in heavier roots to high inter-ring distance which provide less cross-section strength. However, no yearly differences were observed on tissue puncture and compression forces, suggesting that transplanting had no effect on tissue strength.

### Water content

Tissue water status, measured as RWC, has been shown to positively correlate with root tissue turgidity and turgidity, in-turn, has an impact on tissue textural properties like compression and puncture resistance (Robbins and Dinneny, 2015). By maintaining well-watered and deficit irrigated plants we generated sugar beet roots with higher and lower RWC, analogous to findings for carrot by Herppich *et al.* (2001). Following damage assays, we observed that high RWC was associated with increased surface damage, root tip damage and lower compression and puncture forces. Turgid tissues are also more prone to damage in carrots (Herppich *et al.* 2001; McGarry, 1993) and radish (Lockley *et al.* 2021). Lockley *et al.* (2021) reported that an increase in radish hypocotyl water content was associated with a decrease in failure force for both compression and puncture resistances. However, our results are in contrast with Kleuker and Hoffmann (2021) who concluded that tissue strength of sugar beet roots is less determined by the environment. Since, they did not define the soil water content variations in the seven environments prior to harvest, we attribute our findings to the bigger variation in soil moisture content created by the irrigated and non-irrigated treatment.

The negative correlation between RWC and textural properties also supports an 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 results also agree with those of Schafer *et al.* (Schäfer *et al.*, 2020) who concluded that compressive strength of sugar beet tissues increases with decreasing water content of the roots. However, the differences in water content observed by this author were genotypic and not due to soil water content as observed in our study. Our study therefore suggests that apart from the genotypic differences, sugar beets harvested under extreme water conditions may be prone to damage due to tissue frailness that emanates from high water content.

Nause *et al.* (2020) concluded that mechanical properties in sugar beet, like puncture resistance, remain stable throughout the growing period however, this work was carried out under field conditions where water condition was not varied. The significant reduction in tissue strength observed with increased RWC in this current study implies that textural properties are dependent on moisture conditions prior to harvesting (i.e. rainfall prior to harvesting). More extensive surface damage and root tip diameter (an indicator of increased mechanical tissue loss) after damage in irrigated compared to non-irrigated roots, suggest that soil water status is an important factor when considering harvesting plans.

Maintaining water status for irrigated treatment in a period of seven weeks prior to harvest also increased the width and weight of the roots as observed in this current study. Sugar beet varieties with significantly heavier roots usually record high tissue damage compared to light varieties due to increased kinetic energy on impact during cleaning (Van Swaaij *et al.* 2003). Both weight (Kenter *et al.* 2006; 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 agree with those of Hoffmann (2018) who suggested that soil conditions during production such as high or low moisture content may alter the composition of the root's periderm and tissue strength, resulting in a higher susceptibility to damage and abrasions. Our results suggest that crops which receive considerable rain prior to harvesting may be more prone to damage (and sugar loss) at harvest and through handling. These findings, suggest that, where possible, avoiding harvesting following prolonged periods of rain offers an opportunity to minimize root damage.

### Temperature

In the UK, the sugar beet harvest season occurs across a period of several months over autumn and winter. Average temperature data collected at weather stations from 2012 – 2022 within UK sugar beet growing areas range from 6 – 17 °C in autumn and 3 – 10 °C in winter (Met Office, 2023). Adverse conditions such as elevated temperature can exacerbate sugar losses during the clamp stage (Jaggard *et al.* 1997). Such conditions can damage proteins and membranes, thereby weakening cells and reducing tissue strength and resistance to deformation (Guihur *et al.* 2022). This current study found no significant effect of newly harvested root temperature on damage to roots between 4 and 12 °C, suggesting a limited effect of prevailing harvesting temperatures in sugar beet production zones of the UK on root damage. Studies in carrots observed that significant changes in tissue strength occur above 20 °C (Herppich *et al.* 2002; Herppich *et al.* 2005). Therefore, it is possible that increased autumn and winter temperatures due to climate change could lead to increased sugar beet damage susceptibility in future

### Interaction between temperature and water status

There was no significant interaction between root temperature and water status on root damage, puncture or morphological trait, but compression resistance was significantly reduced at the lower tissue temperature of 4 °C in irrigated roots compared to non-irrigated roots. Tissue water content is negatively correlated with resistance in root mechanical properties, especially compression resistance (Lockley *et al.* 2021) and water loss in root crops is reduced at lower temperatures (Herppich *et al.* 2001).

We suggest that high compression resistance observed in non-irrigated plants stored at low temperature was due to low water content in the tissues, while the decreased resistance in compression for roots stored at the same low temperatures in irrigated roots was due to high water content. However, the response in interaction between the temperature and irrigation was neither significant for puncture resistance nor tissue damage. Further work will be needed to separate out the relative contributions of each factor.

### Conclusion

The physiological status of sugar beet at harvest can influence the extent of root tissue damage during the physical action of harvesting. This current study supports previous reports that harvesting sugar beet at a high water content, as may be experienced following an extended period of heavy rain, may lead to significantly higher levels of root damage and therefore yield loss. In contrast, mild (12 °C) and cold (4 °C) root temperatures,

representing realistic autumn and winter temperatures in the UK's sugar beet production zones respectively, do not affect textural properties or root tissue vulnerability to damage for short term durations.

In conclusion, sugar beet damage is not influenced by root temperatures over the range commonly observed at harvest in the UK and temperate production areas. Future climate scenarios, however, may increase damage risk. Higher water status at harvest, such as would be observed in a wet season, increases root tip and surface damage. This suggests that to maximize sugar yield, care is needed when lifting beet in wet conditions. Growers may consider delaying lifting after periods of heavy rain or adjusting the settings on the harvester to reduce damage to the crop.

**Supplementary material.** To view supplementary material for this article, please visit <https://doi.org/10.1017/S0021859625100166>

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**Author contributions.** JM conceived the idea and PZC designed and implemented the study. During implementation, JM and ED played an oversight role mostly on crop physiology and agronomy of the crop. PZC collected data and performed statistical analyses under the guidance of EH. PZC wrote the manuscript which was proofread by JM and ED.

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