Investigation into the agronomic and biological factors affecting post-harvest bruising in *Pastinaca sativa*

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Submitted in accordance with the requirements of Doctor of Philosophy (PhD)

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December 2020

Acknowledgments

I would like to thank my supervisors Professor Jeremy Pritchard and Dr Laura Vickers for their support and insight throughout the project. My thanks is also extended to the members of Lab 203 in Biosciences, and staff at Harper Adams University for their assistance. This PhD would not have been possible without the collaboration of numerous parsnip producers from around the UK and their time, effort and enthusiasm for the project is appreciated. I would like to thank Elsoms, Ltd for part funding the project and for offering so much support and advice over the last 4 years, my thanks is extended to the BBSRC and MIBTP.

I would not have been able to complete this project without the love and support of my parents and sister, for which I shall be eternally appreciative. Finally, my greatest thanks is reserved for Wendy, who has been there every step of the way over the last 4 years. From harvesting roots in the pouring rain, to lab night lab sessions, you have been with me throughout this process and I shall always be grateful.

Manuscripts under preparation

1. Mechanical damage data acquisition and analysis during parsnip harvesting and post-harvest processing by employing an instrumented pseudo-parsnip.

2. Physical and transcriptional changes in parsnip tissue across 3 varieties as a response to mechanical processing damage.

Funding information

This PhD project was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) through the Midlands Integrative Biosciences Training Partnership (MIBTP), at the University of Birmingham. This Icase studentship was part funded by Elsoms, Ltd and the thesis is subject to an embargo as a result.

Abstract

The aim of this study was to provide novel insight into bruising susceptibility in *Pastinaca sativa* (parsnip) roots to facilitate industry-focused solutions. Bruising damage contributes significantly towards pack-house losses, which regularly surpass 50%, and so identifying the causes of post-harvest damage is required to maximise quality and minimise economic losses.

Throughout this study, three varieties of parsnip (V1, V2 and V3) were employed to investigate varietal differences in bruising susceptibility. A bruise replication protocol was developed and bruise severity was calculated, with bruising frequency also contributing towards overall bruising susceptibility. In field trials, variety and harvest date were found to significantly affect bruising severity, whilst irrigation protocol influenced the frequency of bruises, but not bruise severity. Fully irrigated roots harvested later in the year elicited greater bruising than earlier harvested parsnips; V3 elicited the greatest bruise severity (3.09 ± 0.43) across the whole trial during the final harvest, whilst the most bruise resistant variety (V1) elicited a bruise severity of 1.72 ± 0.24. It was found that irrigation scheme did not significantly affect the severity of bruises; however, droughting roots caused the bruising frequency to decrease in all varieties during the first and second harvests.

Post-harvest factors including impact force (g), storage temperature (°C) and storage duration were found to significantly affect bruising susceptibility in parsnips. Bruising severity increased as impact magnitude increased, with the most severe bruising being witnessed in the highest impact group after 72 hours at 20 °C storage (10.58 ± 3.14). In comparison, the lowest impact group elicited a bruising severity of 0.20 ± 0.10 under the same storage conditions. Storing roots post-harvest at 6 °C, as is industry practice, reduced the severity of bruises present compared to higher storage temperatures.

Quantification of impact forces exerted onto roots throughout processing was achieved via employment of a tri-axial accelerometer housed into a 3D-printed shell ("electronic parsnip"). Analysis of impact forces via the electronic parsnip facilitated the identification of destructive processes, comparison of processes across industry, and testing of modifications to find the least destructive working practises. Across packhouse B, polishing exerted the greatest total impact force (g) (197.13 ± 18.03), whilst plastic packing exerted the lowest (64.85 ± 12.30 g). The most destructive process across the entire study was polisher I (734.03 ± 26.89 g), however a significant 55% reduction in force exerted was achieved via modifications to the barrel and brush settings of this polisher.

Scanning electron micrographs indicated that cell rupture and membrane leakage in bruised parsnip tissue were associated with tissue browning. The inherent discolouration potential of parsnip tissue did not vary across varieties, but the bruising response did. V3 was the only variety where damage initiated a significant increase in solute leakage, and across all harvests V3 exhibited greater % tissue solute leakage than the bruise resistant varieties. V3 also had the highest tissue relative water content across all harvest dates during both field trials. This suggests that bruising susceptibility in parsnips is determined not by phenolic and enzymatic activity, but rather solute leakage, concurring with previous research.

Following RNA sequencing and the *denovo* construction of a parsnip transcriptome, a number of damage inducible genes were found to be overexpressed. The phenylpropanoid pathway was significantly upregulated by V1 and V2 following damage, however it was not upregulated by V3. A total of 6 phenylalanine ammonia lyase (*PAL*) genes were upregulated by V1, V2 upregulated 5 *PAL* genes whilst V3 did not upregulate any. V3 also did not upregulate any polyphenol oxidase (*PPO*) genes, whereas V1 upregulated 3 *PPO* genes, 2 of which were homologous with V2. All 3 varieties significantly upregulated tyrosine decarboxylase (*TYDC*) as a response to damage. V1 and V2 also upregulated 12 peroxidase (*POD*) genes, whilst V3 upregulated one *POD* gene (*PNC1*). This indicates differences in the transcriptional response to damage between bruise resistant and susceptible varieties. The molecular mechanism for parsnip tissue browning is thus suggested via PPO, POD, TYDC and PAL following cell rupture and membrane leakage, as a result of mechanical impacts during harvesting and processing.

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Abbreviations and units

ag	Acceleration due to gravity
BD	Bruise depth
BI	Bruise intensity
bp	base pairs
BP	Biological process
BS	Bruise severity
BW	Bruise width
ºC	Degrees Celsius
CC	Cellular component
CD	Crown diameter
cm	Centimetre
DEG	Differentially expressed gene
DW	Dry weight
g	Impact force
$g.s^{-1}$	Impact force per second
h	Height
H	Hours
H1	First harvest
H2	Second harvest
H3	Third harvest
L	Litre
m	Mass
M	Molar
mL	Millilitre
MF	Molecular function
mm	Millimetre
$m.s^{-2}$	Metre per second squared
nm	Nanometre
PAL	Phenylalanine ammonia lyase
PE	Gravitational potential energy
POD	Peroxidase
PPA	Post processing analysis
PPO	Polyphenol oxidase
RTA	Real-time analysis
SD	Standard deviation
SE	Standard error
SWD	Soil water deficit
TSL	Tissue solute leakage
TW	Turgid weight
µm	Micrometre
V1	Variety 1
V2	Variety 2
V3	Variety 3
VC	Velocity change
W	Weight

Chapter 1: Literature review

1.0 Introduction

1.1 Origin and economic importance of parsnips

The parsnip (*Pastinaca sativa)* is a root vegetable crop native to Europe and is closely related to *Petroselinum crispum (*parsley) and *Daucus carota* (carrot), residing within the Apiaeae family (Rubatzky *et al.,* 2017). Parsnip is a monocarpic and facultative biennial (Averill & DiTommaso, 2007), but is usually grown annually in agriculture: its long, tuberous tap root is cream-coloured and is often harvested after winter frosts, thus allowing the vegetable to mature and to become sweeter. Whilst cultivated parsnip is not sufficiently distinct from wild parsnip to justify separate taxonomy (Figure 1.1.0), a number of cultivars have been developed (Cain *et al.,* 2010) and the crop has been grown in Europe since at least the Middle Ages. At present the UK market for parsnips is dominated by the Javelin variety, which commands a market share of approximately 60%. The majority of roots are destined for the pre-pack market (55%) with wholesale and processing 20%, and export 4% (Clarke, 2015).

Whilst FAO, (2015) described parsnip as ‘a minor vegetable crop in temperate parts of the world’, the economic value of parsnip cultivation in the UK and Europe is significant. In 2013, 3000 hectares of land was dedicated for parsnip cultivation, producing 82,500 tonnes of product with a market value of £31 million, with the value of seed estimated to be circa £4 million (per annum) (DEFRA, 2014). Approximately 40% of edible

produce in the UK is deemed below standard for consumption due to misshapen produce, non-uniformity or aesthetic disorders such as bruising discoloration. Whilst the losses for edible produce as a whole are high, the losses for parsnip exceed values accepted in other crops: losses from field to supermarket are estimated at 45% – 55%, with some pack houses reporting rejection levels that surpass 70%. A similarly harvested root crop, *Solanum tuberosum* (potato) was found to be the single most wasted food by UK consumers (WRAP, 2020).

Figure 1.1.0) Image by Dr Richard Tudor (@RichTheBreeder, Twitter, 17/11/2020) illustrating physiological differences between wild parsnip (left) and cultivated parsnip (right).

Reasons for parsnip rejection include scuffing of the crown; presence of parsnip canker (*Itersonilia pastinaceae*); misshapen roots which do not appeal aesthetically to

consumers, and bruising damage. Whilst rejected produce is seldom wasted, the economic value of unacceptable roots does not command the same price when sold for animal feed, composting or as lower quality produce, thus there is an economic incentive for producers to improve the post-harvest quality of roots.

1.2 Physiology

Parsnip seedlings emerge rapidly and develop a significant taproot for storage of nutrients, and large rosette leaves that are continually produced until growth is halted by low temperatures. Flowering usually occurs in May, throughout June and July until October, however this is variable and depends significantly on growing location and conditions. Parsnip is considered a slow growing crop, with roots maturing approximately 120 days after seeding, displaying considerable varietal variation. Whilst the subterranean section is edible, the terrestrial sections are toxic to both livestock and humans; secondary metabolites such as coumarins, furanocoumarins and terpenes are stored terrestrially and play significant roles in anti-predator defence mechanisms (Averill & DiTommasso, 2007).

Similarly to potato, parsnip roots are largely parenchymatous and lacking in specialised thickened tissues (McGarry *et al.,* 1996). Parenchyma plant cells have a large, membrane-enclosed central vacuole and is utilised for maintenance of the optimal pressure within plant cells (Grove & Monaghan, 2018). In root crops, the parenchyma cells form the majority of plant ground tissue and retains dividing ability, making parenchyma cells key to plant growth, and to the response to wounding. Integral for

the exchange of materials between the xylem and phloem, parenchyma cells usually occur as a continuous mass, such as in the cortex in parsnip and make up a large percentage of total mass of the root. This lack of secondary thickened tissues results in tubers being susceptible to damage during harvest and during post-harvest processes through both internal and external damages (McGarry *et al.*, 1996).

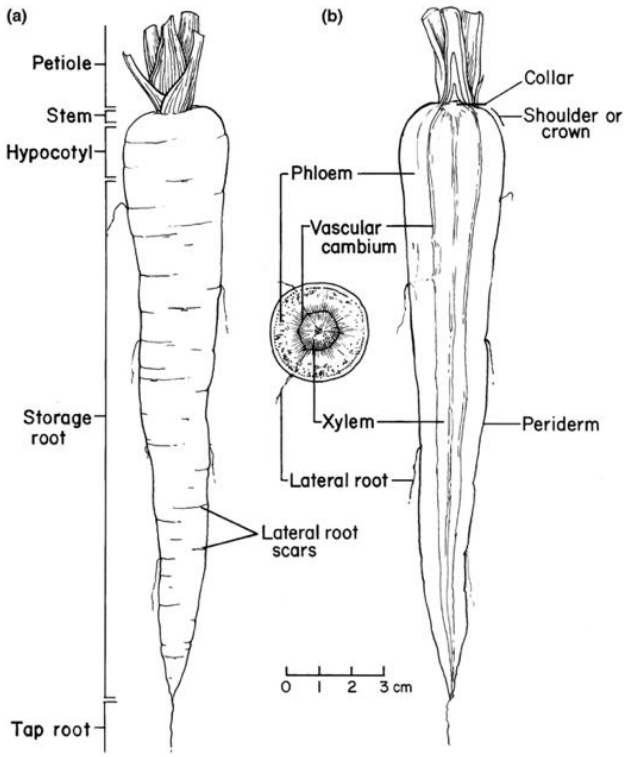


Figure 1.2.0) Illustration of terrestrial and sub subterranean sections of *Apiaceae* roots. Taken from (CABI, 2017).

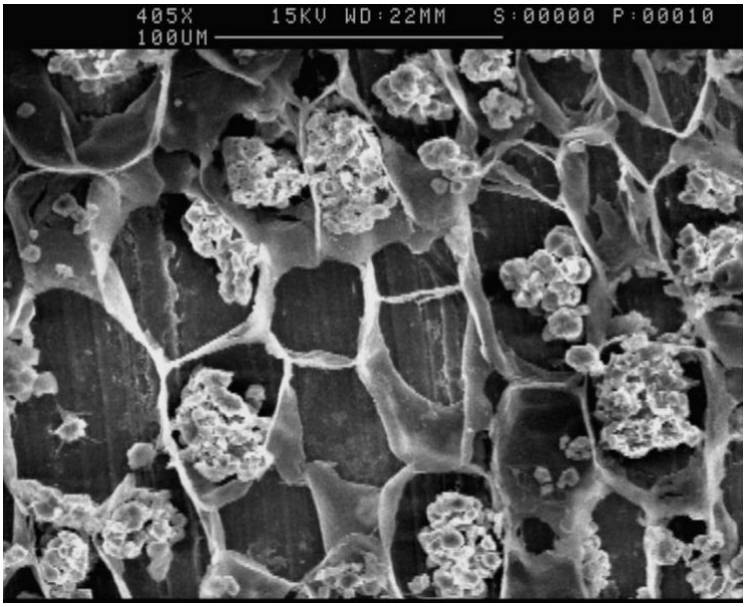


Figure 1.2.1) Authors own scanning electron micrograph of intact subcutaneous parsnip tissue displaying integrity of cell membranes and cell walls with negligible loss of cell compartmentalisation and starch granules, at 405 x magnification. Scale bar = 100 μM.

1.3 Bruising in parsnips

Harvested root crops are subject to damage both during the harvesting process and post-harvest processing (Bentini, Caprara & Martelli, 2006): although very little work has been done on bruising in parsnip. The majority of work focusses on damage to other root crops such as potato, carrot, *Beta vulgaris* (beetroot) and *Raphanus sativus* (radish). However, Chubey & Dorrell, (1972) identified mechanically induced injuries as a major factor affecting post-harvest browning in parsnips in Canada. Furthermore, Toivonen, (1992) studied enzymatic browning in parsnip varieties and found significant varietal differences in the browning response. No significant difference in total oxidative potential was observed, but bruise susceptible tissue displayed greater solute leakage

following injury replication. No research focussing on parsnip processing in the UK or studies on British varieties has been attempted, however one of the varieties (V1) investigated by Toivonen, (1992) is a prominent variety in the UK and is one the most popular varieties by amount of seed sold.

Root crops typically experience greater losses and wastage (40 – 50 %) than cereals (30 %), fish (35 %) and meat or dairy produce (20 %) (Otekunrin and Sawicka, 2019). Post-harvest losses in parsnips are higher than witnessed in similarly harvested and processed root crops. Pack out losses within parsnip processing lines regularly exceed 50% as mechanical damage, pests, misshapen and over/under size roots in combination can cause losses as high as 75% in unfavourable conditions (personal communications with industry participants, Autumn 2019). Due to the need to lift root crops from the ground, they are more susceptible to mechanical damage during harvesting than the majority of other fruit and vegetable crops. Losses immediately at retail purchase for closely related carrot were calculated to be 17.9% (Munhuweyi, 2012), with 50 – 70% of those losses being accounted for by processing damage and the remainder being due to insect damage and tissue decay.

Bruising occurs (Figure 1.3.0 i) when harvest and post-harvest processes exert excessive mechanical, compressive and vibrational forces onto produce resulting in cell breakage and leakage (Opara & Panthare, 2014). Resultant metabolites of the plant's wound response system oxidise to form lignin which frequently presents as a red/brown colour, and is responsible for the bruising/browning of fresh produce (Hussein *et al.,* 2020). Physical damage such as scuffing (Figure 1.3.0 ii) and bruising

remain significant limiting factors affecting the ongoing mechanisation of post-harvest processing (Polat *et al.,* 2012), as there seems to exist a trade-off between ongoing mechanisation, and mechanical damage to produce.

Figure 1.3.0) i & ii (Left to right respectively): i) Author's own photographs illustrating sub peridermal bruise occurrence on parsnip root after removal from processing. ii) illustrating scuffing damage on parsnip root after removal from processing,

Potatoes are prone to the formation of sub peridermal blackspots and bruises within parenchymal tissue following physical impacts (Scharf, 2014) that result in significant losses of quality and therefore economic value. Steensen, (1996) measured the frequency of tubers with bruised areas, with their data indicating that the majority of damage occurred during the harvesting process. Peterson, (1981) demonstrated that the extent of damage during piling at factory intake was two-thirds of the damage caused during the harvesting process. Post-harvest damage was found to account for up for 9 % of market loss in a study focussing on *Ipomoea batatas* (sweet potato) where up to 20 – 35 % of roots were cut, 3 – 5 % were broken and up to 53 % had major scuffing damage following harvesting (Tomlins *et al.,* 2000). However, loading

and unloading roots was found to be the most detrimental process to quality, with up to 19 – 37 % of sweet potato roots suffering from breaks, or scuffing damage respectively. Mechanical impacts may initiate the development of internal tissue discolouration, with browning colouration being a result of the oxidation of endogenous phenolic compounds (Shafie *et al.,* 2015), caused by loss of cell compartmentalisation (Figure 1.3.1) (McGarry *et al.*, 1996). After damage, cytoplasmic poly-phenol oxidases (PPOs) oxidise phenolic compounds residing in vacuoles to quinones in plastids. The non-enzymatic (and peroxidase catalysed) polymerisation of quinones form dark-coloured lignins, and it is this pigmentation which is the main contributor to a unfavourable tissue appearance (Spagna *et al.*, 2005).

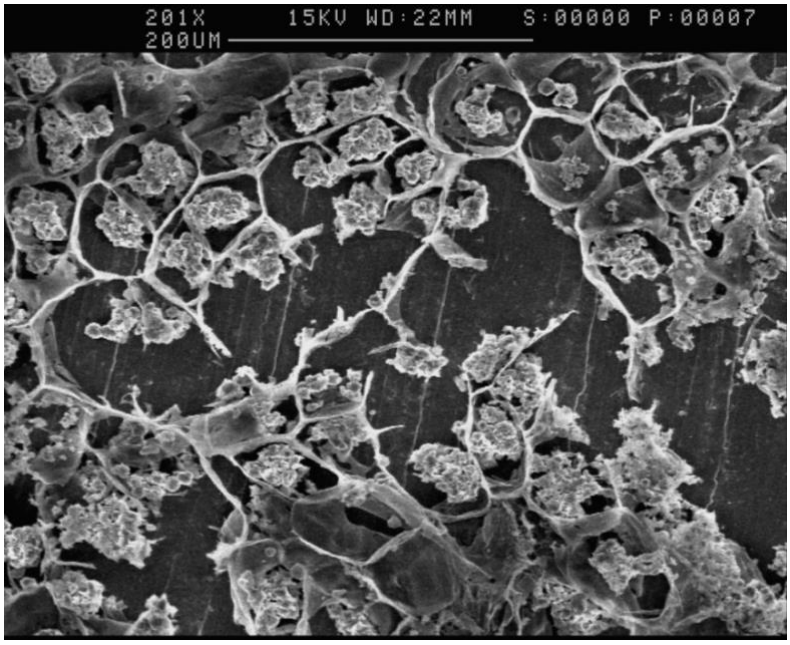


Figure 1.3.1) Author's own scanning electron micrograph of damaged subcutaneous V2 tissue displaying extensive cell membrane and cell wall damage (centre) leading to a significant loss of cell compartmentalisation, at 201x magnification following falling impact replication of 4.49 J. Scale bar = 200 μM.

Visible symptoms of bruising emerge 3 – 72 hours following damage in the majority of root crops (Urbany *et al.,* 2011); this latency period makes the immediate, accurate assessment of damage during the harvesting process difficult. Pack-house operations for parsnip typically lift roots out of the field at dawn, after which they are washed, polished, selected, sorted and bagged before being transported to supermarkets for sale the next day (Telegraph, 2011). The latency period which exists for bruising damage poses a problem for producers, as bruises may not fully develop until they are either already on sale at the supermarket or have already been purchased by consumers.

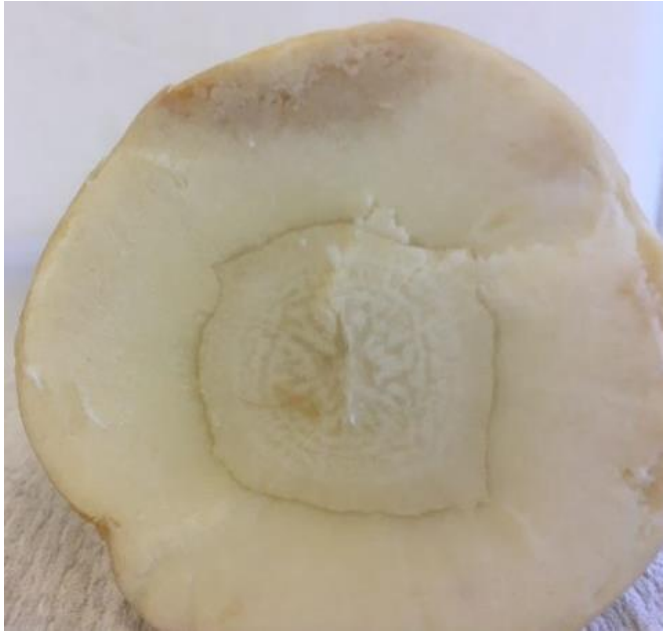


Figure 1.3.2) Author's own image demonstrating the development of bruising damage in V2 tissue following mechanical impact via falling bolt method, following 48 hours of storage at 20 °C.

1.4 Bruising assessment

Quantification of bruising severity during packing and processing in industry is usually performed manually by the naked eye using reference sheets, in order to ascertain whether the damage is sufficient to deem a product unsuitable for consumers (Toivonen *et al*., 2007). The standardised scores used to quantify bruising are highly variable across and between species, with different researchers considering different factors and terminologies. Scores are usually marked on a numerical scale which spans a range of "no bruising" to "unusable", or conversely on a binary system: either the presence or absence of bruising (Van Linden *et al*., (2006a). In research

environments, bruise measurement is achieved by measuring the dimensions of a detected bruise and analysing the severity of colouration to produce a bruising severity score (Scharf, 2014).

It has previously been noted that bruise formation is geometrically variable across crop species with a large range of shapes witnessed (Martinez-Romero *et al.,* 2004). Therefore, the production of a bruising severity score should be specific to the shape and colour of bruises formed after mechanical impacts, specific to that species of fresh produce. Combining the bruise severity values observed with the % likelihood to bruise may be done to provide an overall bruising susceptibility where the likelihood and severity of damage is accounted for.

1.5 Bruise replication

A number of techniques aimed at obtaining controlled bruising damage have been applied to other species of horticultural produce (Opara *et al.,* 2007, Kitthawee *et al.*, 2011, Jimenez & Jimenez, 2013). Pendulum impactors have been employed to replicate impacts, and involve swinging produce from numerous heights onto hard surfaces in order to replicate and measure impact forces experienced during processing (Opara *et al.,* 2009).

Opara, (2007) designed a device for use in *Malus pumila* (apple) where a steel bolt of a known mass is dropped through a perforated PVC pipe from a range of heights, permitting the experimenter to replicate impact energies experienced by fresh produce

during damaging. Jimenez & Jimenez, (2013) designed a methodology facilitating the controlled and replicable impact via dropping produce onto a metal plate allowing impact forces to be accurately measured and impact thresholds calculated. Similarly, Scharf, (2014) employed a falling bolt methodology to replicate bruise formation in potato tubers, which was able to produce quantifiable bruises and significant differences were observed between groups. The falling bolt method is a cheap, replicable, easily transportable and robust method of bruise replication, it allows researchers to easily modify the desired impact force exerted onto produce to study bruising susceptibility under a range of magnitudes.

Previous commercial replication of parsnip bruising was achieved via employment of a cement mixer, where roots were spun for a set period of time, stored and then the number and size of bruises analysed. Whilst effective, other replication methods represent more replicable, exact methodologies where impact force can be easily calculated and manipulated (Stehmel *et al.,* 2010).

1.6 Industrial quantification of impact damage

Over the last 30 years a number of “electronic” fruits and vegetables have been developed in attempts to quantify post-harvest damage and losses (Tennes *et al.,* 1988). Measuring impact magnitudes exerted on fresh produce during harvesting, loading, grading, transport and packaging has facilitated the identification of critical points during processing where significant impact force is exerted (Praeger *et al.,* 2013). Once processes detrimental to produce quality have been identified, methods

aimed at reducing impact force experienced can be tested and compared, in order to identify the least destructive working practices (Emana *et al.,* 2017; Bantayehu & Alemayehu, 2019) and try to reduce mechanical bruising damage and economic losses for producers.

Electronic impact devices measure acceleration forces and velocity change (VC) experienced during falling, impacts and compression. Typically, a produce-shaped shell houses an accelerometer which returns acceleration values via Bluetooth to a computer for later analysis. Devices shaped to mimic fruit such as *Vaccinium myrtillus* (blueberry) (Yu *et al.,* 2012 & Yu *et al.,* 2014, Xu *et al.,* 2015); citrus fruits (*Citrus sinensis* & *Citrus limon*) (Orange and lemon respectively) (Roa *et al.,* 2015); apples (Pang *et al.,* 1992, Herold *et al.,* 1996, Luo *et al.,* 2012), and sugar beet (Bentini *et al.,* 2002) have attempted to quantify impact forces experienced by produce during harvesting and processing by employing a number of different loggers and shells.

Typically the harvesting of fruit is less destructive and employs less mechanical equipment than required for roots crops, therefore the design and specification of the pseudo-fruits reflects this. The IS100 (Techmark Inc. USA) was the first tri-axial accelerometer employed to measure bruising thresholds in fresh produce (apples) (Brown *et al.,* 1990; Schulte *et al.,* 1992). First developed at Michigan State University and the USDA-ARS (Tennes *et al.,* 1988a, 1988b; Zapp *et al.,* 1990), the device was employed to analyse harvest and post-harvest processing peak acceleration and VC values exerted on apples to identify critical points where excessive impacts may exceed the bruising threshold (Pothula *et al.,* 2018).

A number of new loggers have been produced, aimed at measuring either acceleration or pressure data before transmitting this data to a central computer. The IS100 was developed into the IRD (Impact Recording Device) (Techmark Inc. USA) which was been inserted into shells to mirror the shape and weight of fruits such as apples (Ragni & Berardinelli 2001; Luo *et al.,* 2012), *Olea europaea* (olives) (Catania *et al.,* 2015), sugar beet (Bentini *et al.,* 2002) and *Solanum lycopersicum* (tomatoes) (Arazuri *et al.,* 2010). The BIRD (Berry Impact Recording Device) was developed by a team in Georgia, USA and is essentially a miniaturised accelerometer designed to measure impact data during the harvesting and processing of blueberries (Yu *et al.,* 2011; Yu *et al.,* 2014; Xu *et al.,* 2015), apples (Luo *et al.,* 2012) and *Prunus persica* (peaches) (Lin, 1994).

However, as the harvesting and post-harvest processing of root crops employs more mechanical equipment than fruit harvesting (Kumar & Azad, 2020), a number of pseudo-tubers have also been designed which are typically more robust in nature than pseudo-fruits to account for additional impacts. Smaller IRD's may not suitably match the dynamic behaviour of root crops during harvesting and processing; furthermore, locating a small device may prove difficult during all processing stages when processed along with tonnes of normal roots. Parsnips are tapered cylinders with a crown diameter typically between 40 – 70 mm and a desired length of 170 mm, therefore a spherical or egg shaped device would not experience the same number or intensity of impacts as a parsnip shaped shell.

To accurately reflect the acceleration forces and VC exerted on roots during harvesting the device needs to be inserted into a row below the surface resting alongside normal roots; this would prove next to impossible with any devices where the shell is not firm or strong enough to withstand extreme shearing forces exerted during harvesting, sorting and cleaning. Pseudo-potatoes have been analysing potato harvesters and post-harvest processing stages for 25 years, however, the design has evolved from a flattened sphere containing a primitive IS 100 accelerometer device to egg shaped ellipsoids containing more accurate and robust accelerometers.

A comprehensive study and review by Praeger *et al.,* (2013) compared the performance of a number of impact quantification devices across potato harvesting operations. A number of the devices analysed were implanted into egg shaped plastic shells (SmartSpud (Sensor Wireless, Canada), Tuberlog (ESYS GmbH, Germany), PTR 200 (SM Engineering, Denmark)) with a weight mirroring that of potato tubers (200 – 275 grams). They also implanted a miniature device named Mikars (ESYS, GmbH, Germany) into real potato tuber tissue as well as into a synthetic polyurethane dummy with the shape, dimensions and density of both dummy tubers being similar on average to actual tubers. The results for the Mikras devices during potato harvesting returned similar peak acceleration values for both the real and synthetic potato dummies (Praeger *et al.,* 2013). Furthermore, when testing potato processing lines the IRD devices (both the Mikras device and the TuberLog) recorded a very similar total number of impacts experienced during processing, whilst SmartSpud recorded a much lower number of impacts.

It was concluded that assessing bruising potential based on impact values recorded by pseudo-root crops may be suitably achieved by employing either Mikras in real tubers or dummies, the IRD or the Tuberlog. Implanting a data logger into real produce is a viable methodology but introduces a number of potential issues: firstly, the produce is susceptible to being damaged during harvesting or processing, potentially damaging the expensive logger housed inside. Secondly, identifying the dummy root and removing it from processing stages when it is alongside identical looking real produce may prove difficult; whilst it can be painted a bright colour this may be removed during washing or polishing.

Finally, the density of parsnip roots, in contrast to potato tubers, is less than that of water; producers utilise this characteristic to clean and separate parsnip roots using water. Implanting a device such as Mikras into potato tubers increased the density of the tuber dummy to 1.14 $g.cm^{-3}$ (Praeger *et al.,* 2013) – if the density of the dummy parsnip root exceeded 1.00 $g.cm^{-3}$ it would not float, and would become irretrievable from cyclone destoners or other cleaning processes. So whilst an impact logger implanted into a real root may be the most realistic method of quantification, it is not suitable to be run through the entire harvesting and post-harvest operations of multiple parsnip processors, to provide an industry wide comparison of all processing stages. To withstand the extreme shearing pressure exerted on roots during harvesting and processing, a more robust shell is required than any of the potato tuber designed outlined by Praeger *et al.,* (2013) or any other pseudo-fruit described in the literature. It is also clear that to accurately quantify harvesting and processing forces exerted on

roots it is critical that the device mirrors the dimensions and weight of the respective produce.

1.7 Factors affecting bruising susceptibility

1.7.1 Post harvest factors affecting bruising

1.7.1.1 Impact energy

Previous studies have demonstrated that peak impact force is a dominant factor influencing bruise severity (Chen & Yazdani, 1991), with impact force positively affecting bruise formation (Banks & Joseph 1991). Impact force level has been reported to have a substantial effect on bruising in tomatoes (Cui et al., 2018), peaches (Berardinelli *et al.,* 2001), *Pyrus communis L*. (pears) (Celik, 2017), *Punica granatum* (pomegranates) (Shafie *et al.,* 2015; Hussein *et al.,* 2019), *Actinidia deliciosa* (kiwifruit) (Xia *et al.,* 2020), apples (Komarnicki *et al.,* 2017; Afkari-Sayyah *et al.,* 2014) and potatoes (Xie *et al.,* 2020). Reductions to the observed impact forces experienced by produce has been attempted by implementing padding of contact surfaces (Jarimopas *et al.,* 2007; Afkari-Sayyah *et al.,* 2014), or via modifications to root crop processing equipment (Kumar & Azad, 2020).

Idah *et al.,* (2007) found that the amount of energy absorbed by tomato tissue greatly determined the extent of witnessed bruising, observing a positive correlation between drop height and bruise size. Furthermore, Hyde *et al.,* (1993) observed that increasing

impact energy in potato tubers could result in tissue cracking and shatter bruising in addition to the blackspot bruising observed after less severe impacts. Understanding the impact force threshold at which a crop species bruises aids the identification of processes that may exert forces that exceed this threshold. Knowing how tissue responds to variable impact forces provides evidence to producers on how modifications to processing may improve pack-outs and sustainability.

1.7.1.2 Temperature

It has previously been observed that temperature management through the supply chain, from harvesting to supermarket, significantly affects the physical and chemical proprieties of fresh produce (Bill *et al.,* 2014). The effect of temperature has been implicated as a major determining factor for bruise formation in fresh produce (van Zeebroeck *et al.,* 2006). The temperature of tissue at the time of impact affects cell turgidity by altering cell hydration and enzyme activity (Lee *et al.*, 2005).

Ferreira *et al.,* (2009) found that bruise size in *Fragaria* × *ananassa* (strawberries) decreased as tissue temperature declined: this has also been observed in other fruit species, where differential responses to impact types (for example, impact versus compression) has been observed in tissue at low temperatures (Banks & Joseph, 1991; Crisosto *et al.,* 1993). In contrast, Van Zeebroeck *et al.,* (2007) found that apples with a higher tissue temperature at the time of impact suffered from less bruising, with the effect of temperature increasing as impact energy swelled. Thomson *et al.,* (1996) noted that the majority of apple cultivars exhibited greater bruising susceptibility when

tissue temperature was low, and when storage temperature was low (10 °C) in comparison to higher temperatures. However, the evidence is mixed as a number of studies have found no effect of apple tissue temperature at the time of impact and bruise severity (Jung & Watkins, 2009; Bollen, 2005).

Parsnip roots are harvested between 3 – 9 am when temperatures are typically below 10 °C, thus the temperature of parsnip tissue is likely to be low during impacts. Parsnip roots are hydro-cooled during processing before they enter cold storage for < 72 hours before transportation to consumers. The temperature of roots during storage is approximately 6 °C, this inevitably will increase during unrefrigerated transportation and supermarket storage. It has previously been observed in *Persea americana* (avocado) that fruit kept at 5 °C for first 8 hours of storage and then stored at 25 °C, display significantly less bruising than fruits stored at 25 °C for 8 hours then 5 °C for the remainder (Mazhar *et al.,* 2018).

The temperature during storage is a significant factor affecting bruise formation (DeMartino *et al.,* 2002), but its significance differs across studies and species. Shafie *et al.,* (2015) found that storing pomegranates following mechanical impacts at higher temperatures reduced bruise damage; this phenomena has also been observed in apples (Thomson *et al.,* 1996; Zarifneshat *et al.,* 2010). However, Bugaud *et al.,* (2014) found that *Musa acuminata* (bananas) stored at 18 °C displayed greater bruising susceptibility than those stored at 13 °C and positively correlated this with greater membrane leakage. Other temperature responsive fruits such as kiwis have been observed to display larger bruises following storage at higher temperatures, due to low

storage temperatures reducing metabolic activity and increasing tissue firmness (Ehmadi, 2012). Mazhar *et al.,* (2018) found that storing avocadoes at 25 °C in comparison to 5 °C significantly increased bruising; it is hypothesised that higher temperatures have a direct effect on the activity of PPOs and that cell walls are weakened by temperature-sensitive enzymatic activity over the duration of storage (Flitsanov *et al.,* 2000).

1.7.1.3 Storage duration

The relationship between bruise severity and time since impact is typically positive (Opara & Panthare, 2014) as bruise formation occurs over approximately 72 hours. Parsnip roots are presented to the consumer approximately 48 – 72 hours after processing, this period of time is sufficient for sub peridermal bruises to form and remain undetected. Bruise volume has been positively correlated with storage duration in pomegranates (Hussein *et al.,* 2019; Hussein *et al.,* 2020), although over a significantly longer storage period than experienced by parsnips. Azadbakt *et al.,* (2019) found that bruising in pear fruits peaked after 15 days and was greater than the bruising observed after 5 and 10 days. Storage duration following impact has been observed to influence bruising in banana (Banks & Joseph 1991, Bugaud *et al.,* 2014), apples (Samim & Banks, 1993) and potatoes (Xie *et al.,* 2020). Thus, investigating how bruises form over the time spent in storage is important to provide producers with information regarding the optimal storage conditions and duration.

1.7.2 Pre harvest factors affecting bruising

1.7.2.1 Harvest date

The harvest date and time of harvest have previously been observed to influence bruising susceptibility in fresh produce. In bananas, turgor pressure was found to be higher in the early morning than later in the day, which resulted in greater resistance to bruising (Banks & Joseph, 1991). Abbot *et al.,* (2009) found that apples, regardless of variety, were more susceptible to bruising when harvested in the morning in comparison to later in the day. Opara *et al.,* (1997) showed that harvest date affected the physiological properties of apple varieties, and more recently demonstrated significant increases in bruising susceptibility between early and mid-season harvest dates as specific bruise susceptibility increased by 17.9 % (Opara, 2007). This concurs with results by Klein, (1987), Johnson & Dover (1990), and Bollen *et al.,* (2001) who also found that maturity positively affected bruising.

Idah *et al.,* (2007) and Xing *et al.,* (2005) found that maturity positively affected bruising in tomatoes; Hung & Prussia, (1989) observed that fully mature peaches were more susceptible to bruising than immature fruits, however, no significant differences between medium maturity and low maturity groups was found. Opara, (2007) correlated greater bruising in mature apple cultivars to a loss in firmness and skin strength; early harvested fruits were 23% firmer and skin strength was observed to be 21% higher in comparison to the late harvest fruits. Maturity is hypothesised to affect bruising susceptibility as ripening has been implicated with a loss of cell membrane

integrity, thus cells can withstand less impact force before leaking or rupturing (van Zeebroeck *et al.,* 2006). Bugaud *et al.,* (2014) found that as banana tissue matured the peel electrolyte leakage increased, thus ripe tissue was more susceptible to a loss of cell and membrane integrity.

1.7.2.2 Irrigation

Especially dry periods of weather in the UK over the parsnip growing season cause producers to irrigate fields regularly to ensure that plants are sufficiently hydrated. Parsnip cells require water for metabolite transportation and to regulate cell turgidity which provides structure and drives cell expansion (Grove & Monaghan, 2018). Harvesting roots from very dry fields is not usually performed as it damages harvesting equipment; dry fields are irrigated immediately prior to harvest in such circumstances. In some circumstances, fully irrigated carrots have been demonstrated to be more profitable than droughted carrots, as full irrigation treatments increased yield and quality despite the extra water usage (Lellis *et al.,* 2017). Carrots have previously displayed sensitivity to soil water deficits which may result in cracking, splitting and hardening (Kotecha *et al.,* 1998): it is unclear whether closely related parsnip tissue also displays significant tissue sensitivity to droughting, and how irrigation regime affects bruising susceptibility.

Introducing an irrigation deficit to investigate the concurrent effect on bruising has been attempted in olives (Casanova *et al.,* 2017: Casanova *et al.,* 2019) where it has been observed that irrigated fruits bruised significantly more than droughted fruits. Opara, (2007) observed that reducing irrigation frequency in apple orchards caused low

bruising susceptibility in fruit, suggesting that arrested bruising could be accomplished by irrigation and crop load management. The addition of calcium chloride, which has previously shown to increase membrane stability in parsnips (Toivonen, 1992), in irrigation water reduced observed post-harvest browning in *Agaricus bisporus* (mushroom) by increasing membrane integrity, thus reducing the mixing of cytoplasmic substrates with browning enzymes (Kukura *et al.,* 1998).

Mitsuhashi-Gonzalez *et al.,* (2010) found that in apple tissue greater intercellular spaces facilitated more severe bruises as air spaces were observed to weaken tissue: more mature fruits possessed larger intracellular spaces and consequently greater bruising damage. Banana fruit that experienced water loss, and resultant turgidity loss, were found to have a higher threshold for damage than fully turgid fruits (Banks & Joseph, 1991). Maximising turgor pressure was found to increase the threshold at which banana fruit experienced compression damage, thus harvesting earlier in the morning was recommended. Garcia *et al.,* (1995) found that fully irrigated apple trees produced more turgid fruits and fruit at harvest was more bruise susceptible than stored fruit concluding that turgidity and firmness significantly influenced bruising susceptibility. Soil moisture content during the harvesting of potato tubers has been shown to reduce the influence of large mechanical impacts on bruising via increasing tuber dry matter content (Mwanamwenge, 1989).

Given that parsnip fields are always irrigated to some degree when harvested, it is important to assess how soil water content, and consequently root water content, affects bruising susceptibility to assess current working practices. Investigating how

soil water content influences root relative water content and bruising, may reduce the current pack out losses being observed. Irrigation represents a significant cost of production, thus a number of studies in carrot have focused on the most efficient irrigation regime for profitability (Carvalho *et al.,* 2014; Lellis *et al.,* 2017). As carrots have previously been observed to display physical damage as a result of droughting (Kotecha *et al.,* 1998) it is of importance to understand how droughting affects parsnips.

1.7.2.3 Variety

The threshold at which cells rupture and enzymatic browning ensues is determined by physiological factors such as relative water content, cell turgidity, membrane strength and membrane integrity (Opara & Panthare, 2014). Inherent physiological differences in such characteristics exist between varieties within crop species (Scharf, 2014), and varieties often display differential responses to wounding damage (Dwelle et al., 1977; Hussein *et al.,* 2019).

When buying parsnip seed, UK farmers have to consider a number of factors that dictate what variety they grow including desired shape, size, colour, harvest date, pathogen resistance and bruising susceptibility. In the parsnip industry, varietal differences in observed bruising and subsequent pack-out percentages play a significant role in farmer's buying decisions (Personal communications with UK producers, 2018-2019). An especially bad year for post-harvest damage often

motivates producers to grow a greater percentage of a different parsnip variety the following year.

In previous studies, significant differences in browning potential has been observed across parsnip varieties (Kaldy *et al.,* 1976; Toivonen, 1992). Varietal differences in the browning response in parsnips was attributed to differences in cell solute leakage, rather than the inherent browning potential of parsnip tissue. This concurs with observations made by Tudela *et al.,* (2002) who observed that membrane integrity is the determining factor for bruise severity in potato tissue, rather than enzymatic or phenolic content. Goyer & Pelle (2018) also found that phenol and substrate concentration were not the limiting factor in potato bruising across multiple varieties, rather cell compartmentalisation and membrane integrity determined the extent of bruising susceptibility. Investigating the physiological differences between bruise resistant and bruise susceptible varieties may highlight the determinant characteristics that dictate bruising severity, and aid breeders to produce more resistant varieties.

1.7.3 Physiological factors affecting bruising

1.7.3.1 Cell solute leakage

A loss of membrane integrity and cell compartmentalisation (Figure 1.4.3.1.0) releases cell contents into intracellular spaces (Mitsuhashi-Gonzalez *et al.,* 2010) where an oxidative cascade ensues, resulting in discolouration of the local tissue surrounding the impact site (Mazhar *et al.,* 2018). Damage to cell membranes results in enhanced

leakage of solutes into the apoplast; thus plasma membrane integrity is negatively correlated with % solute leakage (Xia *et al.,* 2020) . Bruising is a function of the plant defence response to wounding; the discolouration observed in damaged tissue is a result of lignification, in an attempt to repair and reinforce damaged cells.

Weakened cell walls and membranes reduce the amount of impact energy required to exceed the threshold for cell rupture; encouraging greater mixing of cytoplasmic enzymes and phenolic substrates originating from the vacuole (Hussein *et al.,* 2018). The bruising susceptibility of plant tissue depends on physiological factors such as turgidity, membrane integrity, phenolic content and enzyme activity (Opara & Panthare, 2014). Post-harvest factors such as impact energy, storage temperature and storage duration affect bruising susceptibility by influencing physiological characterises of cells, thus the tissue threshold for rupture and accelerated solute leakage.

Previously in parsnips, Toivonen, (1992) demonstrated that solute leakage, quantified via spectrophotometer, was the determining factor governing browning susceptibility. More recently, Bugaud *et al.,* (2014) observed a positive correlation between peel electrolyte leakage and bruising susceptibility via a conductivity meter in banana tissue. Furthermore, no correlation between bruising and PPO content was witnessed, concurring with previous observations in parsnips Toivonen, (1992) and other crop species (Cantos *et al.,* 2002; Maneenuam *et al.,* 2007).

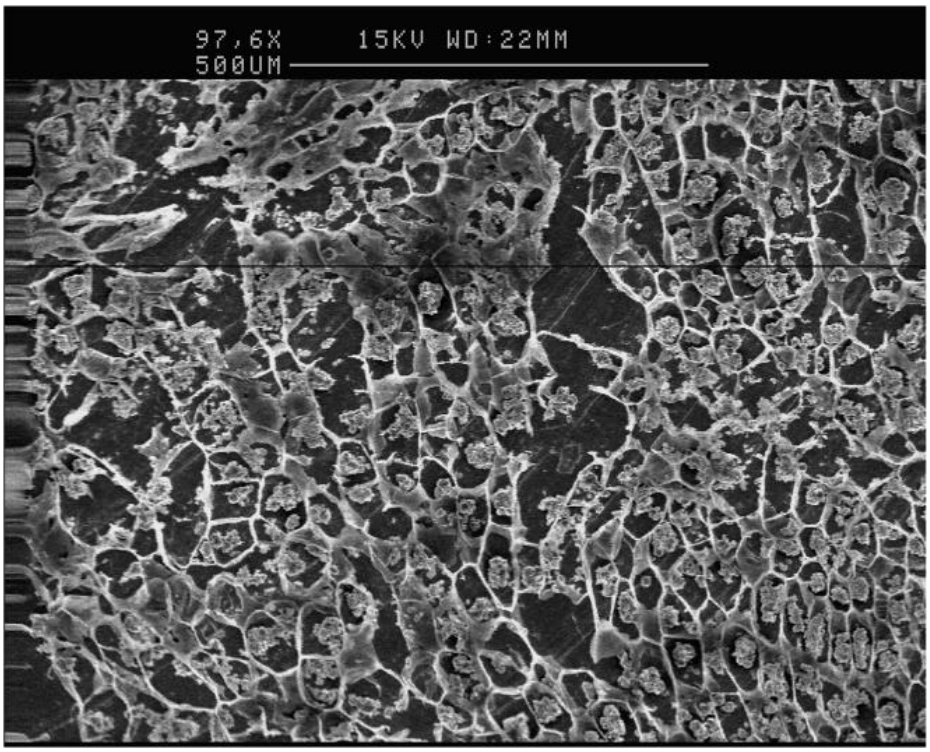


Figure 1.4.3.1.0) Author's own scanning electron micrograph of V2 parenchyma tissue following a falling bolt impact of 4.49 J at 97.6x magnification. Scale bar = 500 μM.

In pomegranates, Hussein *et al.,* (2019) measured a number of physiological characteristics during the bruising response and found that mechanical impacts caused a changed in membrane integrity leading to increased electrolyte leakage. Solute leakage increased over time and was affected by the size of mechanical impacts, with the greatest membrane leakage being observed following the largest impacts (Hussein *et al.,* 2019). Increased solute leakage following mechanical impact to tissue has also been observed in *Diospyros kaki cv. Fuyu* (persimmon fruits) (Lee *et al.,* 2005). Xia *et al.,* (2020) found that electrolyte leakage from kiwifruit tissue

increased over storage time and was greater in damaged tissue compared to control tissue.

The evidence is mixed regarding the effect of temperature on solute leakage, as PPO activity has been positively correlated with temperature but solute leakage is variable in response to temperature changes . Hussein *et al.,* (2019) found that pomegranates stored at lower temperatures exhibited a greater peel electrolyte leakage than those stored at high temperatures following mechanical impacts. In contrast, Bugaud *et al.,* (2014) and Ratule *et al.,* (2006) found that solute leakage was not affected by storage temperature in banana fruits.

1.7.3.2 Enzymatic and phenolic content of tissue

Phenolic compounds such as tyrosine, cinnamic acid and chlorogenic acid exist in low concentrations in healthy tissue and have functions related to antioxidant production and protection from oxidative deterioration (Shakya & Navarre, 2006). In potatoes, Laerke *et al.,* (2002) observed that chlorogenic acid and caffeic acid activity were important in bruise formation whilst Dale *et al.,* (1998) observed varietal differences in phenolic accumulation between potato cultivars. Goyer & Pelle, (2018) found that tyrosine and phenylalanine accounted for up to 80% of the variation in biochemical potential witnessed between potato varieties. Tyrosine has been implicated as an important substrate for enzymatic browning (Dean *et al.,* 1993), with large amounts accumulating in damaged regions of tissue (Borg-Olivier & Monties, 1993).

Concentrations of tyrosine and other phenolic compounds vary throughout tissue types in carrot, with higher concentrations being present in vascular or cortical tissue (Geoffriau & Simon, 2020). Adams & Brown, (2007) found that in potato tubers tyrosine concentrations were greater in the stolon end which correlated with greater bruising susceptibility. A number of studies have suggested that tyrosine content in homogenised potato tissue contributes more towards discolouration than other phenolic compounds (Kim & Dean, 1998; Goyer & Pelle, 2018). However, results gained *in vivo* do often not correlate with results from *in vitro* (Stevens & Davelaar, 1997; Strehmel *et al.,* 2010).

Contrasting findings regarding the significance of phenolic concentration on bruising susceptibility suggest that phenolic and enzymatic concentration are not determining factors in bruise susceptibility (Corsini *et al.,* 2002, Strehmel *et al.,* 2010; Goyer & Pelle, 2018). Similarly, Scharf, (2014) found that one variety of potato tuber (Russell Bank) exhibited a correlation between tyrosine concentration and bruising severity, whilst other potato varieties did not. Phenolic compounds such as tyrosine and chlorogenic acid, produced by the phenylpropanoid pathway are subject to browning via PPO which catalyses the conversion of monophenols to quinones that when oxidised, form melanic pigmentation often referred to as browning or bruising. PPO has been observed to be an enzyme with plant defence functions, as overexpression of PPO results in increased pathogen resistance (Thipyapong *et al.,* 2004). However, PPO activity has also been heavily implicated with tissue browning, as it catalyses the production of dark coloured pigmentation.

In apples, tissue browning susceptibility could be determined by assessing soluble and insoluble brown pigmentation (Amiot *et al.,* 1992), with the degree of browning correlated closely with phenolic degradation. It was observed that in apples, chlorogenic acid was the most suitable substrate for PPO activity and it was suggested that control of apple tissue browning could be controlled most effectively by selecting cultivars with low levels of chlorogenic acid. Lee *et al.,* (2005) found that PPO activity in bruised persimmon fruits was higher than in non-bruised fruits, but observed that it was not the only factor affecting bruise formation and tissue deterioration.

Whilst PPO activity is key for tissue browning, it has been found to not be the only relevant cytoplasmic enzyme associated with enzymatic browning (Richard-Forget &. Gauillard, 1992). Peroxidases (PODs) have previously been implicated in the bruising response, working in parallel with PPO to contribute towards tissue discolouration. A main substrate of POD activity is hydrogen peroxide (H_2O_2), which is a by-product of PPO oxidation of phenolic compounds. Given that concentrations of H_2O_2 in healthy tissue is very low, PPO activity and POD activity are often witnessed in parallel (Huang *et al.,* 1990). The oxidation of diphenolic compounds by PPO produce quinones; which are another substrate for POD activity, as POD typically catalyses the oxidative polymerisation of quinones to form dark pigmentation (Mohapatra *et al.,* 2008). Padding of contact surfaces to successfully reduce bruise damage has been correlated with a reduced accumulation of H_2O_2, O^2- and a reduction in POD activity in kiwifruit (Xia *et al.,* 2020).

Phenylalanine ammonia lyase (PAL) is a crucially important enzyme, regulating the production of phenolic compounds via the phenylpropanoid pathway which are substrates for enzymatic browning. Working in close combination with Trans-Cinnamate 4 monooxygenase (C4H) and 4-coumarate CoA ligase (4CL), PAL regulates the conversion of the essential amino acids tyrosine and phenylalanine to phenylpropanoid compounds, to eventual lignin and antioxidant production. Significant upregulation of the phenylpropanoid pathway has previously been observed as a response to mechanical damage and has been implicated heavily in the bruising response (Belknap *et al.,* 1990; Zhang *et al.,* 2019).

Understanding how physiological factors affecting parsnip bruising may aid the development of diagnostic tools to assess particular traits that may contribute towards reduced, or greater bruising damage (Slater *et al.,* 2014).

1.7.4 Genes involved in the bruising response of fresh produce

Upon sensing wounding damage, plants activate pathways to repair damaged tissue and defence responses to increase pathogen resistance and repair damaged regions (Wang et al., 2020). In carrots, wounding elicits a significant overregulation of phenylpropanoid metabolism as roots switch from sugar metabolism to phenolic metabolism (Han *et al.,* 2017). Upregulation of this pathway, along with the phenylalanine, tyrosine and tryptophan biosynthesis pathway (Figure 1.7.4.0) (including but not limited to the shikimate pathway), and the differential expression of key genes has been described in *Juglans regia* (walnut) (Zhang et al., 2019), tomatoes

(Han et al., 2018) and *Solanum melongena* (eggplant) (Wu et al., 2020). In sweet potatoes, Wang et al., (2020) observed that mechanical damage increased the biosynthesis of phenolics and flavonoids, and that treating roots with benzothiazole effectively increased lignin accumulation whilst decreasing phenolic content.

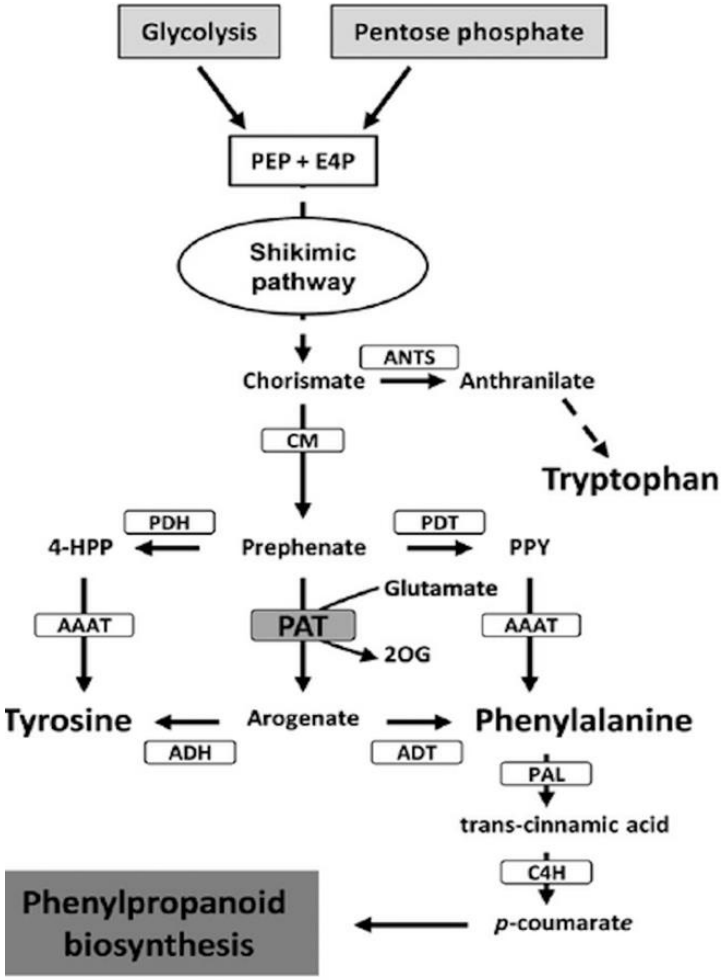


Figure 1.7.4.0) Diagram illustrating biosynthesis of Tyrosine, Phenylalanine and Tryptophan in plants via the Shikimate pathway. Taken from de la Torre *et al.,* (2014).

Genes such as *PAL*, *4CH* (4-courmourate-CoA ligase), and *C4H* regulate the production of phenolic compounds through the phenylpropanoid pathway. Phenylpropanoid biosynthesis produces a number of metabolites that facilitate tissue repair and plant defences (Han et al., 2018): an increase in *PAL* activity is typically associated with the production of downstream phenolic compounds (Figure 1.7.4.2) which then interact with PPO and POD enzymes to cause tissue discolouration.

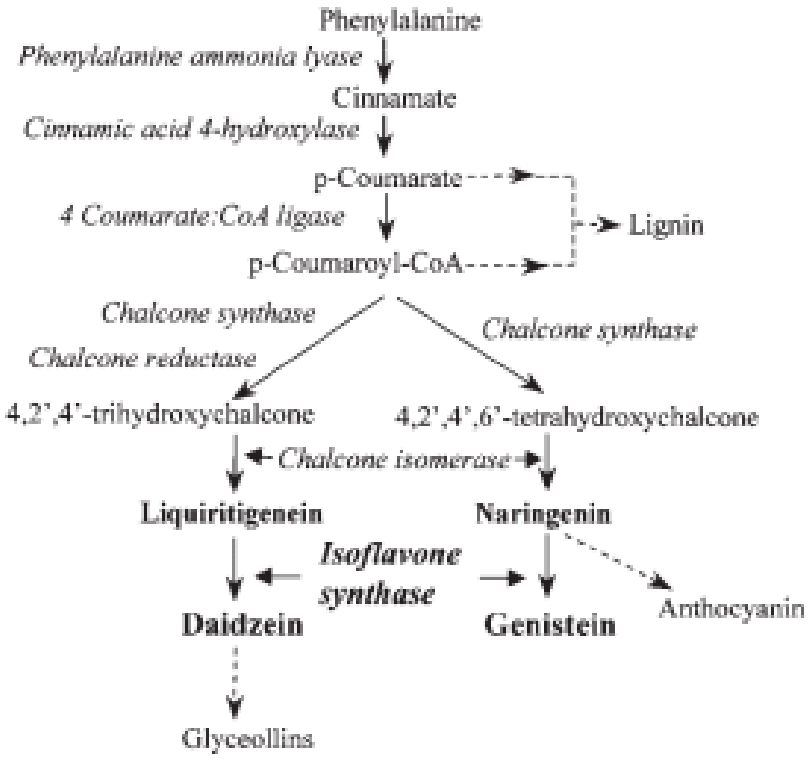


Figure 1.7.4.1) Diagram illustrating simplified phenylpropanoid pathway in plants. Taken from Riyazi *et al.,* (2011).

Different plant species possess a variable number of *PPO* genes, as the *PPO* family ranges from 0 in *Arabidopsis thaliana* (Arabidopsis) to 13 in *Physcomitrella patens* (earthmoss) suggesting a variable range of functions (Tran *et al.,* 2012). Furthermore, in potato tubers Chi *et al.,* (2014) observed that different PPO enzymes contributed variably towards total browning, as just one was responsible for over 50% of oxidative enzymatic activity. Silencing *PPO* genes and concurrent enzyme activity resulted in lower bruising susceptibility in potato and apples (Waltz, 2015) and there is hope that commercial companies can silence *PPO* genes that contribute towards enzymatic browning in bananas, avocadoes, cherries and lettuce.

Over expression of *FAPPO1* in strawberries as a response to wounding was observed by Jia et al., (2015) with 4 *PPO* genes being differentially expressed following damage. Overexpression of *FAPPO1* was found to result in altered expression of *PAL, POD* and other defence related genes. Overexpression of this *PPO* gene also increased PPO activity and delayed fungal infection. Previously in walnut, it was found that silencing of a *PPO* gene (*JrPPO1)* caused the unintended spontaneous development of necrotic spots. Araji *et al.,* (2014) found that in *PPO* silenced walnut lines, tyramine accumulated in large amounts and therefore suggested that *JrPPO1* played a fundamental role in phenolic metabolism.

Damage inducible *POD* genes have been observed in sweet potato (Kim et al., 1999), tomato (Mohan et al., 1993) and potato (Sherf & Kolattukudy, 1993). Li *et al.,* (2010) found that *POD* activity was significantly greater in damaged pear fruit tissue with a chitosan coating enhancing *POD* activity. POD is implicated, in tandem with PPO, as

a key determinant of enzymatic browning in fresh produce, identification of specific *POD* genes in parsnip would be of interest to researchers investigating plant defence responses in general, as well as the more specific bruising response to mechanical impacts. PPO and POD activity have previously been correlated with downstream lignin content, as phenylpropanoids act as substrates for PPO and POD activity (Aquino-Bolanos & Mercado-Sila, 2004).

Identification of key damage inducible genes involved with enzymatic browning in a number of other crop species has facilitated the improvement of cultivars through genomic studies. No previous genetic research relating to enzymatic browning in parsnips has been attempted, thus any information regarding the molecular mechanism of bruising would provide valuable information to breeding programmes.

1.8 Overall objectives of thesis

The overall objectives of this PhD thesis are;

- Identify where post-harvest damage occurs to parsnip roots, and identify the main challenges for post-harvest quality.
- Develop a standard operating procedure for bruise replication capable of reliably exerting industrially relevant bruises.
- Identify the main post-harvest factors driving bruising susceptibility in parsnip roots (e.g. temperature).
- Quantify and analyse post-harvest impact forces to highlight destructive processes and test mitigation strategies to improve post-harvest quality.

- Investigate whether agronomic factors such as variety, harvest date and irrigation significantly affect bruising susceptibility in parsnips and if so, find methods to mitigate the severity of bruising to improve the quality of produce.
- Conduct transcriptomic analysis to identify differentially expressed genes involved in parsnip bruising, to provide information to breeders for selective breeding programmes and cultivar improvement.

Chapter 2. Post-harvest factors affecting bruising in parsnips

2.1 Introduction

Approximately 40% of edible produce in the UK is deemed below marketable quality due to misshapen produce, non-uniformity or aesthetic disorders such as bruising discoloration (FAO, 2015). A number of post-harvest factors have been observed to affect bruising severity and incidence in fruit and vegetables (Opara & Panthare, 2014); factors such as the magnitude of mechanical impact during harvest and processing, post impact storage temperature and storage duration are significant contributors to losses in fresh produce (Van linden *et al.,* 2006), and need to be managed to increase the economic sustainability of the respective crop species.

Fruit wastage from harvest to consumer has been estimated to be as high as 51% by the FAO, (2015) with losses being experiencing along all sections of the supply chain. However, certain processes such as harvesting have been implicated as the main sources of bruising to fresh produce (Aliasgarian *et al.,* 2013). Harvesting costs

typically range from 20-40% of farms total production expenses (He *et al.,* 2007); thus UK parsnip producers have heavily employed mechanisation of harvesting and post-harvest processing to reduce labour costs and increase efficiency. This has had the negative effect of causing additional damage to fresh produce as crops suffer a greater number of mechanical impacts as opposed to hand harvested crops (Brown *et al.,* 1996, Mika *et al.,* 2015).

Roots crops are subject to a multitude of mechanical impact types and sizes (magnitudes) during harvesting, transport, processing and packing. Often mechanical impacts cause visible damage to the outermost regions of produce (i.e. scuffing of the skin), enabling easy identification and removal of damaged produce. However, a lack of visible damage to the outermost skin is not indicative of “perfect” marketable produce. Mechanical impacts are capable of causing subcutaneous tissue rupture leading to bruising (Li & Thomas, 2014) following a loss of cell compartmentalisation and oxidative reactions between cytoplasmic enzymes and vacuole substrates (Mitsuhashi-Gonzalez *et al.,* 2010). Identifying sub-peridermal bruising damage is a challenge for producers, as the damage is often not visible until it has reached the consumer. This latency period in bruise formation has been linked to a delay of polyphenol oxidase activity following mechanical impact and tissue rupture; however, most of the enzymatic reactions causing tissue browning have concluded after approximately 8 hours (Shafie *et al.,* 2015).

Bruise severity typically increases as the time since the impact increases: reducing traveling and storage times is therefore likely to limit bruise formation before produce

reaches the consumer (Hung & Prussia, 1989, Ericsson & Tahir, 1996). This relationship between storage time and bruising severity has been previously observed in apples (Thomson *et al.,* 1996, Yurtle & Erdouan, 2005), pomegranates (Shafie *et al.,* 2015), potatoes (Scharf, 2014) and apricots (Martinez-Romero *et al.,* 2002). Generally, the first incidence of temperature management in parsnip operations is late in the supply chain hours after roots are harvested: the employment of hydrocoolers cool roots to 6 ^{o}C before immediately being packed and entering storage. Kupferman, (2006) noted that harvesting and handling caused apple bruising of up to 35% alone suggesting that the majority of total bruising damage occurs before produce enters temperature controlled storage.

Post impact tissue temperature has previously been observed to influence bruising susceptibility in fresh produce (Dwelle *et al.,* 1977, Thomson *et al.,* 1996, Ahmadi, 2012) with studies demonstrating bruise severity and incidence being stunted following good temperature management (Ferreria *et al.,* 2009, Bugaud *et al.,* 2014). Keeping storage temperatures low (approximately 6 ^{o}C) during processing, storage and transport is employed in avocado fruit to limit bruise formation and delay ripening, thus preserving freshness for the consumer (Mazah *et al.,* 2018), as temperature positively affects enzymatic activity. Prolonging the period of time between harvesting and cold storing produce has been demonstrated to increase senescence and loss of turgidity (Bollen, 2005), which reduces the overall quality of produce.

Previous studies have suggested that cooling produce immediately following harvest may be used to limit bruise formation (Ferreira *et al.,* 2009) and improve shelf life

(Tahir, 2006). Due to the centralisation of the UK parsnip industry, roots now travel further from their harvesting site to packhouses for processing and are transported in 14 tonne lorries for up to 4 hours, before entering processing in temperature-controlled conditions (personal communication with industrial participants, 2018). Assessing how post-harvest temperature management of parsnips affects bruise formation may provide crucial information to producers on how management of procedures and logistics can limit bruise formation and severity. Increasing energy exerted by mechanical impacts from equipment, other produce or stones has a positive effect on bruise formation (Banks *et al.,* 1991). The bruising susceptibility of specific crop species is, by proxy, a measure of their response to mechanical impact (Van linden, 2006) and is dependent on a myriad of factors affecting the biological characteristics of the cell (for example turgor pressure or phenolic compound activity). Reducing the amount of energy exerted onto roots during processing should be a key area of focus for ongoing improvement amongst producers, however it is difficult to quantify impacts experienced by produce reliably to allow identification of destructive procedures and test improvements (Praeger *et al.,* 2013).

Due to the significant variability in processing procedures and equipment witnessed within, but especially between, crop species (Yu *et al*., 2014), in order to test methods to reduce bruising, it is of key importance to produce a species-specific bruising protocol that replicates the respective industry-specific impact magnitudes and produces relevant bruising. Furthermore, replicating bruising over several impact magnitudes witnessed during industrial harvesting and processing would provide producers with evidence and motivation to reduce the impact forces their equipment

exerts on produce, if it becomes clear that industrially relevant impacts can lead to severe bruising, and quantifiable improvements can be achieved (Figure 2.1.0).

When quantifying formed bruises, several characteristics should be considered, such as bruise dimensions, bruise colouration, and the number of bruised regions to measure bruising susceptibility accurately. Furthermore, the size of bruises produced has previously correlated with the size of produce (Opara & Panthare, 2014), therefore normalising bruising scores for produce size must be performed to allow a reliable and respective comparison.

Figure 2.1.0) Authors own images (i & ii); i) left – Fully processed parsnip roots displaying bruising damage in polyethylene storage bags along with electronic parsnip device ; ii) right – Loose parsnip roots for sale at UK supermarket displaying bruising, scuffing and splitting damage.

2.1.1 Aim

This chapter aimed to produce and test a bruise replication protocol and a bruise severity score specific to parsnip roots. Furthermore, this body of work aims to investigate the significance of impact magnitude, post-impact storage duration and post impact storage temperature as factors affecting bruising in parsnips. Producing a reproducible protocol capable of exerting forces witnessed during processing, and reliant quantification of resultant bruising, is of vital importance to this PhD project in order to investigate how a myriad of pre- and post-harvest factors affect bruising.

2.1.2 Objectives

1) To test bolt impact bruise replication protocols and quantify the bruises that form following impact
2) To assess the significance of post-harvest factors (size of impact, storage duration and storage temperature) affecting bruise susceptibility in parsnips

2.1.3 Hypotheses

H_0= Impact magnitude (J) exerted on roots has no significant effect on bruising susceptibility

H_1= Storage duration (hours) following impact has no significant effect on bruising susceptibility

H_2= Storage temperature (°C) has no significant effect on bruising susceptibility

2.2 Materials and methods

2.2.1 Bruise replication

Parsnip plants (V2) were harvested by hand from an Elsoms Seeds Ltd. trial site near Worksop, United Kingdom. V2 was chosen as, during previous variety bruise susceptibility testing, it displayed a moderate susceptibility to bruising ranking 2nd in a 3-way varietal comparison (Authors own data, not shown). Parsnips were manually topped, washed, and then transported to the University of Birmingham for bruise susceptibility testing. Each experimental group contained 10 or 11 roots, all of which had a crown diameter between 40 – 70 mm (commercially acceptable size) and did not exhibit any signs of infection or mechanical damage. Before impact testing, all roots were acclimatised for an hour at room temperature.

To investigate the effect of storage time (at 12, 24, 48, and 72 hours), storage temperature (at 6 ^{o}C or 20 ^{o}C); and impact magnitude (low, medium, high, and extreme impacts) on bruising susceptibility roots underwent a multivariant experiment. Following impact replication and storage, parsnips were analysed to assess bruise size and severity using a potato peeler, scalpel, and callipers to identify and produce a bruising susceptibility ratio. Roots that did not exhibit any bruising were removed from the bruise severity analysis, this is presented in the percentage (%) bruising likelihood comparing the number of roots per treatment that bruised.

To achieve bruise replication, 3 steel weights weighing 100, 200 and 500 grams were dropped from a height of either 0.75 or 1.25 metres through perforated PVC pipes onto the crown's widest point on a parsnip root at the pipe base. This lead to falling bolt impacts with gravitational potential energies (PE) of 0.49 J, 2.45 J, 4.49 J and 6.13 J (respectively categorised as low, medium, high and extreme impact magnitudes) being exerted on roots. The actual impact force exerted onto roots (gravitation potential energy (PE)) was calculated using the following formula:

(PE = m * ag * h)

where m: mass

ag: acceleration due to gravity

h: height above the surface

Each weight had a small circular piece of metal attached to the contact point with the roots to ensure that the contact between falling weight and root was consistent. After testing, roots were stored in polyethylene bags to mirror their treatment in industry, as the experimental groups chosen in this study across all factors have been chosen to generate data that can be applied for the industrial management of bruising in parsnips. Replication of the industry-relevant injury levels and storage conditions experienced by parsnip roots is important to provide industry-applicable data to producers. For each impact magnitude, roots were removed after 12 hours, and then at 24-hour intervals following bruise inducement, for a total of 72 hours. Bruise development was assessed over the 4 time intervals, at 2 different storage temperatures. The effect of storage temperature was investigated as 50% of roots were stored at 6 ^{o}C and the remaining 50% at 20 ^{o}C. Roots in industry are typically

hydro cooled to reduce root temperature to approximately 6 ^{o}C before storage at > 6 ^{o}C for up to 24 hours before transportation. However, not all transportation and supermarket storage units are refrigerated, and roots are displayed to consumers at room temperature, so root temperature is not always consistently low.

2.2.2 Bruise quantification

This study employed a modified methodology described by Scharf, (2014) where in addition to the physical size of the bruise (depth and width), the bruise severity (i.e. colouration) is recorded on a colorimetric scale of 0 – 3 in addition to the crown diameter to accord for variable root size. The score given to each sample was based on the modified colorimetric scale displayed in Figure 2.2.2.0 where a score of 1 indicated very slight discolouration, 2 indicated a darker orange colour, and 3 was given to samples where a dark brown/black was visible in addition to orange regions and 0 given to control regions of tissue. Physical measurements were recorded using a digital set of callipers, accurate to 0.1 mm. Roots that did not bruise were not included in bruise severity analysis; however, the number of roots that did not bruise was recorded and presented as % likelihood to bruise following mechanical impact.

Henceforth, bruising severity (BS) has been calculated using the following formula:

$$BS = ((BD*BW*BI)/CD)$$

where *BD*: Bruise depth (mm)

BW: Bruise Width (mm)

BI: Bruise intensity (0 – 3 colorimetric scale)

CD: Crown diameter (mm)

Figure 2.2.2.0) Colorimetric scale employed for quantifying bruise severity in parsnips.

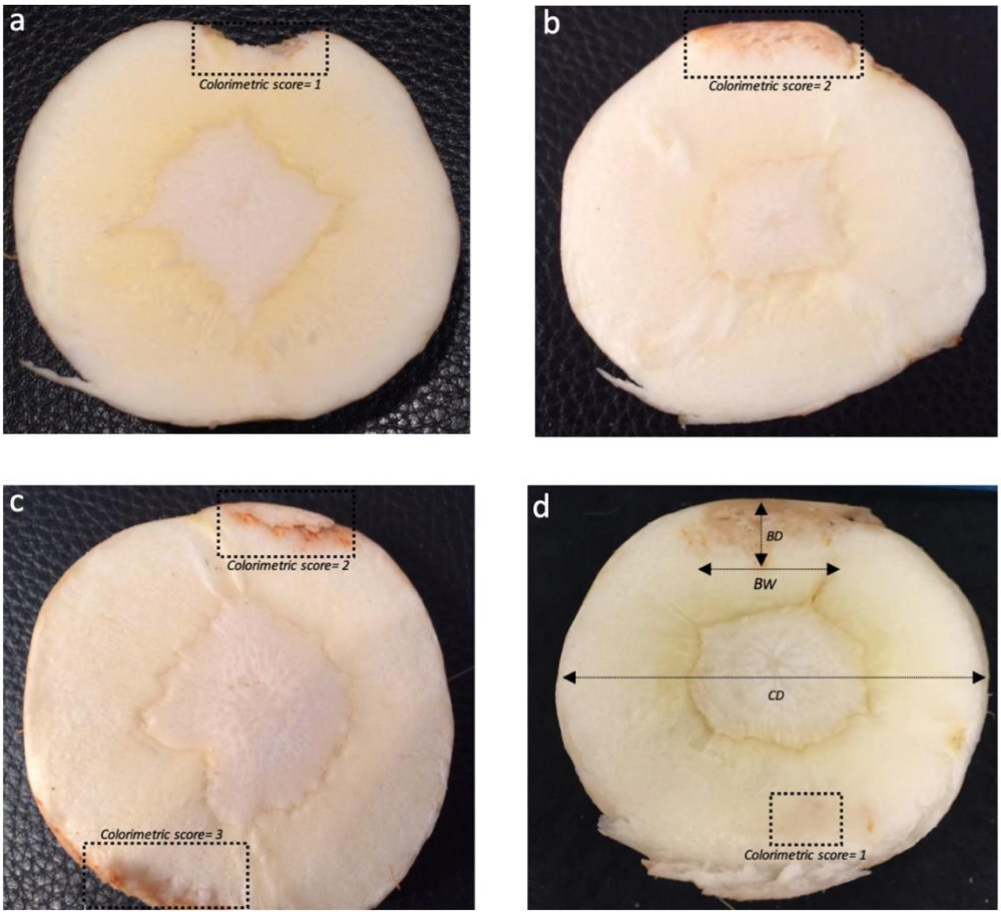


Figure 2.2.2.1) Set of author's own images showing cross sectional slices of parsnip tissue (with bruised subcutaneous regions of tissue highlighted by dashed boxes). (a) Image showing section of bruised tissue with colorimetric score of 1. (b) Image showing section of bruised tissue with colorimetric score of 2. (c) Image showing sections of bruised tissue with colorimetric scores of 2 and 3. (d) Image showing section of bruised tissue with colorimetric score of 1 with *BD* (bruise depth mm), *BW* (bruise width mm) and CD (crown diameter mm) also highlighted.

Statistical analysis and figure production was performed using R Studio Version 0.99.903 (© 2009-2016 RStudio, Inc.) and Prism 8 Version 8.4.3 (471) (June 2020, GraphPad Software, LLC.).

2.3 Results

2.3.1 The effect of impact magnitude, storage time and storage temperature on bruising susceptibility in parsnips

To investigate how impact magnitude (g), time (hours) and storage temperature (ºC) affect bruising susceptibility, analysis of variances (ANOVAs) were employed. Irrespective of storage temperature, impact magnitude (p=<0.001) and storage time (p=<0.0001) were found to significantly affect bruise severity in parsnip roots (Figure 2.3.1.0). For those roots that were stored at 6 ºC; impact magnitude did not significantly affect bruising severity (*p*=0.161) whilst time did (*p*=<0.001), which may indicate that low storage temperatures limit bruising severity following large impacts. In contrast, storing roots at 20 ºC resulted in impact magnitude (*p*=<0.0001) and time (*p*=<0.01) both significantly affecting bruise severity, indicating that storing roots at higher temperatures increases the influence of high and extreme impacts on bruise formation (Figure 2.3.1.1).

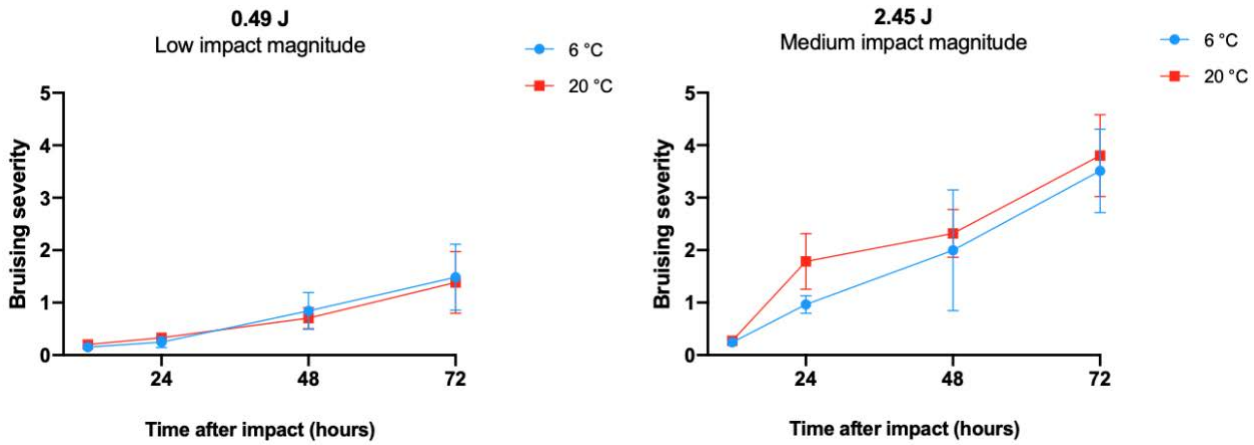


Figure 2.3.1.0) Graphs illustrating the effect of impact magnitude (Low & Medium, 0.49 & 2.45 J respectively) storage duration (12, 24, 48 and 72 hours) and storage temperature (6 ºC and 20 ºC) on bruising severity in V2 roots. (*N*=10-11). Error bars show standard error of the mean (SEM).

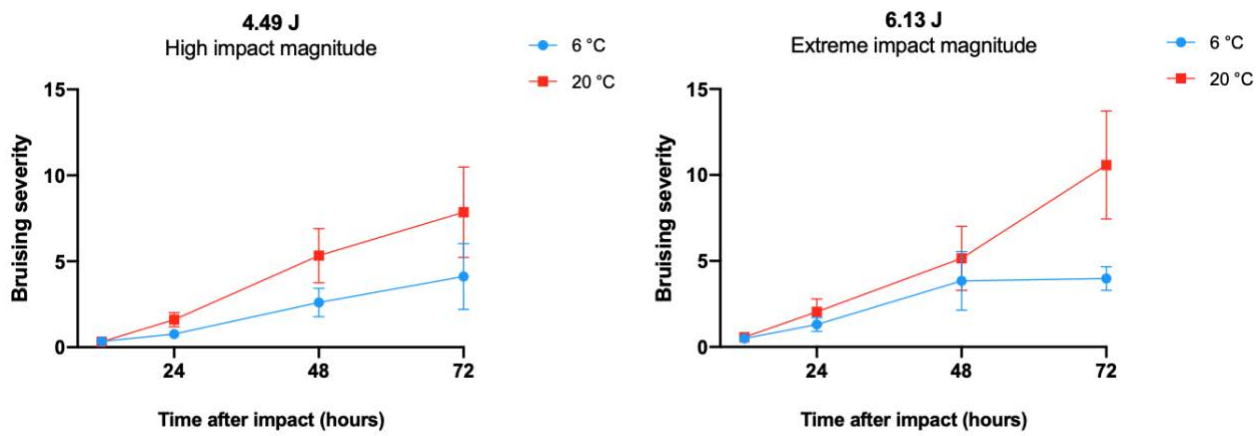


Figure 2.3.1.1) Graphs illustrating the effect of impact magnitude (high & extreme), storage duration (12, 24, 48 and 72 hours) and storage temperature (6 ºC and 20 ºC) on bruising severity in V2 roots. (*N*=10-11). Error bars show standard error of the mean (SEM).

Bruising severity increased as impact magnitude increased (Figure 2.3.1.0), with the most severe bruising being witnessed in the extreme impact group after 72 hours at 20 ^{o}C storage (10.58 ± 3.14). In comparison, low impacts elicited a bruising response of 0.20 ± 0.10, medium 1.60 ± 0.48 and high 5.33 ± 1.78 following identical storage conditions. Bruising severity increased as time progressed across all impact magnitudes and storage temperatures with severe bruises being present after 48 hours of storage at either temperature. This may suggest that post impact, storage duration and temperature play a crucial role in the formation of bruises and that minimizing duration and temperature may limit bruise formation; this confirms trends witnessed in other crop species (Bugaud *et al.,* 2014; Mazhar *et al.,* 2018), where significant reductions in tissue browning has been observed.

Temperature significantly affected bruise severity following large impacts after 48 hours and 72 hours of storage. Storing roots at 6 ^{o}C rather than 20 ^{o}C significantly reduced bruise severity from 10.58 ± 3.14 to 3.98 ± 0.64 following extreme impacts and 72 hours of storage (p=<0.001), whilst after 48 hours of storage following high impacts, bruise severity was significantly reduced from 5.33 ± 1.78 at 20 ^{o}C to 2.60 ± 0.82 (p=0.031) at 6 ^{o}C.

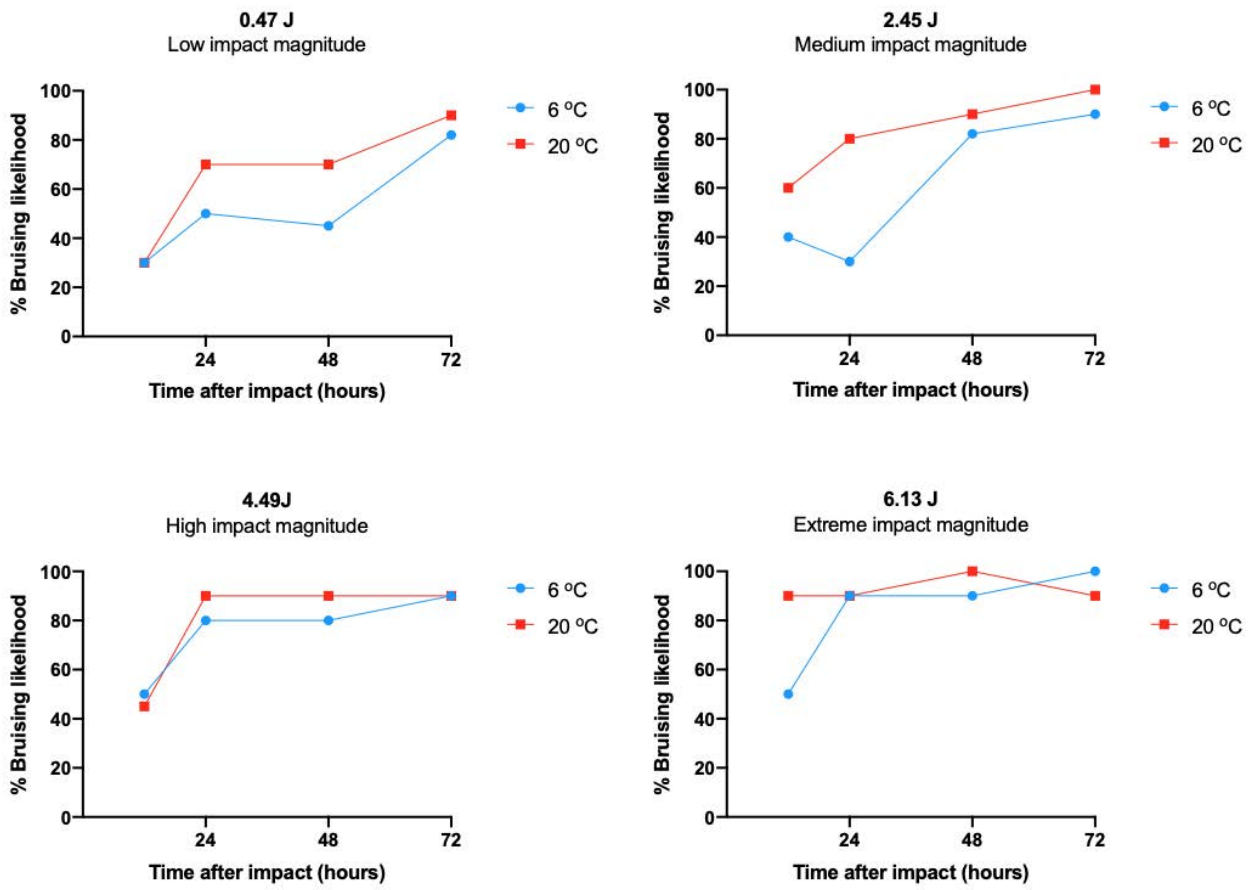


Figure 2.3.1.2) Graphs illustrating the percentage bruising likelihood of parsnip roots following bruise replication testing and storage (*N*=10-11). For this figure the data used were replicated values as percentages were used to compare between groups.

The percentage of parsnips that elicited bruising following impact testing increased as time since impact increased for the majority of groups with few exceptions (Figure 2.3.1.2). At low and medium impact magnitudes, storing roots at 6 °C reduced the likelihood of bruising after 72 hours of storage by 8% and 10% respectively, in comparison to roots stored at 20 °C. Following larger impacts (high and extreme groups) keeping roots at 6 °C instead of 20 °C did not limit the number of roots that bruised as effectively as it did for smaller impacts. Following high impact magnitudes and 72 hours of storage, 90% of roots elicited bruising at both 6 °C and 20 °C, whilst extreme impacts caused 100% and 90% of roots to bruise at 6 °C and 20 °C

respectively. These results suggest that large mechanical impacts may cause 90% + of roots to bruise after 72 hours of storage, with good temperature management contributing little in a reduction of bruising witnessed. Reducing the size and number of impacts experienced by roots is therefore a key factor in reducing postharvest losses across the industry; modifications to equipment and procedures require quantified real time data (Praeger *et al.,* 2013),that is not currently available to UK parsnip producers.

This study suggests that increasing impact magnitude, increasing storage duration and higher storage temperatures contribute to greater bruise formation and severity in parsnips. This provides crucial information to producers so they may employ logistical changes to their operation, and start identifying current processes that are particularly destructive and detrimental to root quality.

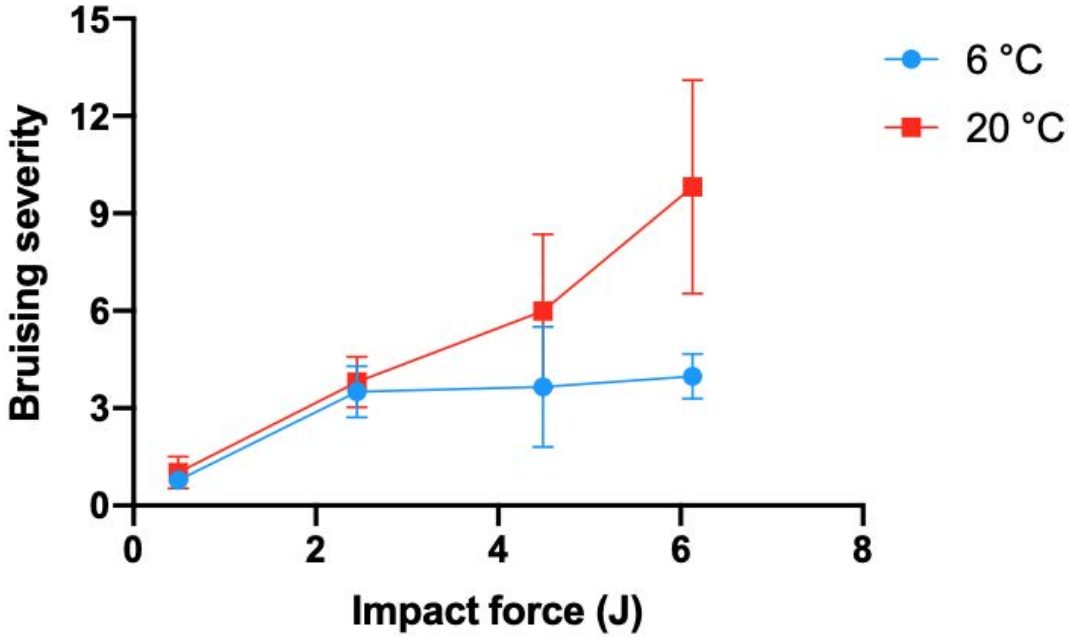


Figure 2.3.1.3) Graph illustrating bruising severity after 72 hours of storage across a range of impact force (J) values at 6 ºC and 20 ºC. (*N*=10-11). Error bars show standard error of the mean (SEM).

2.4 Discussion

Previous research in apples found that increasing impact energy during bruise replication lead to greater bruising damage, but that it was possible to employ mitigating strategies, such as specially designed packaging, to reduce impact forces exerted onto apples during processing (Fadiji *et al*., 2016). Hussein *et al*., (2019) found that bruising susceptibility in pomegranate fruit increased as impact force increased; concurring with the findings from our study. However they also observed, that fruits stored at 5 ºC bruised less than those stored at 20 ºC. Respiration and weight loss increased as storage duration increased, as pomegranate fruits exposed to larger impacts experienced up to 300% greater respiration rate and weight loss than control fruit (Hussein *et al*., 2019). In contrary, this study found that parsnip roots impacted with a force that exceeds 4.49 J (high and extreme) and stored at 6 ºC exhibited a lower bruising severity than roots stored at 20 ºC. Storing roots at low temperatures (6 ºC) did not reduce bruising severity as effectively as for impacts >4.49 J (low and medium) but it did reduce the bruising % likelihood.

For impacts that exceeded 4.49 J, temperature did not affect % bruising likelihood to the same degree as for smaller impacts. Therefore, it would appear that there is a bruising threshold in parsnip tissue that when exceeded, storage temperature can reduce the bruising severity witnessed, but not the % likelihood to bruise. However for bruises under that threshold, temperature can limit the % of roots that bruise, but not the severity of bruises that do form. We suggest that threshold is approximately 4.49 J. Reducing the number of impacts that exceed this value during processing should be

a key area of improvement. This study focussed on the bruising response of V2 roots, thus given the varietal differences witnessed in browning between varieties in parsnips (Toivonen, 1992), future work should include multiple varieties of parsnip to gain variety specific bruising thresholds. A dearth of previous research exists focussing on bruising thresholds in fruit (Pang *et al.,* 1994; Öztekin,& Güngör, 2020) whilst varietal variation in bruising response to different drop heights has also been observed (Hussein *et al.,* 2019).

Parsnip root temperature is controlled once roots are hydrocooled and enter packhouse storage; whilst this will limit bruise formation, this study's results indicate that more needs to be done regarding the temperature of roots during harvesting and processing. Furthermore, the current procedure of transporting and presenting of roots unrefrigerated during transit and in supermarkets may cause greater bruising incidence and severity. The onus is also on supermarkets to expand refrigeration across transportation and supermarket storage to ensure that root temperature is minimised across the whole supply chain to ensure produce quality for consumers. Reducing the amount of time that roots are stored before reaching the consumer is not only beneficial for the general quality of the produce (Bugaud *et al*., 2014), but it may reduce the occurrence and severity of bruising observed by the consumer. Minimising the length of time that roots spend in transportation to and from packhouses, in supermarket storage and on shelves is a logistical solution to a biological problem that may reduce bruise formation and severity in parsnips. Reducing storage time from 72 hours to 24 hours would reduce bruise severity following impacts of all magnitudes, it would also reduce the frequency of roots that bruise.

During processing, there are many pinch points, such as intake, where roots spend significant portions of time not being processed preceding hydrocooling. More efficient logistical planning could reduce the impact bottlenecks currently have on overall production times in parsnip packhouses, by ensuring a smooth flow of roots through processing.

However, even with good temperature management, bruises have been shown to form after infliction of all impact magnitudes tested, at all timepoints, as even roots subjected to low impact magnitudes and then stored at 6 ^{o}C still bruised to some degree (Figure 2.3.1.2). Post-impact root temperature during storage and storage duration are results of current practices that can be easily manipulated, independent of the mechanical processes currently installed in UK packhouses. Whereas, the force of impacts experienced by roots stems from mechanical processes exerting physical forces onto roots throughout harvesting and post-harvest processing. The ongoing centralisation of UK parsnip production both helps and hinders progress; there are fewer packhouses to analyse, thus any changes will have a large effect on the industry as a whole. However, as the acreage each packhouse processes continues to grow, as do the travelling times from harvest site to packhouse increasing the likelihood for bruise formation before roots are processed. Furthermore, ever larger packhouses require ever increasing mechanisation and economies of scale introducing sources of bruising damage for roots with no idea on how this will affect root quality.

Identifying processes that cause roots to bruise, and quantifying the force exerted are steps necessary for producers to reduce the impact magnitudes roots experience

during processing. This approach, when coupled with more efficient temperature and temporal management of roots, offers an opportunity to reduce the occurrence and severity of parsnip bruising. Quantification of impacts experienced by roots during processing is recommended for future study in order to produce a bruise replication protocol that exerts industry specific impact magnitudes onto roots.

After assessing V2 bruising susceptibility under a range of impact magnitudes, it was decided that for future varietal testing (chapter 4 and chapter 5), this study would employ the falling bolt 4.49 J protocol (high impact magnitude), storing roots at 20 ^{o}C for 48 hours. The justification for this decision was that the bruises that formed were not significantly different than bruises formed after replication extreme magnitudes, and this size of impact was found to be commonly exerted onto roots during processing (chapter 3).

Chapter 3 An investigation into post-harvest processing of parsnips in the UK

3.1. Introduction

Bruising damage constitutes the majority of mechanical damage witnessed in fruits (van Zeebroeck *et al.,* 2006; Lu *et al.,* 2010; Fadiji *et al.,* 2016; Bantayehu & Alemayehu, 2019) resulting in significant economic losses for producers (Schulte *et al.,* 1992; Lu *et al.,* 2010), and reduction in appeal and quality for consumers (Manetto *et al.,* 2017). Bruising of subcutaneous tissue is also a significant cause of rejection in root vegetable crops such as potatoes (Praeger *et al.,* 2013), sweet potatoes (Tomlins *et al.,* 2000), carrots (Galati *et al.,* 2005) with enzymatic browning in parsnips previously being observed by Toivonen, (1992). Root crops suffer physical impacts whilst undergoing harvesting, transport, intake, cleaning, grading, polishing, bagging and storage.

Mechanisation is employed during harvesting and post-harvest processing of parsnips to reduce labour costs and improve capacity and efficiency. The employment of heavy machinery and conveyers to transfer roots between processing stages introduce further sources of damage, as roots collide with machinery; experience drops between conveyers, and are hit by other roots and stones. Compressive forces are also exerted onto produce during transport, with typically 14 tonnes of roots transported in a single transport lorry. Roots are compressed against each other, stones or the metal sides and floor of the transporter. Furthermore, transportation exerts unavoidable vibration

forces on produce (Fernando *et al.,* 2019), perhaps severe enough to cause membrane leakage, cell rupture and enzymatic discolouration.

Parsnip processing in the UK has become progressively more monopolised and centralised, with the vast majority of UK parsnips being processed in one out of approximately 15 pack houses. This drive for efficiency has drastically increased the flow rate of roots being processed by each producer, causing a demand for an increase in productive capacity in the processing plants. It is clear that a systems approach is required to identify critical processes where excessive impact forces are exerted onto roots that may cause bruising damage, in order to increase the sustainability of parsnip processing.

Directly quantifying the impact forces experienced by produce within harvesting and packhouse operations has been achieved in a plethora of crops by employing various instrumented devices that log acceleration and velocity change (VC) data (Praeger et al., 2013). Previous studies highlight critical processes that exert significant forces onto produce and attempt to test modifications applied to reduce impact forces exerted and compare results between producers to produce guidelines to best working practices (Roa *et al.,* 2015). There has been no previous coordinated effort to quantify parsnip processing damage across the UK parsnip industry, however individual packhouses have tested equipment and modifications before but found it difficult to reliably quantify impact data.

As the consumer demand for high quality, blemish free and uniform produce increases, producers are required to match that demand by increasing parsnip processing's economic sustainability via damage mitigation methods. Mechanical damage, pests, pathogens and non-uniformity are characteristics that lower the economic value of the produce to the producer as consumers view such produce as inferior (Matzinger & Tong 1993). To quantify processing damage inflicted onto produce is the first step to identify destructive processes or transfer points, modifications can then be tested to find least destructive working practises and equipment.

Quality control report- Strawson's Ltd		
Reason for rejection	07/11/2019, *N*=87	08/11/2019, *N*=73
Defect	%	%
Bruising	22.39	19.79
Sliced crown	2.77	2.99
Pests	0.00	8.03
Misshapen	11.59	5.25
Undersize	3.84	3.36
Oversize	3.98	7.14
Packout	44.87 %	48.75 %
Defect	55.13 %	51.23 %

Table 3.1.0) Table illustrating quality control data from parsnip packhouse for two dates in November 2019 providing reasoning for rejection at Strawson's Ltd packhouse.

Given that packhouse losses regularly exceed 50 % (Table 3.1.0) with bruising often representing that greatest contributor to rejection, it is clear that post-harvest management strategies are required to improve the sustainability and profitability of the industry.

3.1. Harvest and post-harvest processing of parsnips in the UK

3.1.1. *Harvesting*

Figure 3.1.1.0) Authors own image illustrating electronic parsnip device (A) inserted into freshly topped row of parsnips. Also shown is loading elevator chute (B) and loading trailer (C).

Parsnips are typically harvested between 3 – 9 am to ensure that root temperature is as low as possible during harvesting and transportation to limit spoilage. The vegetation from the plants are removed firstly row by row by a topper (Figure 3.1.1.0). Once topped, the harvester runs down the planted rows and roots are lifted out of the soil by metal “tongues” acting as elevators, then the produce runs up sieve agitation webbing to remove soil and stones from the crop. The roots are carried up an agitation webbing series before reaching the loading chute, where they are propelled from a

conveyor into a transport lorry travelling parallel to the harvester (Figure 3.1.1.1). A proportion of harvesting operations utilise an additional soil removal stage before transportation to the packhouse (Figure 3.1.1.2). During soil removal the harvested roots are unloaded into loading hoppers and ran up another series of agitation webbing before being transferred into a transport lorry.

Figure 3.1.1.1) Image from Grimme Ltd. (2020) illustrating roots being lifted from ground (A) by lifting equipment and running up agitation webbing (B) to remove stones and soil before being elevated by loading conveyor C).

Figure 3.1.1.2) Authors own image illustrating whole harvesting and transport operation at a parsnip producer in the UK. Shown is A) the hydraulic lifting of the loading lorry into B) the soil removal stage, C) before roots are elevated and dropped into the transport lorry.

3.1.2. Intake

After transportation from field to packhouse, roots await entry into the packhouse in transport trailers (Figure 3.1.2.0). Parsnip intake procedures vary significantly between packhouses across the UK with some employing water and gravity to remove roots from transporters, whilst others simply use gravity. Roots are removed from the transport trailers by lifting the trailer up vertically via the use of hydraulics; utilising gravity to remove roots. Most packhouses also employ overhead hanging pipes (Figure 3.1.2.1) which propel water onto roots, flushing them from the trailer onto conveyors or water pools. Unloaded roots are typically transported up conveyors into a cyclone

destoner, where roots are cleaned and separated from soil and stones in a rotating pool of water which controls the flow of roots into the packhouse (Figure 3.1.2.2).

Figure 3.1.2.0) Authors own image illustrating parsnip roots and electronic parsnip in 14 ton transport trailer awaiting unloading and intake into the packhouse.

Figure 3.1.2.1) Image by Haith Ltd. (2020) illustrating unloading roots (carrots in this case) from transport trailer via use of gravity and overhead water pipes.

Figure 3.1.2.2) Image by tmicltd UK, 2020 illustrating parsnip roots being cleaned in a cyclone destoner following unloading from transport and intake into the packhouse.

3.1.3. Manual inspection

Following cleaning, roots are subject to manual inspection and trimming to remove diseased, damaged or misshapen roots. Roots that pass inspection are trimmed to a length of 170mm. Conveyors carry roots from the previous process to a manual inspection area where a number of stations with accompanying staff hand pick roots to inspect, cut to length and remove any remaining foliage attached to the crown (Figure 3.1.3.0). Roots that do not conform to specifications are dropped onto waste conveyor belts. Once cut, roots are allowed to continue on the main conveyors to the next processing stage. A number of packhouses use a large machine (a trimmomatic) to trim roots to length: however, this still requires a significant amount of man power as each parsnip has to be manually loaded into the trimmomatic machine.

Figure 3.1.3.0) Image by Poskitts Ltd, 2020 illustrating manual inspection stations in a parsnip processing packhouse in the UK.

3.1.4 Polishing

Roots are transported by conveyor and dropped into a loading hopper before entry into the polisher. Typically polishers are employed by producers for two reasons: firstly, polishing removes the first few layers of outer skin and any small potential blemishes, and secondly to clean roots to ensure that they are as lightly coloured and smooth to the touch as possible.

Figure 3.1.4.0) Image by Haith Ltd, 2020 illustrating parsnips exiting rotating drum of the polisher into a pool of circulating water.

The polisher in Figure 3.1.4.0 works by employing a rotating metal drum which forces roots to move along the bottom of the polisher, and the resulting friction caused between root and metal drum polishes the root. Not shown in is the exit door of polishers, which facilitates the control of the exit flow of roots via manipulation of the angle of the exit door (partially shown in Figure 3.1.4.1). If roots are unable to exit the polisher due to a blockage of roots or a partially closed door, they are picked up by the rotating drum and moved back to the starting end of the polisher. Other polisher types (Figure 3.1.4.2) have a number of independently moving brushes on the inside of the rotating drum causing friction between the brushes and root skin (Figure 1.1.4.1). The speed of the rotating barrel/drum and brushes can be changed in response to the flow of roots in the packhouse.

Figure 3.1.4.1) Image by Haith Ltd 2020 illustrating empty root vegetable polisher with independently moving brushes lining the inside of the rotating drum . Not shown is exit door which facilitates users to increase or decrease the period of time roots spend in the polisher and therefore the flow of roots out of the polisher.

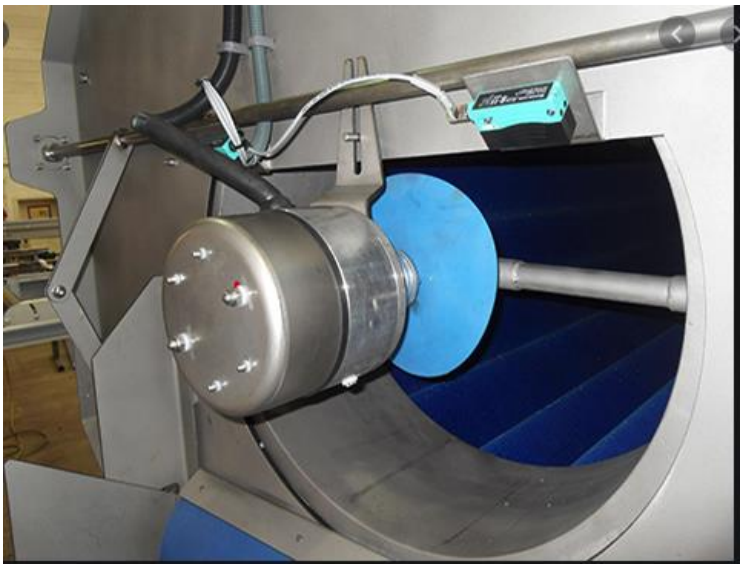


Figure 3.1.4.2) Image by Haith, 2020 showing the inside of a root vegetable polisher.

3.1.5 Grading

Illustrated in Figure 3.1.5.0 is a typical parsnip grader used by producers to separate roots based on crown width. Roots move across the metal rollers, with the smallest roots falling through first onto the first conveyor below the grader. Larger roots fall through later and land on the second conveyor below the grader.

Figure 3.1.5.0) Image by Haith Ltd, (2020) illustrating grading of parsnip roots by crown diameter (mm) in a UK parsnip packhouse.

3.1.6 Hydrocooling

Before storage, root temperature is dramatically reduced via employment of a hydrocooler (Figure 3.1.6.0) where cold water (approximately 6 ^{o}C) falls onto a conveyor loaded with roots. The conveyor moves slowly, thus roots spend approximately 15 minutes being cooled before entering the next processing stage.

Figure 3.1.6.0) Image by Haith Ltd (2020) showing root hydrocooler reducing the temperature of carrot roots before they enter cold storage.

3.1.7 *Packing*

Each conveyor takes the corresponding roots to their respective packing areas; typically larger roots are sold loose in crates, whilst smaller roots are sold in 500 gram supermarket packets or in stew packs (Figure 3.1.6.0 (left)). One of the reasons why smaller parsnips typically make up the supermarket packs is due to overweighing – if producers packed two larger roots into a “500 gram” pack they may pack significantly more than the 500 grams they are paid for by the supermarket. It is therefore easier to get as close as possible to whatever weight designated using smaller roots that weigh approximately 100 – 150 grams rather than larger roots that are sold loose (200 grams +). Roots are elevated on a Newtec conveyor to enter each respective packing

process. For supermarket bagging (Figure 3.1.6.1 (right)) 3 – 4 roots fall through a chute into a waiting bag below, where an automated machine then weighs and heat seals the bag.

Figure 3.1.7.0 (left)): Authors own image of parsnip roots for sale in a UK supermarket in 500g plastic packs. Figure 3.1.7.1 (right): Image by Haith, 2020 of parsnip roots exiting the hydrocooler and collecting in the hopper that precedes Newtec lifting for packing.

During loose crate packing, roots enter a number of loading hoppers suspended above an empty crate. Once the weight required has been reached, all hoppers open causing roots to fall typically a few feet into the crate below. The roots fall into a larger bag inside the crate, which is then sealed until roots are presented to the consumer (Figure 3.1.6.2). However, in several packhouses roots were instead allowed to fall over 3 feet

into an empty crate with no interior bagging, before being moved into storage (Figure 3.1.6.3).

Figures 3.1.6.2 & 3.1.6.3 (from left to right); Authors own images illustrating bagged and loose parsnips for sale in a UK supermarket. Also shown is the electronic parsnip inside storage plastic bag along with roots, a number of which possess large bruises. Loose parsnips for sale are typically the largest roots. Significant amount of mechanical damage is present on roots in both images.

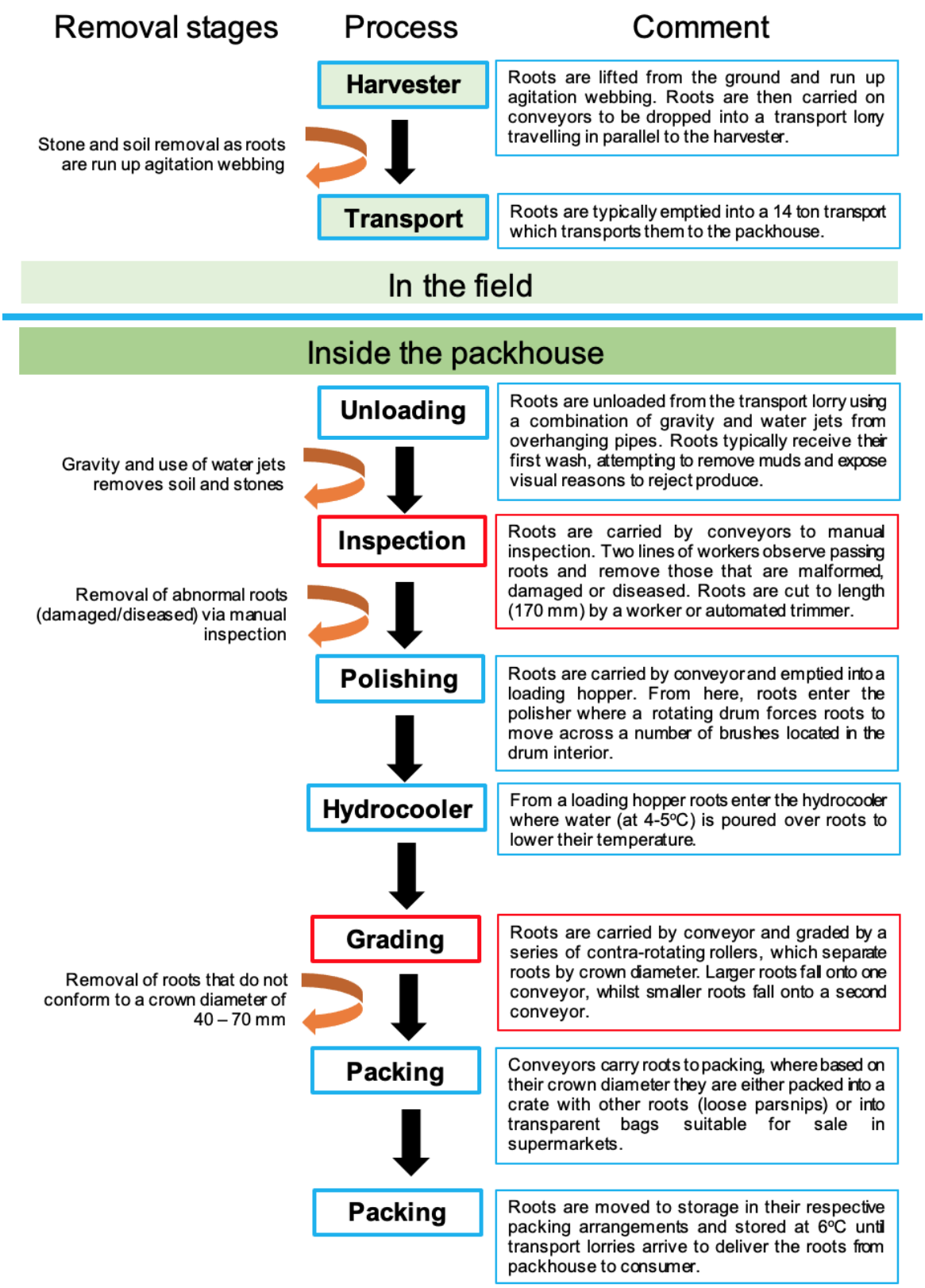


Figure 3.1.6.4) Authors own workflow detailing processes harvesting-packing.

The objective of this research is to identify critical processes, equipment or transfer points where impact forces may exceed the bruising threshold for parsnips roots in a nationwide study across the UK, participating with the majority of the parsnip processing industry. Comparing results from specific processing stages across industry may highlight working practices or equipment that are less detrimental to root quality, and modifications to equipment can be tested to identify the least destructive practices.

The hypotheses of this chapter were as follows;

***H_0*:** There is no significant difference in the incidence of bruising below the peridermal layer between stages of post-harvest processing.

***H_1*:** There is no significant difference in the incidence of scuffing between stages of post-harvest processing.

***H_2*:** There is no significant difference in the impact damage data witnessed between processing stages across an individual parsnip packhouse (Packhouse B).

***H_3*:** There is no significant difference in the impact damage data witnessed between specific processing stages across the parsnip processing industry.

H_4: No significant reductions in impact damage during processing can be achieved through modifications to polishers.

3.2. Materials and methods

3.2.1. Section A) Identification of post-harvest processing procedures that cause mechanical damage in parsnips at packhouse A

In order to analyse which procedures and equipment cause bruising damage and breakage of the perdiermal layer (scuffing) damage throughout the post-harvest and packaging process, an exploratory study was conducted at a participating packhouse in October 2018. The partipiating processor is based in the north of England and is one of the largest producers of carrots and parsnips in the United Kingdom.

A total of 120 roots were removed from six stages of processing (post harvesting; post-soil removal; post-destoner; post-manual inspection; post-grading and post-packing). Only roots that conformed to the size and shape of supermarket standards were removed (40 - 70 mm crown diameter at its widest point) as it was deemed unnecessary to analyse the damage present in roots that are commericially irrelavent.

Roots were transported to the University of Birmingham where they were stored in a temperature controlled room at 4 °C for 24 hours after being washed by hand. Following cold storage, roots were left for a further 24 hours at a room temperature of approximately 10 °C before any analysis occurred. The rationale for storing roots in this manner was to generate a methodology that replicates the storage conditions witnessed in industry. Following incubation, roots were cut transversely at 10 mm

intersections to allow visual analysis of tissue and whether and where damage occurred. The location and type of damage was recorded as; crown/taper and bruising/scuffing (Figure 3.2.1.0) after 48 hours of storage.

Figure 3.2.1.0) Author's own images illustrating post-harvest damage in parsnips. Clockwise from top left (A-C); A) Scuffing of parsnip root, B) Parsnip bruising around circumference of crown and C) Sub peridermal bruising and defoliation damage.

In order to provide statistical evidence an analysis of variance (ANOVA) was employed to compare the average incidence of scuffing, and average incidence of bruising

across the six processing stages, and to investigate whether any significant differences were present between groups.

3.2.1 Section B) Impact damage data acquisition and analysis during parsnip harvesting and post-harvest processing by employing an instrumented pseudo-parsnip.

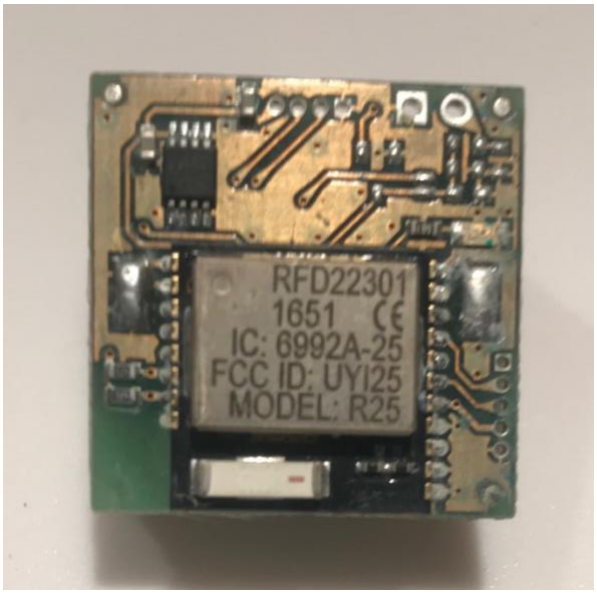
In order to quantify and analyse impact damage data on a national scale the harvesting operations of five, and the packhouse processing operations of eight UK parsnip operations was analysed (Figure 3.2.2.0). The electronic parsnip was processed along with parsnip roots through the entire harvesting, pre cleaning and packhouse processes. For each harvesting or processing stage we performed a minimum of 3 replicates.

Figure 3.2.2.0) Map illustrating location of visited packhouses throughout the mainland United Kingdom and Northern Ireland, Blue dot represents location of University of Birmingham.

The electronic parsnip is a modification of the Tuberlog, first developed in 2001 by ESYS GmbH. Traditionally used for measurement of the mechanical load of potato tubers throughout harvesting and post-harvest processing, the Tuberlog contains and triaxial impact acceleration sensor embedded in a potato shaped synthetic device. The electronic parsnip device is comprised of an Impactrac sensor (Martin Lishman Ltd, 2020) housed within a polyurethane 3D printed shell which when sealed, is watertight and robust enough to withstand extreme shearing pressures.

The sensor (Figure 3.2.2.1) has been designed to analyse harvest, and post-harvest damage of parsnips in the most accurate way possible. The shell and sensor weigh 200 grams and possess a crown diameter of 64 mm, a length of 170 mm and a tail

diameter of 25 mm (Figure 3.2.2.2). The weight, shape and density of the casing has been designed to mirror marketable produce, it is of importance that the device floats as destoners commonly utilize this characteristic to separate hubris from roots.



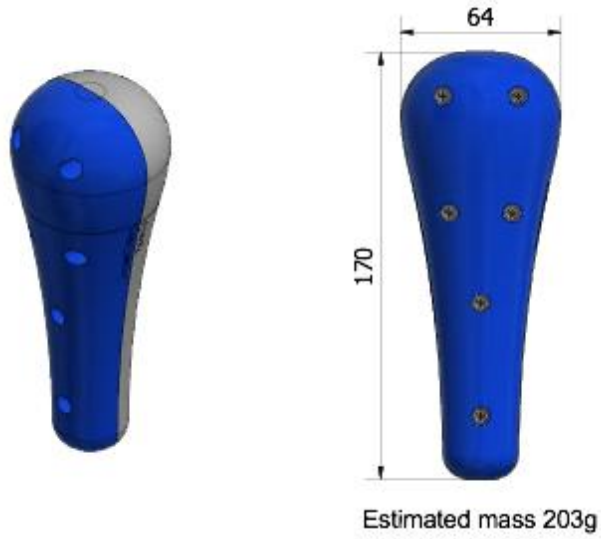
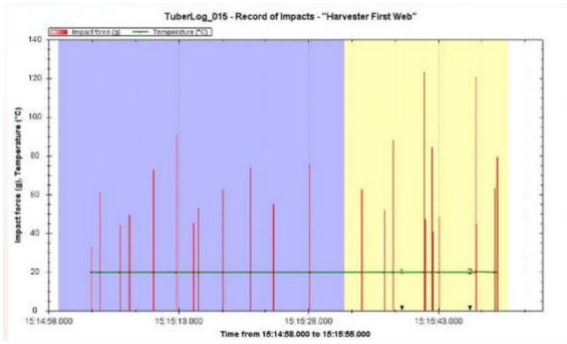


Figure 3.2.2.1 (left) shows computer chip board used by the Tuberlog (Martin Lishman, 2020) Image is authors own. Figure 3.2.2.2 (right) shows design of electronic parsnip shell (dimensions in mm) in preparation for the 3D printing (Martin Lishman Ltd, 2018).

The device employs microelectromechanical (MEM) acceleration sensors (Roa *et al.,* 2015) on a tri-axis to measure acceleration in the X, Y & Z axes. The chip board is powered by a ½ sized AA lithium battery (3.6 V, Saft Ltd, 2020) housed within a plastic case. The impact magnitude range for the electronic parsnip is 1 g to 28 g where 1 g = 9.8 $m.s^{-2}$ as the peak value per second is recorded by the Martin Lishman Ltd software. During testing, the exact time that the device entered and exited each

processing stage was recorded across all replicates, this was later matched to the time stamp on each reading taken by the device.

The software is comprised of two junctures. Firstly, firmware inside the Impactrack sensor records, processes and transmits peak impact magnitude (g) and temperature (°C) data to the second phase, an Impactrack application developed by Martin Lishman Ltd. This facilitates both real time analysis (RTA) utilizing the Impactrack app (Figure 3.2.2.3), and post processing analysis (PPA) as the raw data is transmitted via Bluetooth to laptop where R studio and Prism 8 are then used for data organisation and analysis (Figure 3.2.2.4). For this study we focussed on PPA as this facilitated a nationwide comparison of impact damage data. Whilst RTA of observed impact magnitude per second ($g.s^{-1}$) is useful to instantly highlight critical points during processing where large impact magnitudes are observed, it does not provide the ability to compare equipment and procedures across industry. PPA also allows a greater number of impact parameters to be analysed rather than just impact magnitude per second ($g.s^{-1}$) which RTA facilitates. PPA was performed by employing R Studio Version 0.99.903 (© 2009-2016 RStudio, Inc.) and Prism 8 Version 8.4.3 (471) (June 2020, GraphPad Software, LLC.).



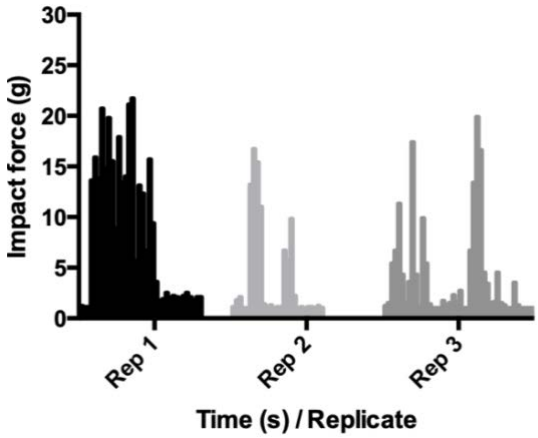


Figure 3.2.2.3 & Figure 3.2.2.4) Comparison of real time analysis (left) employing Martin Lishman app (Martin Lishman Ltd 2020) and post processing analysis (right).

The device, combined with our PPA, permits the analysis and presentation of total process acceleration magnitude (g), mean total velocity change ($m.s^{-2}$), detection of number of and size of peaks per process and temperature fluctuations (degrees). The impact magnitude (g) values observed whilst the device was in each processing stage were isolated and sorted to allow statistical analysis and comparison.

Identifying destructive processing stages in each pack house, in addition to testing new protocols to reduce the size and number of large impact magnitudes, and comparing specific processes across the industry introduces a novel and innovative methodology. Using the data collected from each packhouse (described above) processes from each packhouse were compared with regard to the physical impacts those processes had on the device and roots (Figure 3.2.2.5). The data for each processing stage from each location was pooled allowing comparison of harvesters, pre cleaners, intake procedures, polishers, graders and packaging machines. This information was

presented in a confidential report to each packhouse and used to identify parts of processing that would need refinement to reduce the detrimental impact of processing on parsnip quality.

In order to investigate the relationship between the output from the electronic parsnip (g) and the actual energy exerted by impacts (J) the 4 impact magnitudes inflicted on roots included in chapter 2 were replicated and recorded by the electronic parsnip. Briefly, 3 steel bolt weighing 100, 200 and 500 grams respectively were dropped from a height of either 0.75 or 1.25 metres through perforated PVC pipes (of each height) onto the crown of the electronic parsnip at the pipe base for a total of 10 replicates per impact size, mirroring the exact treatment roots experienced previously. This lead to falling bolt impacts of 0.49 J, 2.45 J, 4.49 J and 6.13 J (respectively categorised as low, medium, high and extreme impacts) being exerted on roots.

Figure 3.2.2.5) Authors own images illustrating the electronic parsnip in situ; Left: Device and roots wait to be unloaded from transport lorry into packhouse; Right: Device and roots stationary on conveyer waiting to enter next processing stage.

The device was employed in a number of packhouses across the UK to analyse the following parameters:

1. **Cumulative mean impact force (g)**
    - The total impact force (g) experienced by the device was summed for each replicate and the mean calculated across all replicates for each group.

2. **Cumulative mean velocity change ($m.s^{-2}$)**
    - The total VC ($m.s^{-2}$) experienced by the device was summed for each replicate and the mean calculated across all replicates for each group.

3. **Time spent in each process (seconds)**
    - The amount of time the device spent in each process was precisely measured by employing a stopwatch marking the exact time the device entered and exited each stage.

4. **Mean peak impact magnitude (g)**
    - The peak impact magnitude (g) witnessed in each replicate was recorded and a mean across all replicates for each processing stage calculated.

5. **Size and number of impacts sorted by impact size (0-5 g, 5-10 g, 10-20 g, 20+ g)**

- The impacts (g) experienced by the device for each replicate was sorted by impact size into 4 groups (0-5 g, 5-10 g, 10-20 g, 20+ g) and a mean calculated for each group across all processing stages.

Statistical analysis was performed using R Studio Version 0.99.903 (© 2009-2016 RStudio, Inc.) and Prism 8 Version 8.4.3 (471) (June 2020, GraphPad Software, LLC.). to compare differences in the mean between groups an Analysis Of Variance test (ANOVA) was employed. If the *p* value of the one-way ANOVA was less than 0.05 (significant), a Duncan's' multiple range test was employed. The use of a standardized range distribution test, such as Duncan's multiple range test, was employed to determine critical values for comparisons between means. If the means between 2 groups do not differ significantly, they are assigned with an identical letter above their associated bar in the bar graph. In contrary, if two groups are found to be significantly different, they will not share a letter above the graph, indicating statistical significance. Error bars shown as the standard error of the mean.

A Wilcoxon test was employed to analyse differences in impact distribution across impact sizes, whilst a Chi squared test analysed distribution of scuffs and bruises. Error bars shown as the standard error of the mean.

An agreement was reached between participants of this study that information would be anonymised to facilitate an industry wide comparison of operating procedures and machinery, and how detrimental these are to parsnip quality.

3.3 Results

3.3.1 *Experiment one: Identification of post-harvest processing procedures that cause mechanical damage in parsnips at Packhouse A*

3.3.1 Location of and number of bruises found on parsnip roots removed from a post-harvest processing operation

3.3.1.1 Average number of bruises found on removed parsnip root

The number of bruises found on removed roots was recorded for six processing stages incorporating all harvesting and post harvesting processes that roots undergo from field to cold storage. The average number of bruises found per parsnip increased from the first processing stage (harvest) to the final (packing) from 0.55 ± 0.17 bruises exerted following harvesting, to 1.25 ± 0.30 bruises exerted following packing (Figure 3.3.1.1.0). It would be expected the total number of bruises increases throughout processing as roots suffer impacts from each additional operating procedure. Although the average number of bruises per root increased throughout processing, no significant difference in the average number of bruises was found between processing stages (p=0.418). This suggests that there may be equipment or procedures that contribute to bruising, however this methodology is not refined enough to detect significant differences between individual processing stages and identify destructive processes.

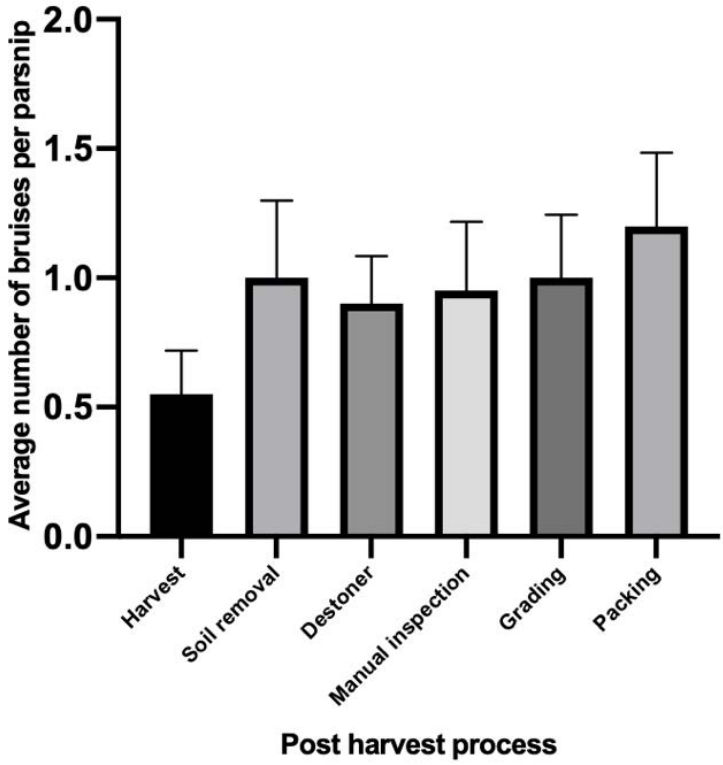


Figure 3.3.1.1.0) Average number of bruises found on each removed parsnip after six post-harvest processing stages at a UK packhouse. (*N*=20) (*p*=0.418). Error bars show standard error of the mean (SEM).

3.3.1.2 Location of bruises found on parsnip roots removed from post-harvest processing

The location of bruises observed on removed parsnip roots was recorded as either Crown half or tail half, a Chi squared test was performed to determine whether roots were bruised equally on the tail and crown halves. Figure 3.3.1.2.0 illustrates the bias that was observed for roots to be bruised on their crown half, rather than the tail half of the root (p=<0.001). The largest discrepancy between damage on either half of the root was observed following removal from grading where 80.95 % of bruises were found on the crown end of the root, in comparison; following destoning 53.85 % of bruises were found on the crown end.

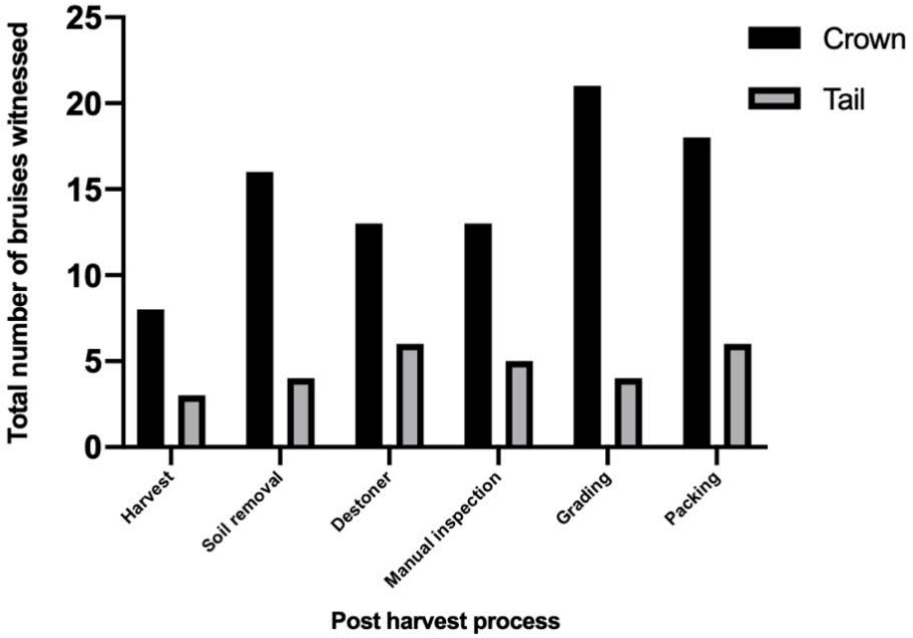


Figure 3.3.1.2.0) Location of bruises witnessed on removed roots per root Crown or tail halves) from six post-harvest processing stages at a UK packhouse. (N=20) (p=<0.001).

3.3.2 Location of and number of peridermal scuffs found on parsnip roots removed from a post-harvest processing operation

3.3.2.1 Number of peridermal scuffs found on parsnip roots removed from a post-harvest processing operation

The number of peridermal scuffs found on removed roots was recorded for six processing stages incorporating all harvesting and post harvesting processes that roots undergo (Figure 3.3.2.1.0). No significant difference was found for the number of scuffs exerted by processing stages (p=0.239). The average number of scuffs found per parsnip increased from the first processing stage to the third stage from 0.20 ± 0.09 scuffs exerted following harvesting, to 0.45 ± 0.17 bruises exerted following destoning. Following manual inspection the number of scuffs observed on removed roots fell to 0.05 ± 0.05, this may be expected as workers on the packhouse line remove roots with visible damage. Scuffing occurs to the periderm, and is thus easier to spot and remove in comparison to bruising that occurs beneath the skin of roots. The average number of scuffs per roots increased following manual inspection to the final processing stage however the number observed was lower than was found before manual inspection.

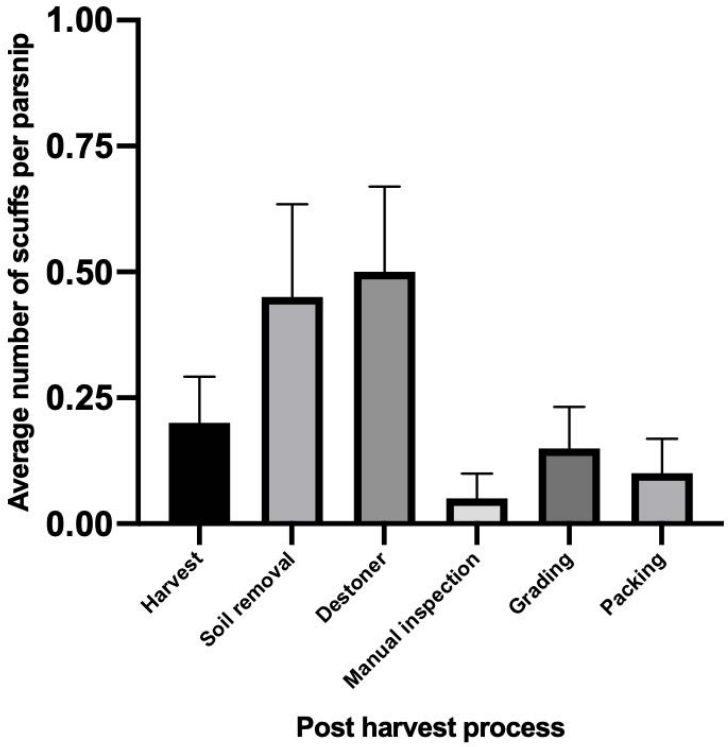


Figure 3.3.2.1.0) Average number of scuffs per removed parsnip following six post-harvest processing stages at a UK packhouse. (*N*=20) (*p*=0.239). Error bars show standard error of the mean (SEM).

3.3.2.2 Location of scuffs found on parsnip roots removed from post-harvest processing

The location of scuffs observed on removed parsnip roots was recorded as either crown half or tail half, a Chi squared test was performed to determine whether roots were scuffed equally on the tail and crown halves. Figure 3.3.2.2.0 illustrates that no bias that was observed for roots to be scuffed on their Crown half, rather than the tail half of the root (*p*=0.158).

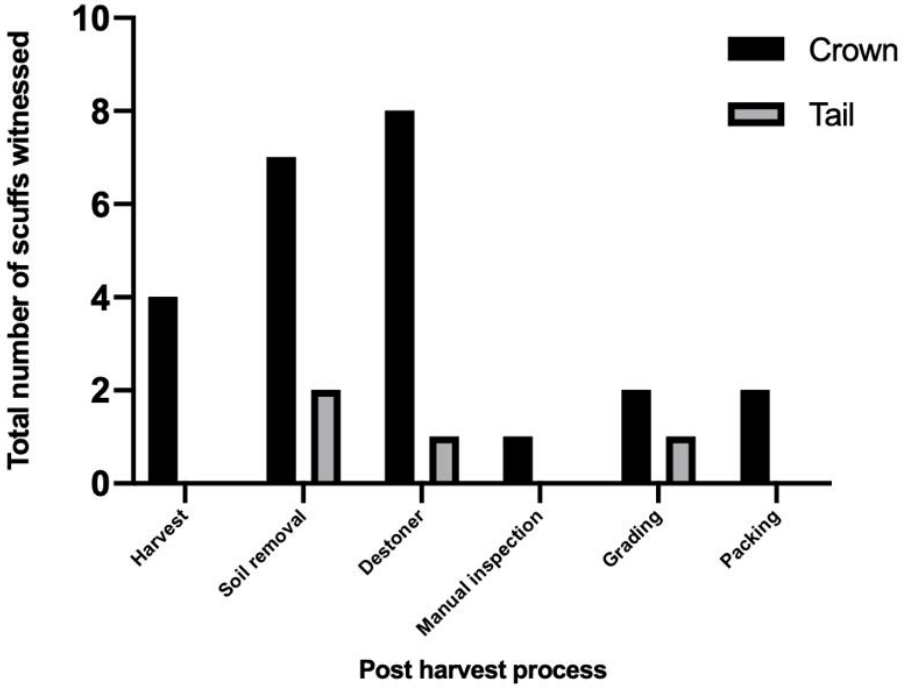


Figure 3.3.2.2.0) Location of cumulative scuffs witnessed on removed roots (Crown or tail halves) from six post-harvest processing stages at a UK packhouse. (*N*=20) (*p*=0.158).

The largest discrepancy between scuffing on either half of the root was observed following harvesting where 100.00 % of scuffs were found on the crown end of the root. In comparison; following grading 50.00 % of scuffs were found on the crown end. However, the employed Chi squared test did not find a bias for roots to scuff on one side or the other across the packhouse as a whole.

3.3.3 Section B) Impact damage data acquisition and analysis during parsnip harvesting and post-harvest processing by employing an instrumented pseudo-parsnip.

Actual impact energy (J) exerted during testing vs device output (g)

The 4 impact magnitudes described in chapter 2 (low, medium, high and extreme) were replicated on the electronic parsnip to investigate the correlation between actual impact energy (J) and device output (g) (Figure 3.3.3.0). Significant differences (p =<0.001) in device output (g) between impact groups were found following employment of a one-way analysis of variance (ANOVA) test. The largest mean output by the device was 15.15 g, experienced during the extreme impact magnitude group (6.13 J) with the smallest mean output (1.84 g) observed during the low impact magnitude group (0.49 J). The results of this study indicate that the electronic parsnip device is capable of differentiating between different impact magnitudes inflicted. Correlation between device output and bruising susceptibility may therefore provide producers with a predictive tool for managing post-harvest losses of parsnips based on quantitative data.

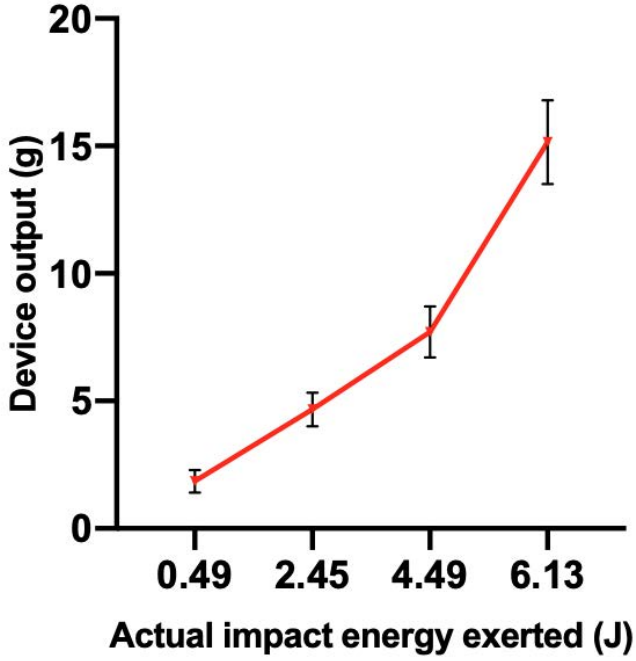


Figure 3.3.3.0) Graph displaying actual impact energy (J) vs device output (g) following falling bolt bruise testing. (*N*=10) (*p* =<0.001). Error bars show standard error of the mean (SEM).

Furthermore, these results provide information on how impacts witnessed during industrial processing translate into bruise formation. In previous work (Chapter 2) it was observed that impacts that exerted 15+ g (extreme impact magnitude) formed severe bruises, and that bruising may be reduced if the impact magnitude exerted is lower, the storage duration is shorter and if storage temperature management is consistent in keeping root temperature low.

3.3.3.1 Hypothesis one: Investigating the ability of the electronic parsnip to find significant differences in impact parameters between 8 different processing stages across Packhouse B

Overview of harvesting and post-harvest processing across Packhouse B

The electronic parsnip analysed all harvesting and post-harvest processing stages in one of the largest packhouse operations in the UK. The processes that the device analysed were; harvesting, intake, polishing (#1 & #2), grading (#1 & #2) and packing (loose or bagged). To further understand why any differences in impact force (g) experienced by roots was observed across post-harvest processes it was deemed necessary to apply PPA to the raw acceleration magnitude values (g) to generate mean total impact force (g), total VC ($m.s^{-2}$), time (s) and peak impact magnitude (g). Investigating these additional parameters may provide information on why certain processes are less destructive than others or vice versa. Here, data is presented from those 4 parameters across all postharvest processes from one of the biggest parsnip packhouses in the world to investigate whether significant differences in parameter values between processes occur.

3.3.3.1.1 Cumulative mean impact force (g) experienced by roots across Packhouse B

The device recorded cumulative impact force (g) for each process across 3 replicates to provide an overview (Figure 3.3.3.1.1.0) of cumulative mean impact force (g) experienced by roots during processing where 1 g = 9.8 $m.s^{-2}$ as measured by the

device . Significant differences in mean impact force (g) per process were found across this operation (p=<0.001) with the second polisher contributing the greatest amount of total impact force (g) (197.13 ± 18.03) per replicate on average. Packing roots into plastic bags suitable for supermarkets was the process that contributed least to overall total impact force (g) (64.85 ± 12.30), loose packing was found to exert greater cumulative impact force (g) (134.56 ± 7.76) than bagged packing did, however this was not observed to be significant following a Duncan's multiple range test. During loose packing roots are dropped from a conveyor and deposited into crates for storage in comparison to bagged packing for supermarkets where 3-4 roots are dropped down a chute into plastic bags breaking their fall.

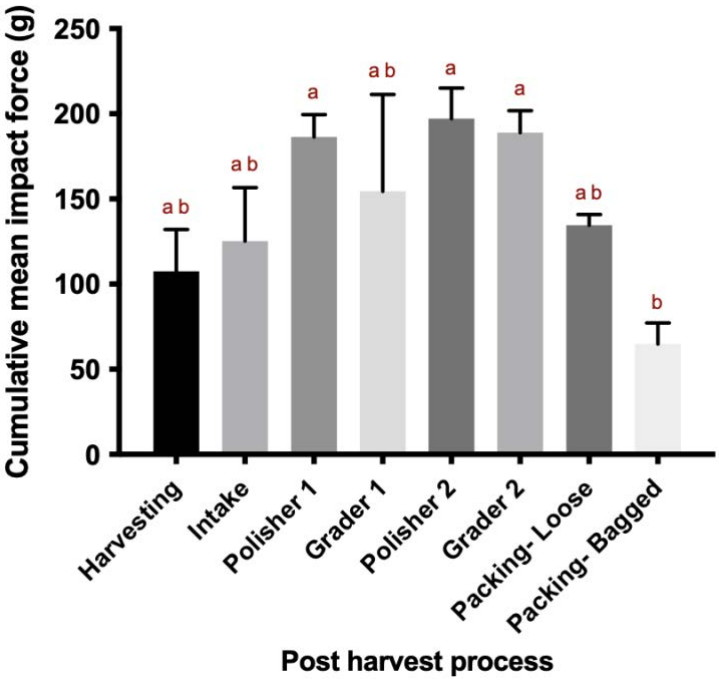


Figure 3.3.3.1.1.0) Cumulative mean impact force (g) measured by the electronic parsnip across 8 post-harvest processing stages in a UK packhouse. (N=3) (p=<0.001). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at p<0.05 according to Duncan's' multiple range test.

Prior to the results of this study producers assumed that the majority of damage to roots occurred during harvesting and transport, and not in the packhouse (personal communication with company 2). The results from this analysis suggest that certain processes in the packhouse actually exceed the cumulative impact forces (g) witnessed during harvesting. In packhouse B, polishing and grading were the processes that contributed most to total impact force (g) experienced during processing, the detrimental nature of these processes to roots is compounded as this packhouse runs two polishers and two graders. This practice of polishing and grading roots twice does not occur in all packhouses; only two packhouses that we studied utilized a double polishing system however it is clear that these processes introduce significant impacts to roots that may cause bruising damage.

The ability to quantify impact force (g) witnessed during parsnip harvesting, and post-harvest processing is novel but further PPA may disseminate these values and provide evidence as to what causes underlying differences in performance in respect to maintaining product quality.

3.3.3.1.2 Cumulative mean velocity change ($m.s^{-2}$) experienced by roots in Packhouse B

The cumulative mean VC ($m.s^{-2}$) experienced by the device during each processing stage was calculated by summing the individual velocity changes ($m.s^{-2}$) between time points for each replicate, then calculating the average across all 3 replicates for each of the processes under investigation (Figure 3.3.3.1.2.0). Across harvesting and post-

harvest processing stages significant differences in cumulative mean VC ($m.s^{-2}$) were observed (p=0.029). A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.045) that was calculated. On average, grader 2 exerted the greatest total VC ($m.s^{-2}$) (1261.30 ± 155.79). The lowest value for total VC ($m.s^{-2}$) was witnessed during packing into supermarket bound plastic bags (316.21 ± 137.64), the total velocity change exerted on roots during loose packing (855.54 ± 137.97) was greater than plastic packing.

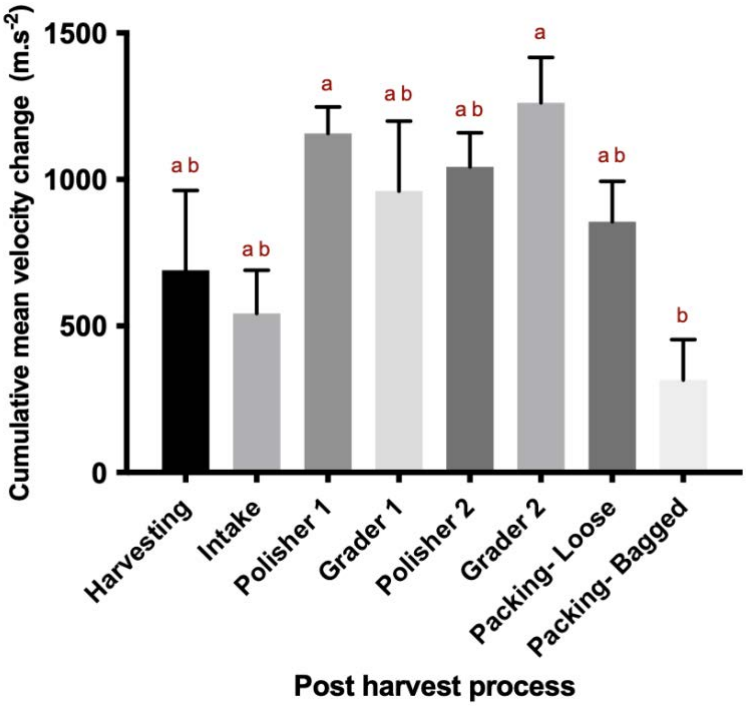


Figure 3.3.3.1.2.0) Cumulative mean velocity change ($m.s^{-2}$) experienced by the device during 8 processing stages in an UK packhouse. (N=3) (p=0.029). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at p<0.05 according to Duncan's' multiple range test.

3.3.3.1.3 Mean time spent being processed (seconds) across packhouse B

No significant difference in time spent in each process across packhouse B was observed (*p*=0.075). The device spent the longest period of time (seconds) in grader 2 (72.67 ± 12.17) whilst the second longest time witnessed was during intake (67.33 ± 18.22) (Figure 3.3.3.1.3.0). The significant amount of time roots spent during intake may explain the discrepancy observed between cumulative mean VC roots experienced during intake procedures. Significant variability in the time spent within specific processes was found (e.g Intake) and this is reflected in the size of the standard error of the mean bars observed, this was attributed to the device being stuck or clogged at a pinch point during processing, which normal parsnip roots also experience.

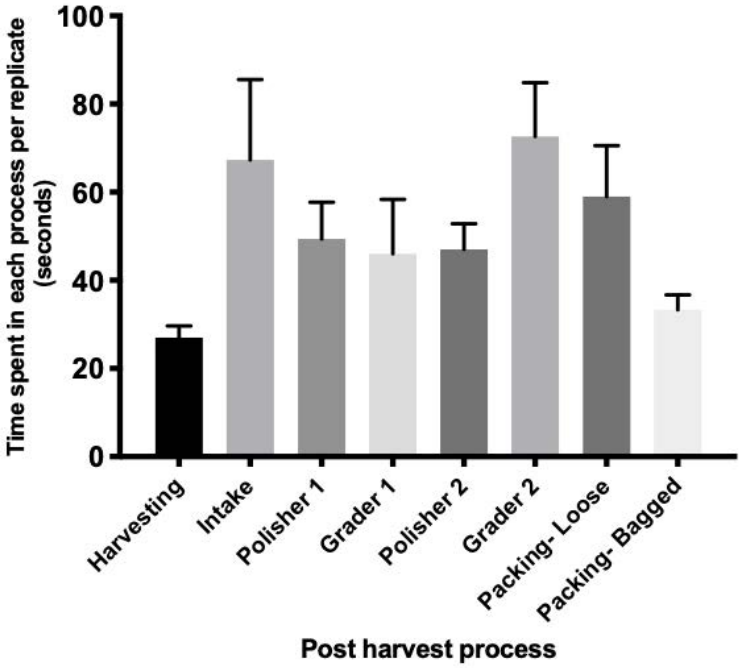


Figure 3.3.3.1.3.0) Mean time the device spent being processed (seconds) in 8 processing stages in a UK packhouse. (*N*=3) (*p*=0.075). Error bars show standard error of the mean (SEM).

3.3.3.1.4 Mean peak impact magnitudes (g) experienced by roots across Packhouse B

The peak impact magnitude (g) witnessed per replicate, per process was calculated and used to produce a mean peak impact that roots could expect to experience during each processing stage. Such information is important as peak values observed on the device during processing can be replicated in the laboratory on parsnip roots to calculate bruising susceptibility values based on size of impact magnitude. Significant differences in the size of the peak impact (g) exerted on roots was observed across processing stages (p=0.045) following employment of a ANOVA. A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.045) that was calculated.

The largest mean peak impact magnitude (g) witnessed for a process was polisher 1 (19.72 ± 2.80) however this was very closely followed by polisher 2 (19.33 ± 3.00), grader 2 (19.23 ± 2.39) and loose packing (18.90 ± 3.74) (Figure 3.3.3.1.4.0). The lowest mean peak impact (g) roots could expect to experience during processing was during packing into supermarket plastic bags (6.50 ± 1.70) which was lower (than loose packing into crates.

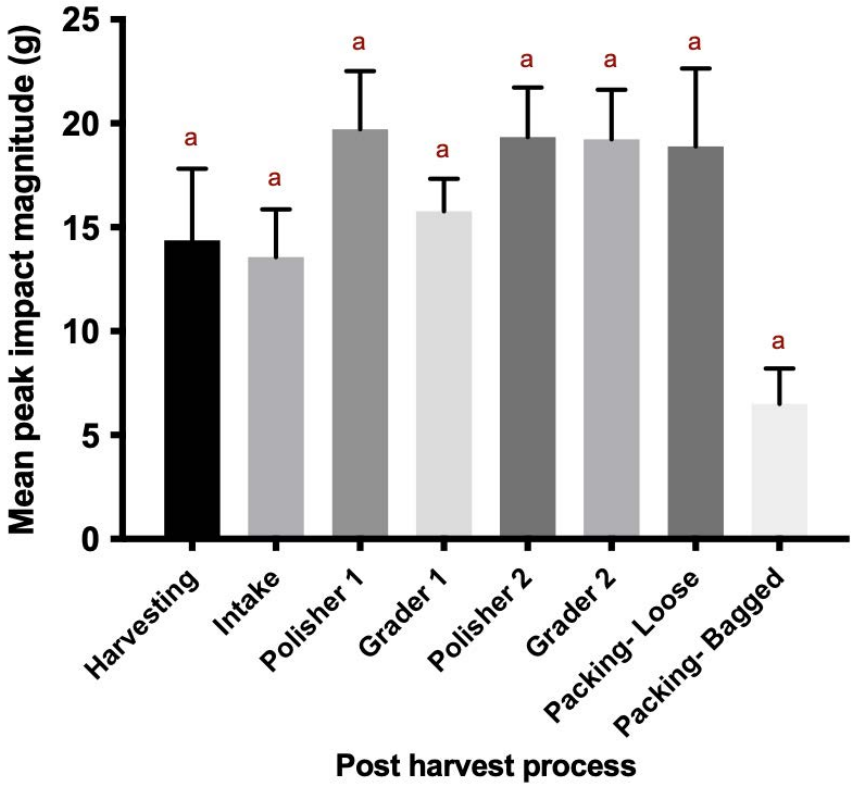


Figure 3.3.3.1.4.0) Mean peak magnitude (g) experienced by the device across 8 processing stages in a UK operation. (*N*=3) (*p*=0.045). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

3.3.3.1.5 Size and number of impacts (g) witnessed across Packhouse B

The comparison of the distribution of the witnessed impact force (g) values across processing in packhouse B was significantly different for 3 of the 4 impact ranges following employment of a Wilcoxon test. No significant difference in the number of impacts that exceeded 20g was observed between the 8 processing stages (*p*=0.062), however impacts sized between 0-5 g (*p*=0.008), 5-10 g (*p*=0.008) and 10-15g (*p*=0.008) all displayed significant discrepancies (Figure 3.3.3.1.5.0).

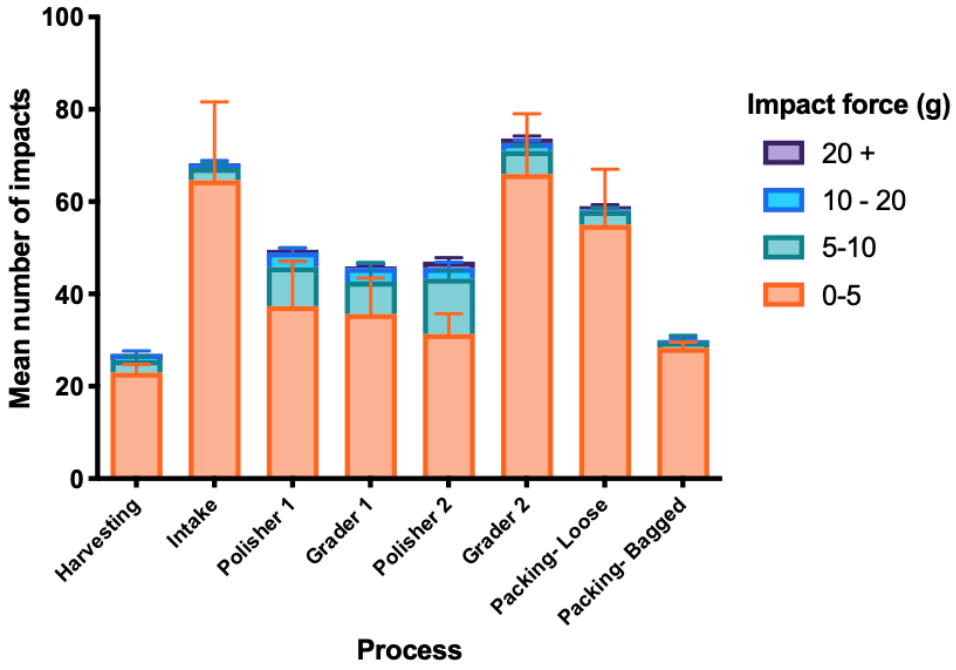


Figure 3.3.3.1.5.0) Total number of impacts recorded during harvesting and post-harvest processing of parsnip roots at Packhouse B sorted by impact size (0-5 g, 5-10 g, 10-20 g and 20 g+). (*N*=3). Error bars show standard error of the mean (SEM).

Polisher 2 exerted the greatest number of impacts per replicate (1.33 ± 0.88) that exceeded 20 g, whereas harvesting, intake and bagged packing exerted no impacts that exceeded 20 g in any of the replicates performed. For impacts that ranged between 10-20 g, Polisher 1 exerted the greatest number per replicate (3.20 ± 1.25) whereas both methods of packing roots exerted the lowest (0.33 ± 0.58). Polisher 2 inflicted the greatest number of impacts for those ranged 5-10 g (12.00 ± 1.73) whilst bagged packing again scored lowest with 1.33 ± 1.31.

Finally for impacts that ranged from 0-5 g, Grader 2 inflicted 66.00 ± 12.72 whilst harvesting exerted the lowest number of impacts between 0-5 g (23.00 ± 1.71). These results indicate that significant differences in the number of large impacts inflicted on

roots exists between processes; with some exerting few to no impacts that exceeded 10 g, whilst others (Polishing) consistently exerted impacts that exceeded 10-15 g. Previous research (chapter 2) has shown that impacts of this size are sufficient to cause severe bruising to parsnips, thus producers should aim to reduce the number of impacts that exceed 10 g across all processing stages.

The electronic parsnip has displayed the ability to detect significant differences in impact force (g), VC($m.s^{-2}$) and peak impact magnitudes, although no significant difference in time (p=0.075) was observed between stages. The device found that 3 of the 4 impact size categories (0-5, 5-10 & 10-15 g) differed significantly in their distribution of impact forces, however none was witnessed for impacts that exceeded 20 g. Analysing differences in impact forces, VC, peak magnitudes and time across specific processes (e.g polishing) may provide producers with information on which models perform best, and if modifications can improve performance.

3.3.3.2 *Hypothesis 2: Investigating the ability of the electronic parsnip to find significant differences in impact parameters within specific processing stages across industry*

The objective of this section of study was to determine whether or not the electronic parsnip could detect significant differences in measured impact forces experienced in specific processing stages across multiple harvesting and packhouse operations.

The device was employed to analyse the previously described impact parameters across each of the industrial processing stages:

- Cumulative mean impact force (g)
- Cumulative mean velocity change ($m.s^{-2}$)
- Time spent in each process (seconds)
- Mean peak impact magnitude (g)
- Size and number of impacts sorted by size (0-5 g, 5-10 g, 10-20 g, 20 g+)

3.3.3.2.1 *Quantitative evaluation of mechanical parsnip harvesting across 7 harvesting operations in the UK*

In this study the 7 separate harvesting operations analysed across 4 producers and are represented by A1-D, with the post-harvest soil removal procedure analysed being represented by SR. A1 is an older harvester model whilst A2 is the newly updated model (both were ran in the same fields, under the same conditions using the same transport lorry). Both B and C represent harvesting into either a full 14 ton transport lorry (B1 and C1 respectively) or into an empty transport lorry (B2 and C2 respectively) at two separate producers. Unlike A, the same harvester at each location. The device was inserted into a row of freshly topped parsnip roots to record its interactions during the mechanical harvesting process . An example of real time analysis from the raw data of a harvesting replicate has been converted so that the first movement of the device and surrounding roots is relative time zero (Figure 3.3.3.2.1.0). A single replicate was categorised from the first movement of device in the row, to the final movement in the transport trailer. During harvesting a large number of large impacts

were observed across all 7 harvesters and one soil removal procedure across all replicates performed (Table 3.3.3.2.2.0).

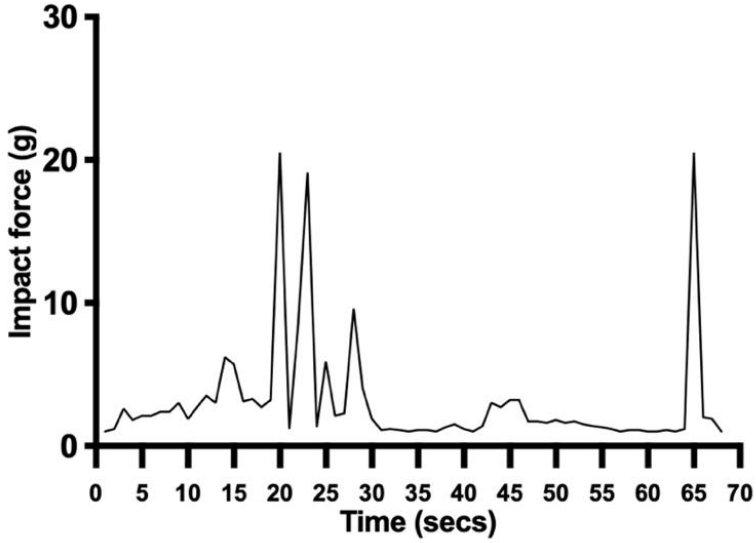


Figure 3.3.3.2.1.0) Real time analysis illustrating impacts recorded by device during mechanical parsnip harvesting during one replicate over a time period of 68 secs in harvester A1. Each peak in the figure represents an impact with “g” standing for gravitational acceleration, defined as impact force.

3.3.3.2.2 *Cumulative mean impact force (g) during mechanical harvesting of parsnips*

Impact parameter	Industry harvester								
	A1	A2	B1	B2	C1	C2	D	SR	*ANOVA result*
Mean total impact force (g)	149.60 ± 9.75	109.07 ± 10.48	87.76 ± 9.22	100.70 ± 3.84	138.5 ± 24.50	183.66 ± 3.02	245.70 ± 33.49	122.30 ± 8.94	***p=<0.001***
Mean total velocity change ($m.s^{-2}$)	1078.74 ± 106.01	752.64 ± 109.39	503.39 ± 108.65	589.63 ± 53.05	819.61 ± 110.42	708.87 ± 341.05	1427.53 ± 48.64	680.77 ± 165.69	*p*=0.074
Mean time (s)	25.75 ± 1.79	26.25 ± 1.44	27.00 ± 2.64	24.33 ± 1.20	51.00 ± 3.05	54.33 ± 2.60	62.00 ± 2.51	38.00 ± 2.68	***p=<0.001***
Mean peak impact magnitude (g)	22.18 ± 0.67	18.03 ± 3.38	16.07 ± 1.71	16.10 ± 1.98	15.33 ± 1.73	20.83 ± 2.55	18.60 ± 1.72	15.73 ± 1.51	*p*=0.253

Table 3.3.3.2.2.0) Results of mean impact parameters recorded by electronic parsnip across industrial parsnip harvesters in the United Kingdom with the standard error of the mean also shown. Of the 4 parameters analysed, 2 were found to significantly differ in performance across those investigated (mean total impact force (g), mean total velocity change ($m.s^{-2}$), time (s) and peak impact magnitude (g)) following analysis of variance testing.

The cumulative total impact force (g) experienced by the device during harvesting was recorded across 7 harvesters and 1 soil removal stage, the cumulative mean impact force across 3-4 replicates is shown in Figure 3.3.3.2.2.0. The device recorded the highest cumulative mean impact force (g) during harvester D (245.70 ± 44.49) and the lowest during harvester B1 (87.77 ± 9.22) with significant differences in cumulative mean impact force (g) being observed across industry harvesters (*p*=<0.001*)*. A

Duncan's multiple range test was employed following the significant ANOVA *p* value (*p*=<0.001) that was calculated. The total impact force (g) exerted by post-harvest soil removal (122.30 ± 9.84) was not significantly different from the lowest value observed across harvesting (seen in below graph as SR and harvester B1 are denoted by the same letter (b)). This suggests that introducing a post-harvest soil removal stage before transport to the packhouse introduces a source of total impact (g) that is at least as detrimental to root quality as harvesting.

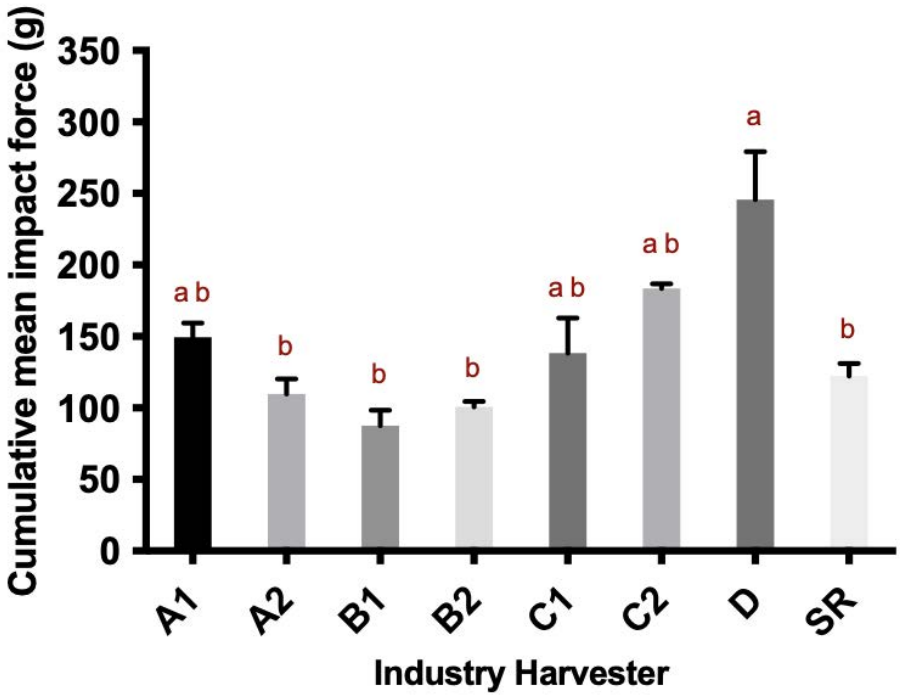


Figure 3.3.3.2.2.0) Cumulative mean impact force (g) recorded by electronic parsnip across mechanical parsnip harvesters (A1-D) and soil removal (SR) in the United Kingdom. (*N*=3-4) (*p*=<0.001). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

The cumulative mean impact force (g) experienced during A1 was greater than during A2 indicating that changing harvesters can lead to a greater performance in terms of

reducing impact forces when run by the same operator in the same field. Such analysis on how a producers' specific current harvester performs in comparison to newer models provides crucial information for growers when making significant buying decisions. No significant difference between either B1 (full) and B2 (empty), or between C1 (full) and C2 (empty) was found despite roots falling into full lorries have a shorter distance to drop from the harvester conveyer, to their final resting position in the transport lorry. It may be expected that roots that fall from the elevator chute into an empty transport lorry would experience greater total impact forces as full lorries have roots already loaded that break the fall of falling roots.

3.3.3.2.3 Cumulative mean velocity change ($m.s^{-2}$) during harvesting

The total mean VC ($m.s^{-2}$) experienced by the device during harvesting was calculated by summing the individual VC ($m.s^{-2}$) between time points for each replicate, then the average across all 3 or 4 replicates was calculated and is presented in Figure 3.3.3.2.3.0. Across all harvesters, no significant difference in cumulative mean VC ($m.s^{-2}$) was observed (p=0.074). The greatest total mean VC was witnessed during harvesting in harvester D (1427.53 ± 48.64) with the lowest cumulative mean VC being witnessed in B1 (503.39 ± 108.65).

No significant differences in mean total VC ($m.s^{-2}$) were witnessed between A1 and A2 (p=0.076), between B1 and B2 (p=0.529) or between C1 and C2 (p=0.546). The cumulative mean VC ($m.s^{-2}$) witnessed during soil removal (680.77 ± 165.69) did not differ significantly from the lowest harvester value (B1) (p=0.400) indicating that in

terms of the amount of VC experienced by roots, soil removal is at least as detrimental to root quality as harvesting.

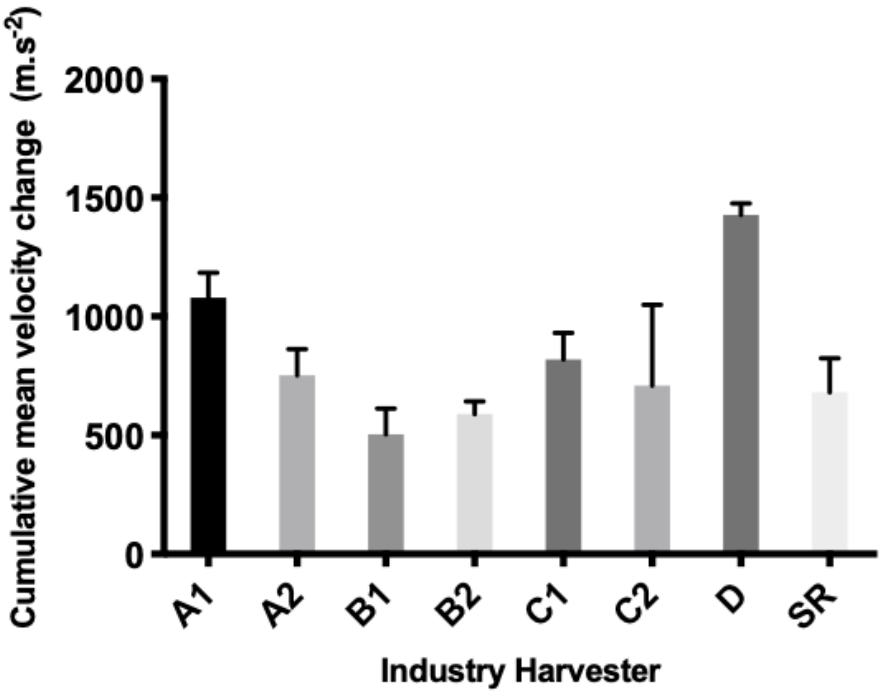


Figure 3.3.3.2.3.0) Cumulative mean velocity change ($m.s^{-2}$) recorded by electronic parsnip across mechanical parsnip harvesters (A1-D) and soil removal (SR) in the United Kingdom. (*N*=3-4) (*p*=0.074). Error bars show standard error of the mean (SEM).

3.3.3.2.4 Time (seconds) spent being mechanically harvested

The mean time being mechanically harvested was calculated by precisely recording the time that the device first moved during harvesting, and came to its final resting place in the transport lorry. Significant differences in the mean time spent being harvested were found (*p*=<0.001) with harvester D recording the slowest time (62.00 ± 2.51 secs) in comparison to the fastest harvester, B2 which on average took 24.33

± 1.20 seconds (Figure 3.3.3.2.4.0). *)*. A Duncan's multiple range test was employed following the significant ANOVA *p* value (*p*=<0.001) that was calculated.

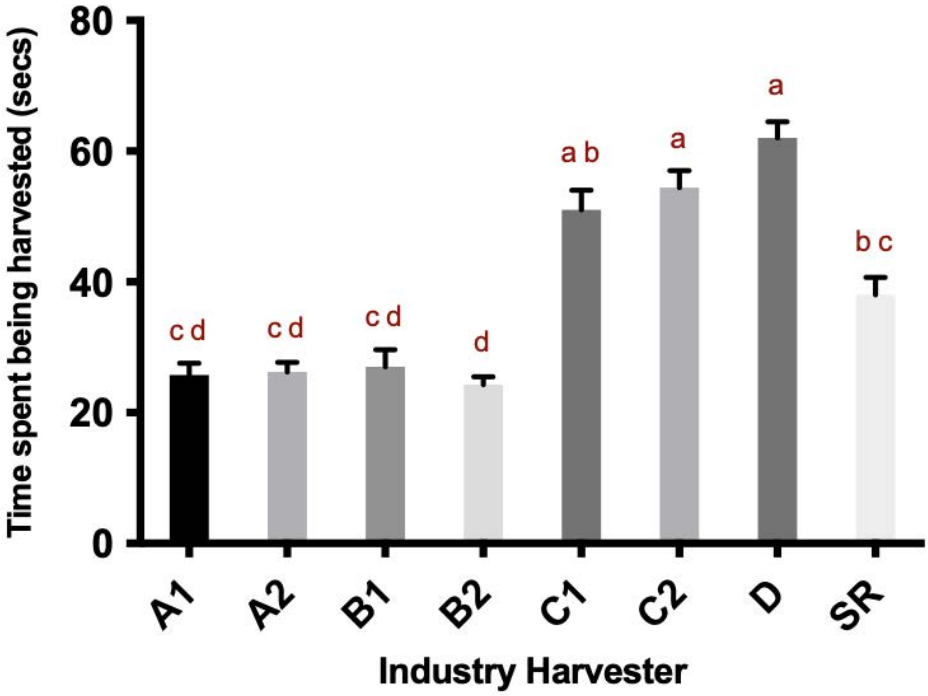


Figure 3.3.3.2.4.0) Time spent being harvested recorded by electronic parsnip across mechanical parsnip harvesters (A1-D) and soil removal (SR) in the United Kingdom. (*N*=3-4) (*p*=<0.001). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

3.3.3.2.5 Mean peak magnitude (g) witnessed during harvesting

The mean peak impact magnitude (g) was calculated by recording the peak value of each replicate for all harvesters, then an average of the replicates was used to represent the likely peak magnitude experienced during harvesting roots. No significant difference in mean peak magnitude (g) was witnessed across all industry

harvesters (p=0.253) despite A1 recording an average peak impact (g) of 22.18 ± 0.67 and the lowest, C1 recording a mean peak impact (g) of 15.33 ± 1.73 (Figure 3.3.3.2.5.0). The peak impact exerted by soil removal (15.72 ± 1.51) did not differ significantly (p=0.8916) from the lowest peak magnitude (g) witnessed during harvesting (C1). No significant difference was observed between A1 & A2 (p=0.309) indicating that in this study the newer model of harvesting equipment was no better or worse than the old equipment. Furthermore, emptying roots into an empty or full transport lorry had no effect on mean peak impact magnitude between B1 & B2 (p=0.9913) or between C1 and C2 (p=0.1367).

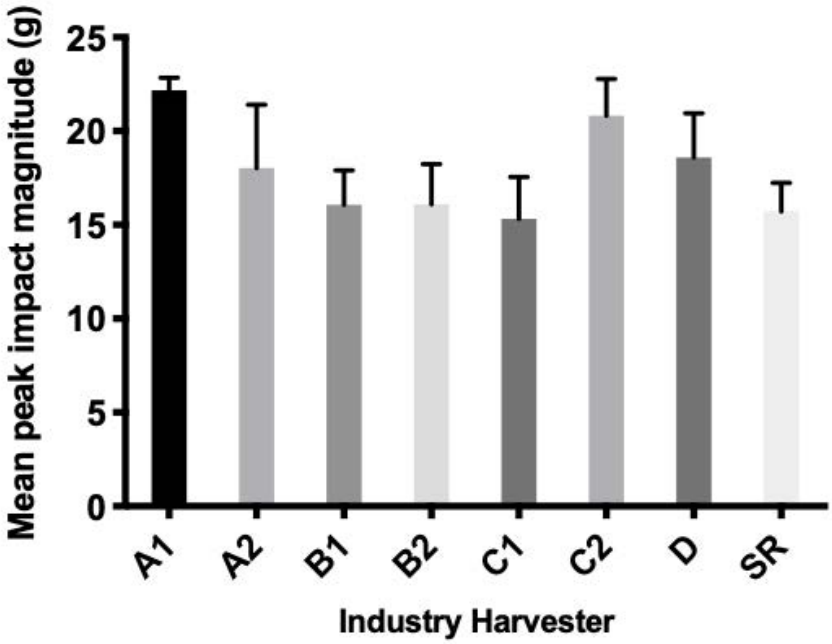


Figure 3.3.3.2.5.0) Mean peak magnitude (g) recorded by electronic parsnip across mechanical parsnip harvesters (A1-D) and soil removal (SR) in the United Kingdom. (N=3-4) (p=0.253). Error bars show standard error of the mean (SEM).

3.3.3.2.6 Size of impacts (g) witnessed during harvesting

The comparison of the distribution of the witnessed impact force (g) values across harvesters and soil removal was significantly different for 3 of the 4 impact ranges following employment of a Wilcoxon test (Figure 3.3.3.2.6.0). No significant differences in the distribution of impacts that exceeded 20g were observed (p=0.125) with 4 of the 7 methods not exerting any impacts over 20g. Within the 0-5, 5-10 and 10-20 g impact groups, significant differences were observed (p=0.008). Harvester C2 exerted an average of 47.33 ± 5.98 impacts sized between 0-5 g, whilst the fewest number of similarly sized impacts were inflicted by harvester B2 (18.33 ± 3.13). The greatest mean number of impacts sized between 5-10 g were inflicted by harvester D (11.33 ± 2.85) whilst, in contrast to 0-5 g impacts, harvester C2 inflicted the fewest number of 5-10 g impacts (3.00 ± 0.67). Soil removal exerted the highest number of impacts sized between 10-20 g (4.33 ± 2.06) whilst Harvester B only exerted 1.33 ± 0.64 per replicate, the fewest witnessed across the 7 seven harvesters and soil removal.

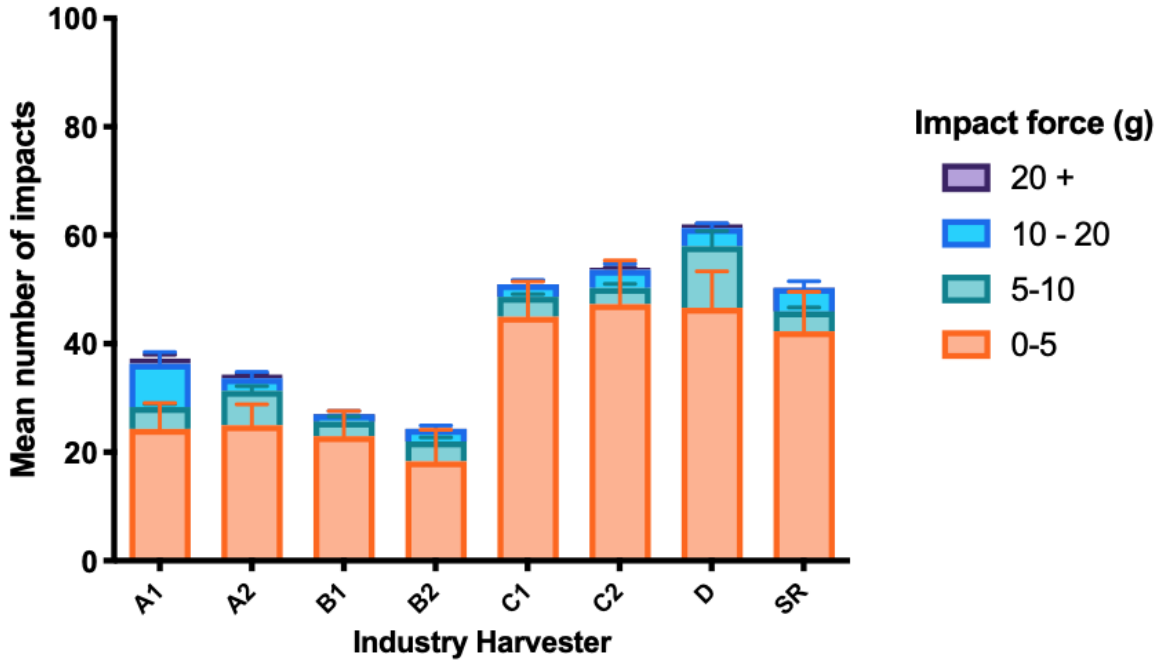


Figure 3.3.3.2.6.0) Total number of impacts recorded during parsnip harvesting across 7 harvesters and one soil removal sorted by impact size (0-5 g, 5-10 g, 10-20 g and 20 g+) normalised for 3 replicates per harvester or soil removal stage. (*N*=3-4). Error bars show standard error of the mean (SEM).

3.3.3.3 Quantitative evaluation of parsnip intake procedures

In order to investigate whether the device can detect significant differences in impact parameters between intake procedures the device was inserted onto the top of roots in the transport lorry at the entrance to the packhouse. Due to the extreme variability witnessed across operations in terms of how parsnips are unloaded from the transport lorries into packhouse facilities, in this study results are presented for just two methods of intake recorded at packhouse A. Typically, this operation utilizes 800 gallons of water per minute, sprayed onto roots from overhead in the lorry whilst the trailer is

hydraulically lifted leading to a combination of water pressure and gravity being employed to unload roots. To investigate whether less destructive methods of intake were available we reduced the water flow by 75% to 200 gallons a minute and compared the mean results for each parameter analysed across the two methods.

3.3.3.3.1 Cumulative mean impact force (g) experienced during parsnip intake

The cumulative mean impact force (g) experienced by the device during intake into the packhouse was recorded employing two levels of water across 3 replicates, the total mean impact force experienced by the device during each intake method is shown in Figure 3.3.3.3.1.0. The device recorded the highest total impact force (g) whilst employing the 800 gallons a minute intake method (125.83 ± 31.32) whilst removing roots from transport employing a quarter of the water exerted a mean total impact force (g) of 92.33 ± 9.92. No significant difference in cumulative mean impact force (g) was observed between intake methods (p=0.396) despite a reduction in water usage of 75% resulting in a reduction in impact force of 26.62 %, however this was only tested over 3 replicates due to industrial limitations.

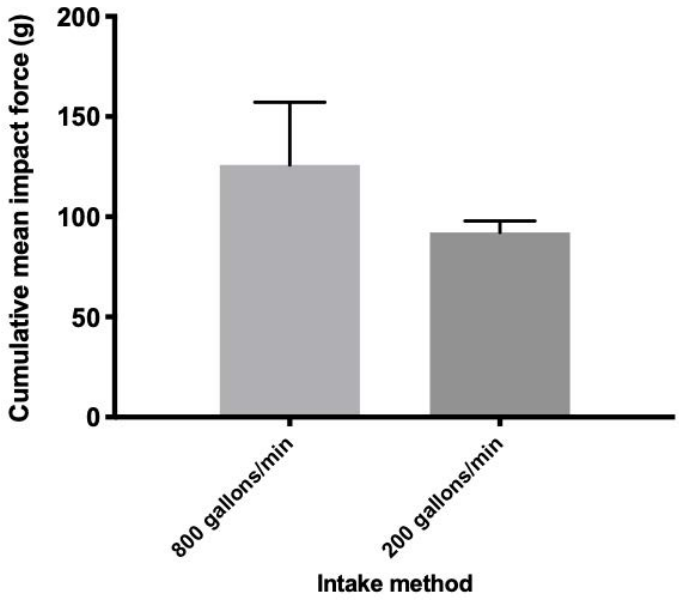


Figure 3.3.3.3.1.0) Mean total impact force (g) experienced by the device during testing of intake methods in a UK parsnip packhouse. (*N*=3) (*p*=0.396). Error bars show standard error of the mean (SEM).

3.3.3.3.2 Cumulative mean velocity change ($m.s^{-2}$) during parsnip intake

The cumulative mean VC ($m.s^{-2}$) experienced by the device during each intake method was calculated by summing the individual VC ($m.s^{-2}$) between time points for each replicate, then the average across all 3 replicates was calculated and is presented in Figure 3.3.3.3.2.0. No significant difference in cumulative mean VC ($m.s^{-2}$) was observed between intake methods (*p*=0.427). The higher value ($m.s^{-2}$) was observed whilst employing a greater volume of water to dislodge roots from the trailer (543.20 ± 49.01) in comparison to what was observed whilst employing 200 gallons per minute (376.97 ± 38.03).

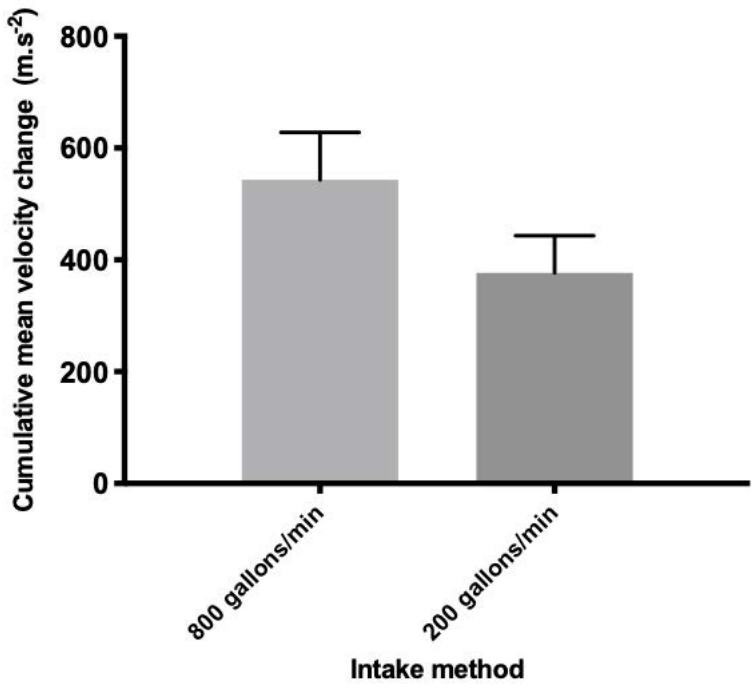


Figure 3.3.3.3.2.0) Cumulative mean velocity change ($m.s^{-2}$) recorded by electronic parsnip across two intake methods at a UK packhouse. (*N*=3) (*p*=0.427). Error bars show standard error of the mean (SEM).

3.3.3.3.3 Time (seconds) spent during parsnip intake

No significant difference in time spent during intake was found between the methods (Figure 3.3.3.3.3.0) (*p*=0.479). Employing 800 gallons of water a minute on average led to roots completing the intake process in 67.33 ± 18.22 seconds, whilst employing a quarter of the water lead to a reduction in time taken to 51.00 ± 8.54 seconds. Despite no significant difference being observed between methods, the difference in time taken could explain why in terms of total impact force (g) and total VC ($m.s^{-2}$) the 800 gallon method exerts greater values on roots and how this does not translate into mean values witnessed per second ($g\ s^{-1}$ & $m.s^{-2}$ / s^{-1}).

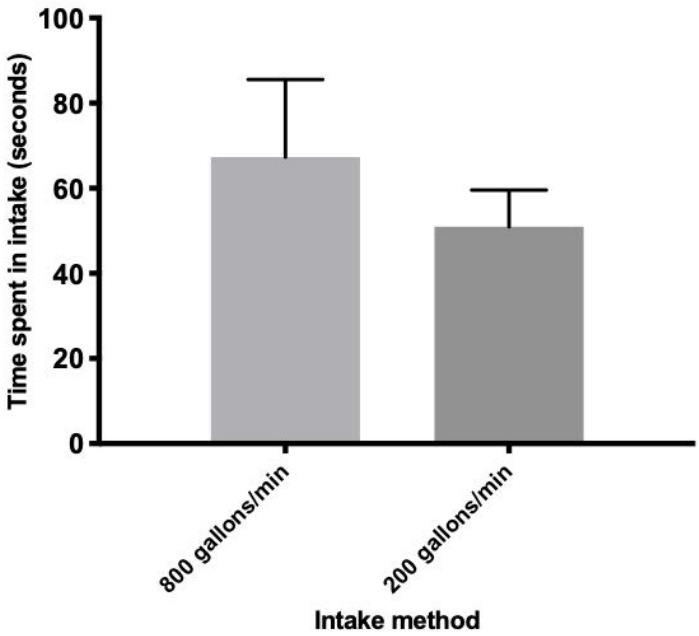


Figure 3.3.3.3.3.0) Time taken for the device to complete two intake methods in one UK packhouse. (*N*=3) (*p*=0.479). Error bars show standard error of the mean (SEM).

3.3.3.3.4 Mean peak magnitude (g) witnessed during parsnip intake

In order to analyse the peak impact magnitudes (g) roots can expect to experience during intake we calculated the peak impact (g) for each replicate, and took the average across all replicates per method. No significant difference in mean peak impact magnitude (g) was observed between intake methods (*p*=0.327) despite the 800 gallon method exerting a mean peak impact of 13.57 ± 2.28 g per replicate in comparison to 9.70 ± 2.71 g exerted by the 200 gallon method (Figure 3.3.3.3.4.0).

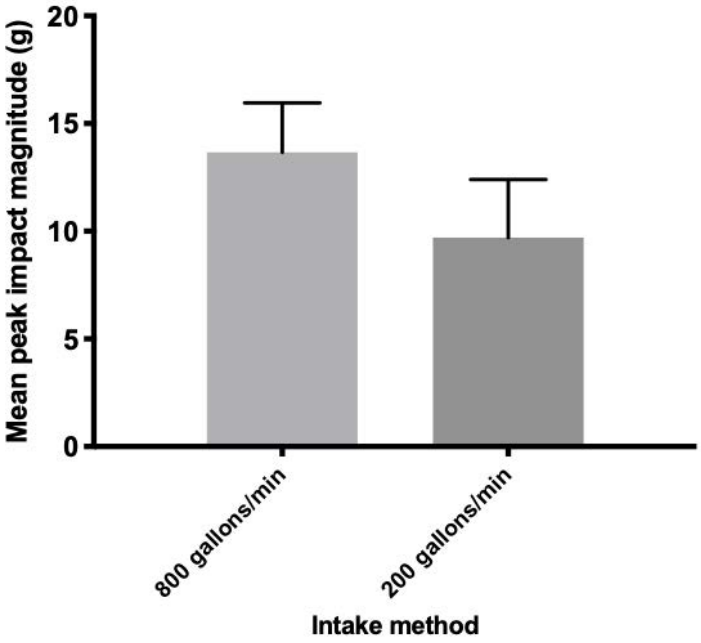


Figure 3.3.3.3.4.0) Mean peak impact size (g) experienced by the device per replicate across 2 intake methods in a UK packhouse. (*N*=3) (*p*=0.327). Error bars show standard error of the mean (SEM).

3.3.3.3.5 Size of impacts (g) witnessed during parsnip intake

Following the employment of a Wilcoxon test; significant differences in the distribution of impacts was observed for 1 of the 4 impact magnitudes (5-10 g) was observed whilst 3 did not exhibit significant differences between intake methods (0-5 (*p*=0.09) & 10-20 (*p*=0.057)), furthermore neither intake method inflicted any impacts that exceeded 20 g (Figure 3.3.3.3.5.0). Significant differences were found between intake methods for impacts sized between 5-10 g (*p*=0.042) as 800 gallons a minute exerted 64.67 ± 14.56 whilst 200 gallons inflicted an average of 48.00 ± 13.67 impacts between 5-10 g per replicate.

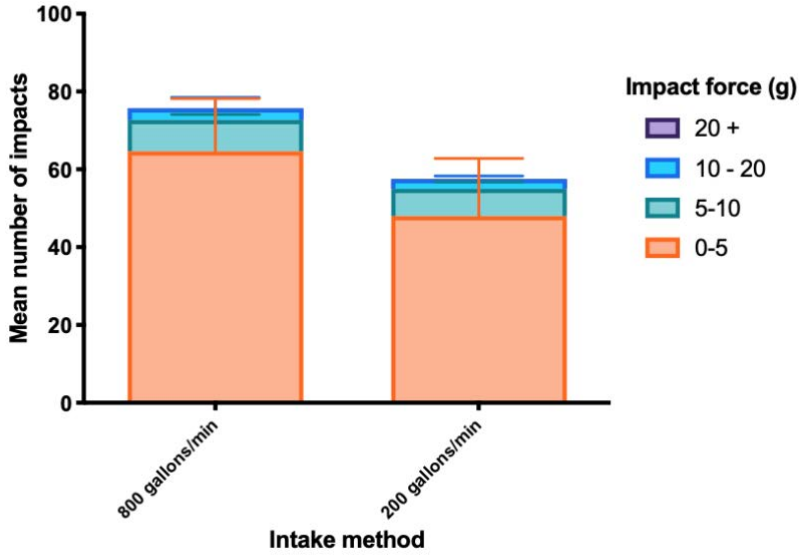


Figure 3.3.3.3.5.0) Total number of impacts recorded during parsnip intake sorted by impact size (0-5 g, 5-10 g, 10-20 g and 20 g+) normalised for 3 replicates per harvester or soil removal stage. (*N*=3). Error bars show standard error of the mean (SEM).

3.3.3.4 Quantitative evaluation of 10 industry parsnip polishers

In order to investigate whether the device can detect significant differences in impact parameters between polishers, the impact forces exerted during polishing in 10 industry parsnip polishers from across the UK were analysed. All the polishers listed below were run on their standard, parsnip settings with normal running procedures so that a comparison of current practice across the industry could be produced. Then at a later date, modifications to polisher settings and procedures could be tested to investigate whether impact forces can be reduced by good management of postharvest technology and procedures. The device was put on the conveyor leading to the intake hopper for all polishers, it then ran through the polisher along with normal parsnip roots, before being collected from the conveyor at the exit end of the polisher (Figure 2.2.3.2.3.0). All polishers were analysed at least 3 times, the results from all impact parameters across all 10 industry polishers is summarised in table 3.4.3.0.

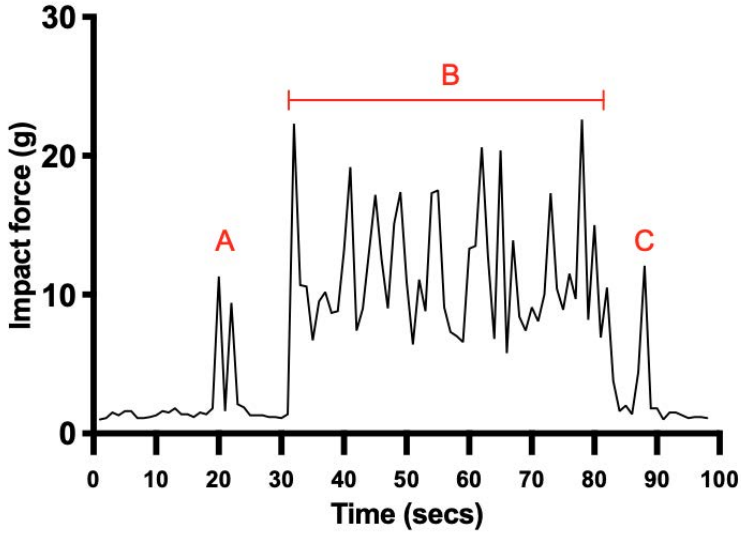


Figure 3.3.3.4.0) RTA graph illustrating impact force (g) experienced per second by the device whilst being processed by an industry polisher H across 1 replicate.

The impact force (g) peaks shown in Figure 3.4.3.0 occurred during one replicate of processing in polisher H, A highlights the force experienced as the device falls into the loading hopper from the preceding conveyor. B illustrates the peaks experienced whilst the device is inside the rotating drum of the polisher being polished by moving brushes along the inside of the drum. Finally, C highlights the forces experienced upon exit of the polisher, where roots fall down onto a conveyor to be taking to the next processing stage.

Impact parameter	**Industry polisher**										
	A	B	C	D	E	F	G	H	I	J	*ANOVA p value*
Mean total impact force (g)	335.90 ± 43.03	186.56 ± 11.80	197.13 ± 10.03	185.86 ± 78.70	193.80 ± 60.06	400.90 ± 20.98	655.36 ± 95.06	443.13 ± 31.76	734.03 ± 26.89	475.80 ± 44.14	***p=<0.001***
Mean total velocity change ($m.s^{-2}$)	1999.85 ± 212.86	1210.10 ± 98.64	1043.70 ± 116.25	1062.64 ± 359.62	1035.23 ± 247.36	1813.33 ± 437.40	2629.67 ± 542.18	2442.81 ± 404.87	3333.96 ± 90.30	2329.79 ± 176.49	***p=0.027***
Mean time (s)	73.33 ± 10.69	55.20 ± 6.54	48.67 ± 5.85	56.67 ± 8.98	40.33 ± 4.41	49.00 ± 5.29	60.00 ± 3.21	107.67 ± 7.79	96.67 ± 5.81	73.33 ± 3.71	***p=<0.001***
Mean peak impact magnitude (g)	19.80 ± 0.87	20.54 ± 2.15	14.05 ± 0.69	25.20 ± 0.45	17.97 ± 2.75	22.00 ± 1.43	23.45 ± 0.62	19.93 ± 0.99	22.27 ± 0.49	21.40 ± 1.35	***p=0.002***

Table 3.4.3.0) Table displaying mean and SEM values for all parameters across 10 industry polishers.

3.3.3.4.1 Cumulative mean impact force (g) experienced during polishing across industry polishers

In order to analyse the cumulative mean impact magnitudes (g) roots can expect to experience during polishing the total cumulative impact force (g) exerted for each replicate was calculated, and the average across all replicates for the 10 polishers.

Highly significant differences (p=<0.001) in cumulative mean impact force (g) were observed. A Duncan's multiple range test was employed following the significant ANOVA p value (p=<0.001) that was calculated. The greatest cumulative mean impact force (g) was exerted by polisher G (746.90 ± 35.226) whilst polisher I ranked second exerting 734.03 ± 26.88 G on roots. The lowest cumulative mean impact force (g) was exerted by Polisher D (185.87 ± 78.70). These results indicate that significant variance in performance in terms of impact forces (g) exists across the parsnip industry, with some polishers reliably exerted lower cumulative total impact forces (g) on roots that are being processed (Figure 3.4.3.1.0). Whilst the inherent design of polishers causes an amount of force and movement that is unavoidable, these results suggest that producers may learn from one another best practises and equipment that are less detrimental to product quality. Performing further analysis into VC, time spent being polished, peak magnitudes and number of sized impacts may provide further information into why certain polishers seem less destructive than others.

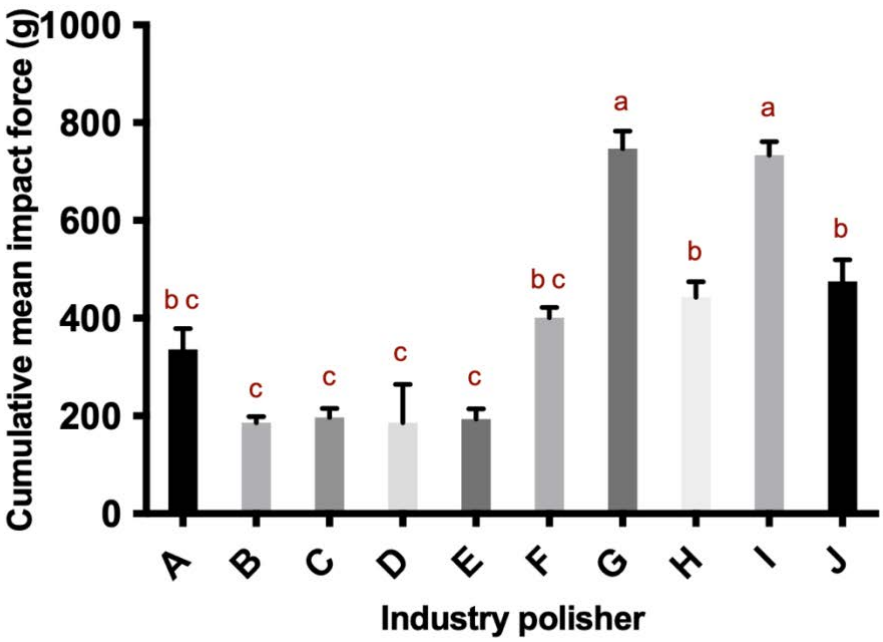


Figure 3.3.3.4.1.0) Cumulative mean impact force (g) experienced by the device across 10 industry parsnip polishers run on standard settings. (*N*=3-4) (*p*=<0.001). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

3.3.3.4.2. Cumulative mean velocity change (m.s^{-2}) experienced during polishing across 10 parsnip polishers

The cumulative mean VC (m.s^{-2}) experienced by the device during polishing was found to significantly differ (*p*=0.027) across industry polishers with huge variation in performance being witnessed (Figure 3.4.3.2.0). A Duncan's multiple range test was employed following the significant ANOVA *p* value (*p*=<0.027) that was calculated. Polisher I exerted the greatest mean VC (m.s^{-2}) on roots during polishing (333.96 ± 90.30) whilst ranking second for highest total impact force (g), Polisher C was the least destructive in terms of VC, exerting 1035.23 ± 247.36 m.s^{-2} onto roots during an

average run. Polisher G exerted the second greatest mean VC on roots but the greatest total mean impact force (g).

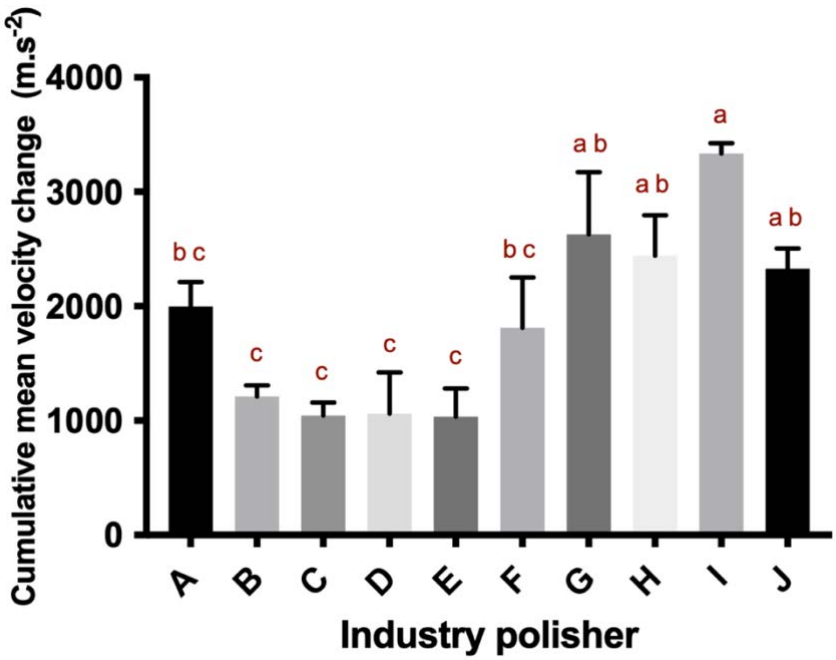


Figure 3.3.3.4.2.0) Cumulative mean velocity change ($m.s^{-2}$) experienced during polishing across 10 parsnip polishers. (*N*=3-4) (*p*=0.027). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

3.3.3.4.3 Time (seconds) spent being polished in 10 industry parsnip polishers

Investigating how long the device spent in each of the industry polishers may provide explanations for why certain polishers exerted large impact forces (g) or velocity changes ($m.s^{-2}$) on roots during processing. Significant differences in time (seconds) spent being polished was observed across the 10 industry polishers (*p*=<0.001) with the device and roots spending 107.67 ± 7.79 seconds in Polisher H , whilst Polisher E processed roots in 40.33± 4.41 seconds on average (Figure 3.3.3.4.3.0). A Duncan's

multiple range test was employed following the significant ANOVA *p* value (*p*=<0.001) that was calculated.

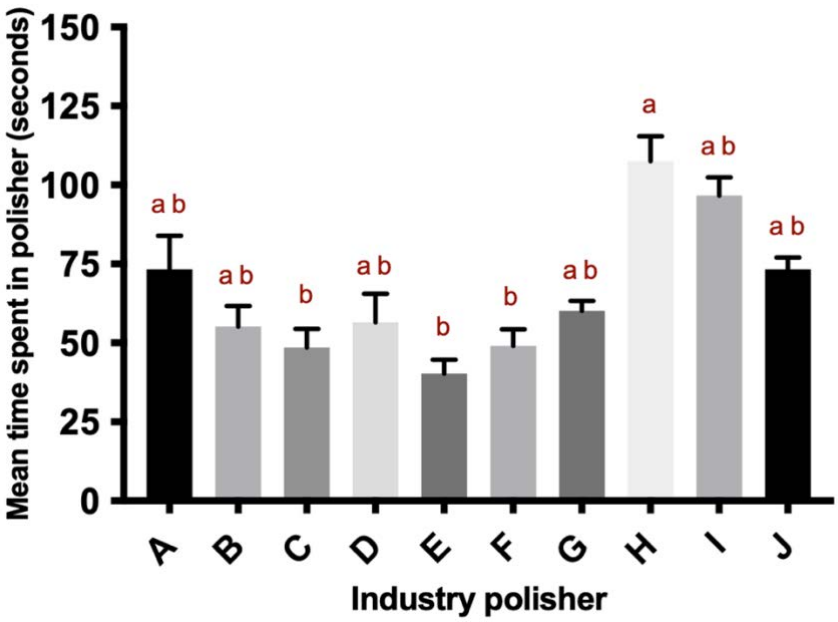


Figure 3.3.3.4.3.0) Time (seconds) spent being polished in 10 industry parsnip polishers. (*N*=3-4) (*p*=<0.001). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

Despite roots spending more time in polisher H compared in polisher G, polisher G exerted greater impact force (g) and VC $m.s^{-2}$ onto the device. This suggests that the amount of time spent being polished is not always indicative of polisher performance in terms of impact force and VC; the design of polisher G exerts more force onto roots in 60 seconds than polisher H does in 107 seconds (Figure 3.4.3.4.0). Whilst reducing the amount of time spent being polished would reduce the impact forces and VC experienced by roots, making modifications to current polishers may provide improvements to relatively destructive polishers such as polisher G.

3.3.3.4.4 Mean peak impact magnitudes (g) experienced by device in 10 industry parsnip polishers

The mean peak impact (g) experienced by the device was found to significantly differ across polishers (p=0.002) with polisher C exerting a peak impact of 14.05 ± 0.69 g per replicate (Figure 3.3.3.4.4.0). A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.002) that was calculated. Polisher D exerted the largest peak impact (25.20 ± 0.45 g) with remaining 8 polishers applying a mean peak impact ranging from 17.97 g- to 23.45 g to roots during polishing.

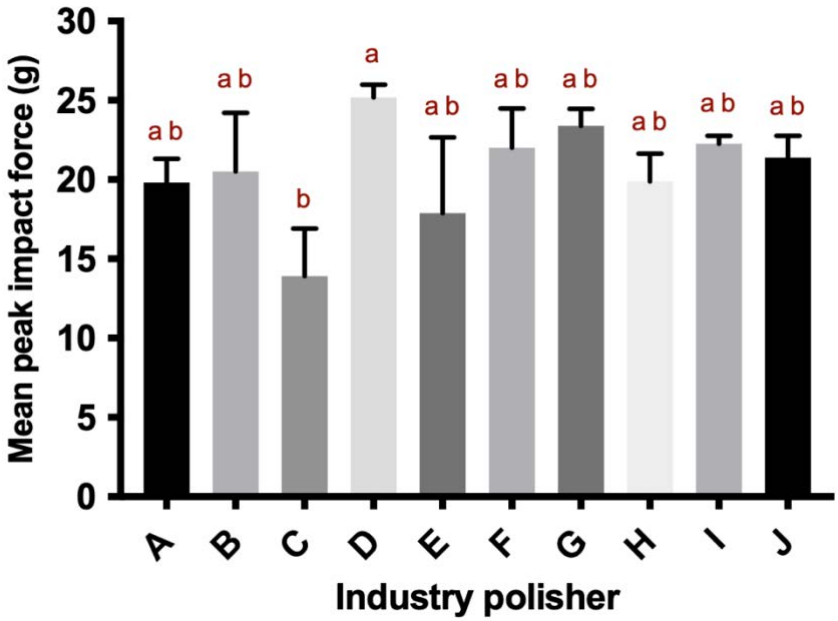


Figure 3.3.3.4.4.0) Mean peak impact size (g) experienced by the device per replicate across 10 industry polishers. (N=3-4) (p=0.002). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at p<0.05 according to Duncan's' multiple range test.

3.3.3.4.5 Size of impacts (g) witnessed during polishing across 10 industry parsnip polishers

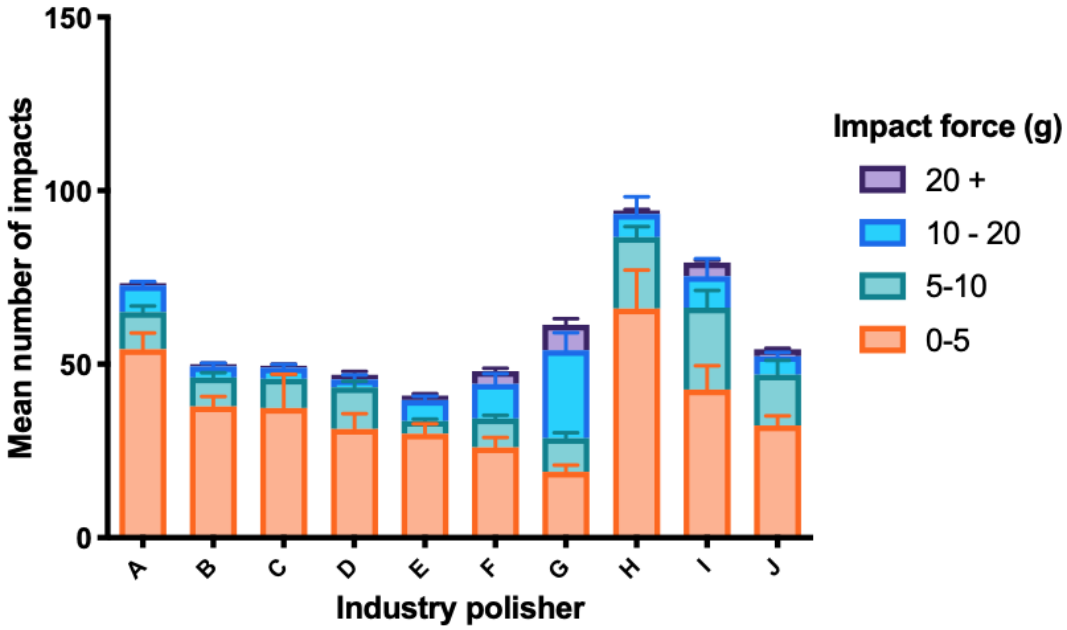


Figure 3.3.3.4.5.0) Total number of impacts recorded during parsnip intake sorted by impact size (0-5 g, 5-10 g, 10-20 g and 20 g+) across 10 industry polishers. (*N*=3-4). Error bars show standard error of the mean (SEM).

The comparison of the distribution of observed impact force (g) values across industrial polishers was significantly different for 3 of the 4 impact ranges following employment of a Wilcoxon test; with impacts exceeding 20 g experiencing no significant difference between polishers (Figure 3.3.3.4.5.0). Impacts sized between 0-5, 5-10 and 10-20 g exhibited significant differences (p=0.002). Polisher H inflicted the greatest mean number of impacts sized between 0-5 g (66.00 ± 11.13) as polisher G exerted only 19.00 ± 1.76, the lowest amount witnessed. Polisher I inflicted an average of 23.67 ± 4.89 impacts sized between 5-10 g in contrast to the fewest exerted (3.66 ± 0.45) by polisher E. For impacts sized between 10-20 g polisher G scored highest (25.33 ±

4.87), in contrast to impacts between 0-5 g where this polisher exerted the fewest. Polisher D exerted an average of 2.33 ± 1.67 g impacts sized between 10-20 g which was the lowest amount witnessed across industry polishers.

3.3.3.5 Quantitative evaluation of 7 industry parsnip graders

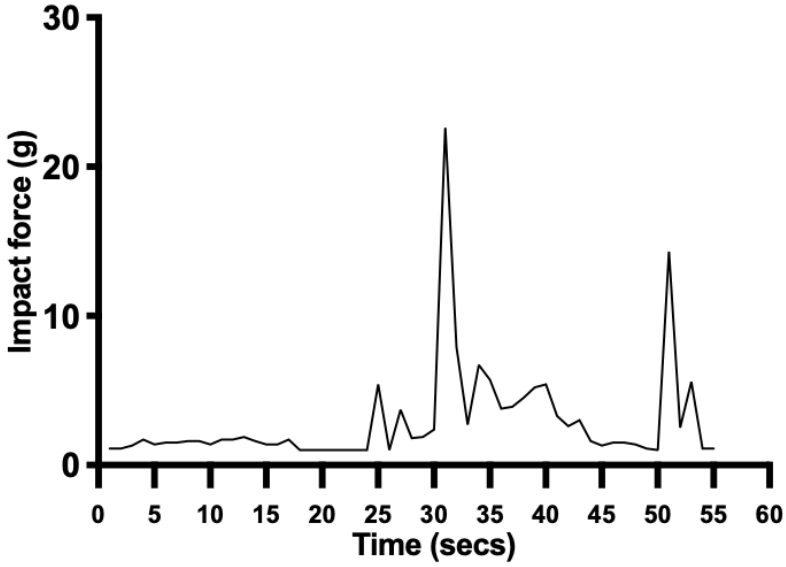


Figure 3.3.3.5.0) RTA graph illustrating impact force (g) experienced per second by the device whilst being processed by grader B across one replicate.

In order to investigate whether the device can detect significant differences in impact parameters between graders, the impact forces exerted during polishing in 7 parsnip graders from across the UK were recorded and analysed. All graders were run on their standard, settings with normal running procedures with a minimum of 3 replicates per grader. The device was entered into the preceding hopper before grading, the device then ran across the rollers with other roots before dropping approximately 90cm onto

a conveyor below (peak can be seen in Figure 3.4.4.0. Graders typically sort parsnips into 2 sizes based on crown diameter which determines their future as either packed (smaller roots) or loose (larger roots). The device was then transferred by conveyor into a hopper preceding packing, where it was removed.

3.3.3.5.1 Cumulative mean impact force (g) experienced during grading

The mean total impact force (g) experienced by the device and roots during grading was found to be significantly different (p=0.022) across industry graders (Figure 3.3.3.5.1.0). A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.022) that was calculated. Grader B exerted the lowest mean total impact force (104.43 ± 30.61 g) whilst Grader F inflicted 217.40 ± 26.30 g per replicate, the highest value witnessed across industrial graders. These results suggest that employing different models of graders can reduce the total impact force (g) exerted onto roots.

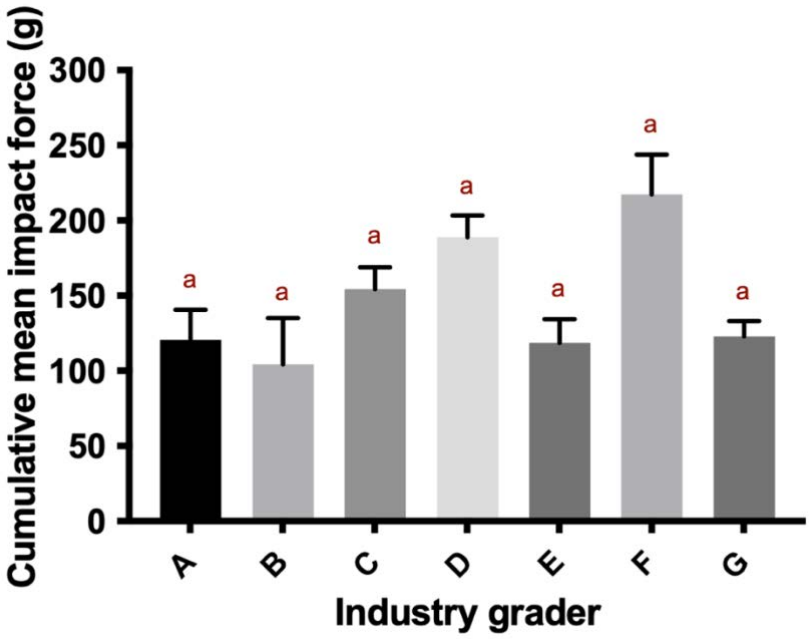


Figure 3.3.3.5.1.0) Cumulative mean impact force (g) experienced during grading across 7 industry parsnip graders. (*N*=3) (*p*=0.022). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at $p<0.05$ according to Duncan's' multiple range test.

3.3.3.5.2 Cumulative mean velocity change (m.s^{-2}) experienced across 7 parsnip graders

No significant difference in cumulative mean VC (m.s^{-2}) was observed across industrial graders (*p*=0.223) despite variation being observed (Figure 3.3.3.5.2.0). In correlation with total mean impact force (g), Grader F again scored highest across graders exerting a total mean VC of 126.90 ± 31.56 m.s^{-2} whilst Grader E exerted the lowest

total VC per replicate (57.46 ± 31.12 $m.s^{-2}$). Grader D exerted the second greatest total VC ($m.s^{-2}$) and total impact force (g) .

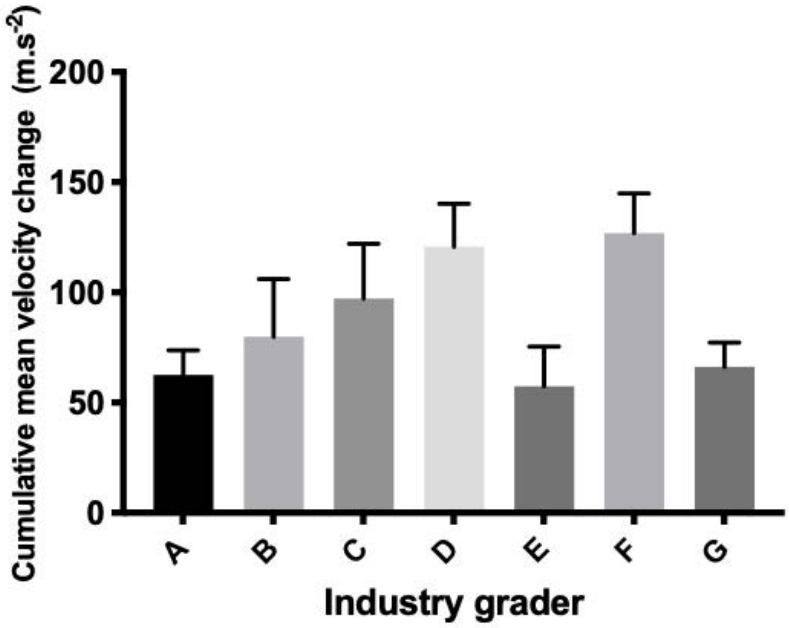


Figure 3.3.3.5.2.0) Cumulative mean velocity change ($m.s^{-2}$) experienced across 7 industrial parsnip graders. (*N*=3) (*p*=0.223). Error bars show standard error of the mean (SEM).

3.3.3.5.3 Time (seconds) spent being graded across 7 graders

The amount of time (secs) it took roots to complete the grading process was found to significantly different across industry graders (*p*=0.0014) with Grader D taking the longest (73.33 ± 12.81 seconds) to process roots (Figure 3.3.3.5.3.0). A Duncan's multiple range test was employed following the significant ANOVA *p* value (*p*=0.0014) that was calculated. Grader B completed the process in 29.33 ± 3.84 seconds which

was the fastest grader to process roots in addition to the grader which exerted the lowest total impact force (g). Grader D exerted the second greatest total impact force (g) and total VC whilst Grader F scored highest for both categories. Grader F took the second longest to process roots (57.33 ± 2.33 secs) whilst Grader D took the longest; this indicates that graders that take the longest to process roots inflict the greatest impact and velocity forces on roots.

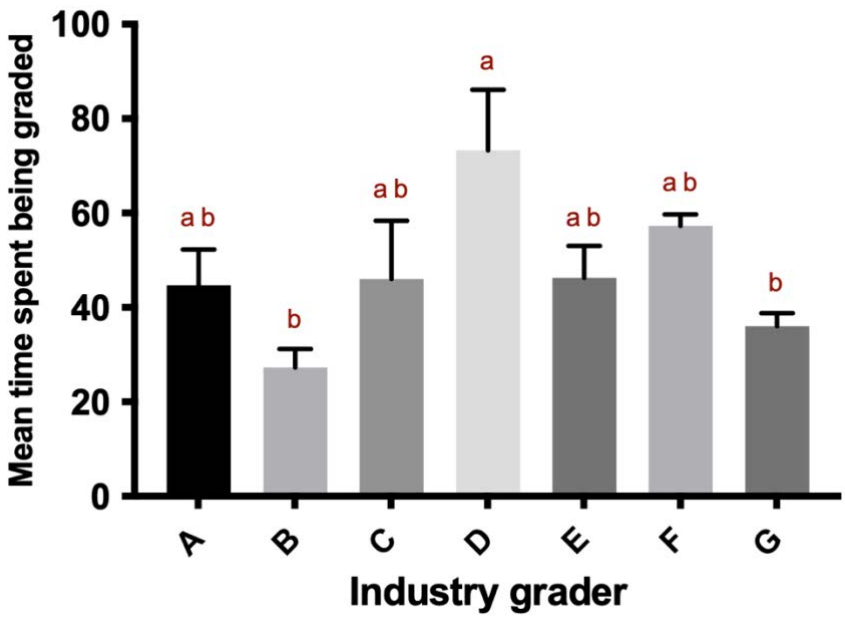


Figure 3.3.3.5.3.0) Mean time (secs) for roots to be processed by 7 industrial parsnip graders. (*N*=3) (*p*=0.0014). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at *p*<0.05 according to Duncan's' multiple range test.

3.3.3.5.4 Mean peak impact magnitudes (g) experienced by device during grading

No significant differences in mean peak impact magnitude inflicted onto the device were found across industry graders (p=0.189) (Figure 3.3.3.5.4.0). Grader B inflicted the highest peak impact (g) per replicate (19.16 ± 6.80) whilst Grader G inflicted a peak mean impact (g) of 11.33 ± 1.30, the lowest witnessed. The peak impact witnessed across all graders was a result of the device dropping through the rollers onto the conveyor below; reducing the height of the drop of introducing padding below the drop surface may reduce the peak impact exerted on roots thus the likelihood of mechanical damage and bruising.

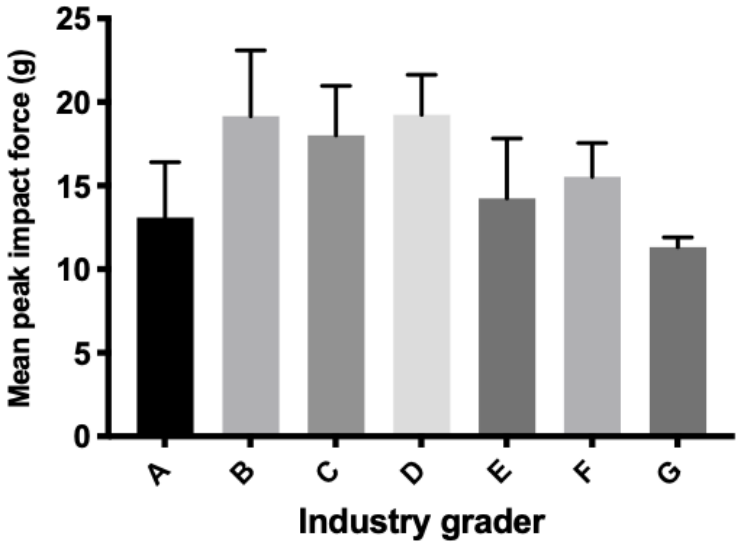


Figure 3.3.3.5.4.0) Mean peak impact force (g) experienced by the device during grading across 7 industrial parsnip graders. (N=3) (p=0.189). Error bars show standard error of the mean (SEM).

3.3.3.5.5 Size of impacts (g) witnessed during grading across 7 parsnip graders

The comparison of the distribution of the witnessed impact force (g) values across the 7 industrial parsnip graders was significantly difference (p=0.0156) for 3 of the 4 impact ranges (0-5, 5-10 and 10-20 g) following employment of a Wilcoxon test. No significant difference was observed for impacts that exceeded 20 g (Figure 3.3.3.5.5.0) (p=0.625). Grader D inflicted the greatest mean number of impacts that ranged between 0-5 g (65.66 ± 14.23) whilst Grader B inflicted the lowest number (23.33 ± 16.12) in this range. Both Graders C & F exerted the greatest number of impacts between 5-10 g (7.00 ± 2.54 & 7.00 ± 2.39 respectively) whilst G exerted 2.00 ± 0.96 impacts sized between 5-10 g per replicate. For impacts ranged between 10-20 g Grader F exerted the greatest mean number (5.00 ± 2.02) whilst Grader A only exerted 1.00 ± 0.58 impact between 10-20 g per replicate. Two of the industry graders (A & G) exerted no impacts that exceeded 20 g in any of the replicates whilst Grader D inflicted an average of 1.00 ± 0.58 impacts of 20g or larger per replicate, however no significant difference for this range of impact was observed (p=0.625).

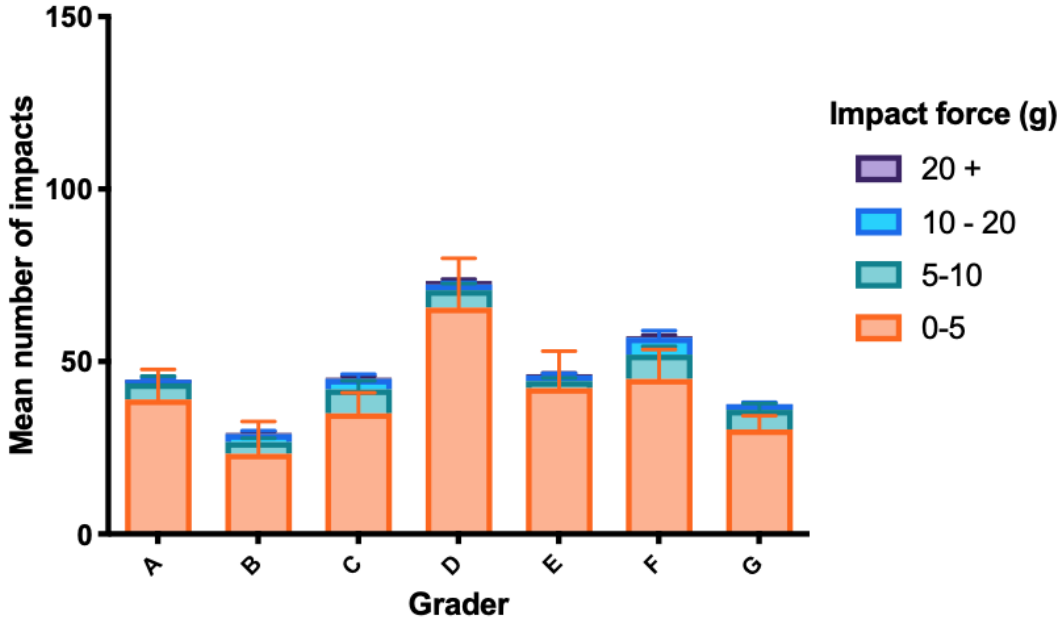


Figure 3.3.3.5.5.0) Size of impacts (g) witnessed during grading across 7 industrial parsnip graders. (*N*=3). Error bars show standard error of the mean (SEM).

3.3.4 Hypothesis 3: Investigating the ability of the electronic parsnip analyse modifications to destructive processes

The most destructive processing stage in regards to impact force (g) and VC ($m.s^{-2}$) in packhouse B was polishing. Furthermore the two most destructive individual processes witnessed across hypothesis 2 were exerted by polisher G and polisher I; in relation to impact force (g) and VC ($m.s^{-2}$) respectively. As a potential major source of impact forces and thus mechanical injury, modifications that can be made to polisher settings that reduce stress experienced by roots may be beneficial for product quality.

This section of study involved revisiting the packhouse where polishers F & G reside; hereby referred to as polisher 1 (P1) and polisher 2 (P2) respectively. Across industrial

polishers, P1 and P2 scored 4th and 1st highest in terms of mean total force (g) exerted (400.90 ± 20.98 & 655.36 ± 95.06 g respectively). P1 scored 6th highest (813.33 ± 437.40) for total VC (m.s^{-2}) whilst P2 scored 2nd highest, exerting 2629.67 ± 542.18 m.s^{-2}. Therefore P1 may be regarded as an average polisher in terms of inflicting mechanical damage, however P2 exerted some of the greatest impact forces across industrial polishers and is one of the two most destructive.

To investigate whether quantifiable improvements could be made to already installed polishers, the electronic parsnip analysed P1 under 3 settings, whilst P2 was tested under 4 settings, with 4 replicates for each group. In table 3.5.0 the mean total impact force (g), mean total VC (m.s^{-2}), mean time (secs) and mean peak impact magnitude (g) are listed with the accompanying ANOVA *p* value. Settings are shown as Barrel speed and then Brush speed, illustratively, B40/B60 represents Barrel speed at 40 and Brush speed at 60. P2 had the option of closing or opening the exit door to control the flow of roots; this is represented by U (up) and D (down) (Figure 3.3.4.0); The exit door to P1 is always down thus ensuring roots exit the polisher and are not kept inside for significant periods of time.

Figure 3.3.4.0) Image by Haith Ltd 2020 illustrating the inside of a Haith root vegetable polisher with carrots being polished on the inside as they progress through the rotating barrel. Brushes are clearly visible, whose speed can be changed, along with the speed of the rotating barrel.

Impact parameter	Polisher 1			ANOVA *p* value across P1	Polisher 2				ANOVA *p* value across P2
	B40/B60	**B60/B35**	**B50/B60**		**B10/B60 U**	**B10/B60 D**	**B40/B80 U**	**B40/B80 D**	
Mean total impact force (g)	241.59 ± 28.55	327.47 ± 9.82	257.20 ± 10.64	*p= 0.138*	644.60 ± 38.18	382.97 ± 97.90	456.30 ± 75.37	289.87 ± 19.55	***p= 0.042***
Mean total velocity change ($m.s^{-2}$)	1482.74 ± 111.72	1721.41 ± 110.82	1420.67 ± 193.63	*p= 0.366*	3528.98 ± 152.39	1839.46 ± 557.03	1905.12 ± 402.13	1446.48 ± 268.44	***p= 0.043***
Mean time (s)	35.33 ± 5.03	42.00 ± 1.73	31.00 ± 1.52	*p= 0.061*	75.33 ± 6.17	45.67 ± 11.68	49.67 ± 4.33	31.67 ± 2.73	**p= <0.001**
Mean peak impact magnitude (g)	22.43 ± 2.45	19.57 ±1.42	23.10 ± 1.51	*p= 0.183*	20.50 ± 2.51	21.30 ± 1.80	19.93 ± 1.48	23.23 ± 2.97	*p= 0.379*

Table 3.5.0) Table displaying means and SEM values for total impact force (g) total velocity change ($m.s^{-2}$), mean time (seconds) and mean peak impact magnitude experienced by the device across polisher settings. (*N*=4).

3.3.4.1 Cumulative mean impact force (g) experienced during polishing across polisher settings

Across P1 settings no significant differences in total mean impact force (g) were found (p=0.138) with the original setting of B40/B60 ranking lowest exerting a total of 241.59 ± 28.55 g onto roots per replicate (Figure 3.3.4.1.0). Running P1 on B60/B35 inflicted the greatest total impact force (g) per replicate across P1 settings. In contrast, significant differences in mean impact force (g) were witnessed across P2 settings (p=0.042) indicating that modifications to polisher settings may reduce impact forces exerted onto roots. A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.042) that was calculated for the second polisher. P2 running on it's original settings (B10/B60 U) inflicted 644.60 ± 38.18 g per replicate which was greater than the other P2 settings. Closing the exit door reduced total impact force (g) on the B10/B60 setting to 382.97 ± 97.90 per replicate,. Running P2 on settings B40/B80 with the door down inflicted the lowest total impact force (g) (289.87 ± 19.55) whilst the same settings with the door up inflicted 456.30 ± 75.47 g on average, however this difference was not significant (p=0.129).

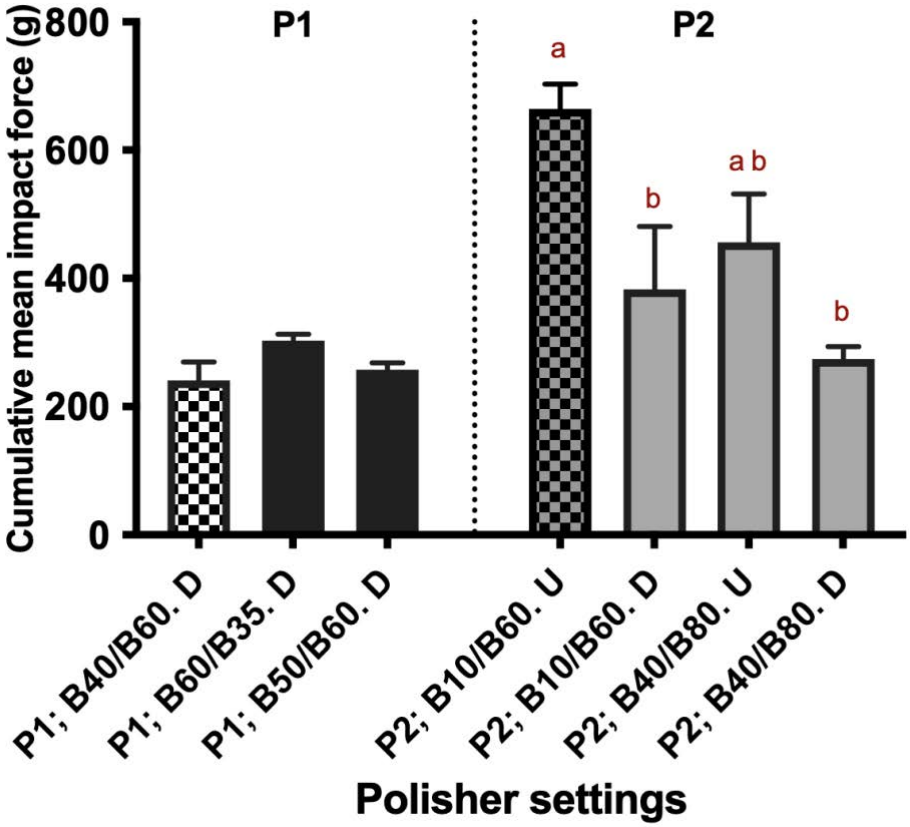


Figure 3.3.4.1.0) Cumulative mean total impact force (g) across 2 polishers (P1 & P2) on multiple settings with normal operational settings illustrated by patterned bars. (*N*=4). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at $p<0.05$ according to Duncan's' multiple range test.

Roots that are processed by this packhouse are polished by both P1 & P2 before entering hydrocooling and storage, therefore a worst case scenario of running P1 on B60/B35 and P2 on B10/B60 U may inflict a total impact force of 972.07 g. This combination was actually the settings being employed when analysis was initiated (shown by patterned bars in Figure 3.3.4.1.0); thus modification to current practises in terms of P2 settings are recommended. Operating parsnip polishers with the door down results in roots exiting the polisher into hoppers when they reach the exit end. In

contrast, the door being up during operation only allows a proportion of roots to exit the polisher, the remainder are held inside until there is space for them for exit which results in greater total impact forces (g) being exerted on roots.

3.3.4.2 Cumulative mean velocity change ($m.s^{-2}$) experienced during polishing across polisher settings

No significant difference in cumulative mean VC ($m.s^{-2}$) were observed across the 3 P1 settings (Figure 3.3.4.2.0) (p=0.366) with the normal operating setting (B40/B60) exerting a mean total VC of 1482.74 ± 111.72 $m.s^{-2}$ ranking 2nd. Running P1 on B60/B35 again ranked 1st exerting 1721.41 ± 110.82 $m.s^{-2}$ onto roots whilst B50/B60 inflicted the lowest total VC (1420.67 ± 190.63 $m.s^{-2}$). In contrast to P1, once again significant differences in mean VC were found across P2 settings (p=0.043) with B10/B60 U exerting 3528.98 ± 152.39 $m.s^{-2}$; the largest value witnessed, and in correlation with total force (g). A Duncan's multiple range test was employed following the significant ANOVA p value (p=0.043) that was calculated. B40/B80 D exerted the lowest total mean VC (1446.48 ± 268.44 $m.s^{-2}$) witnessed across P2 settings correlating with total impact force (g).

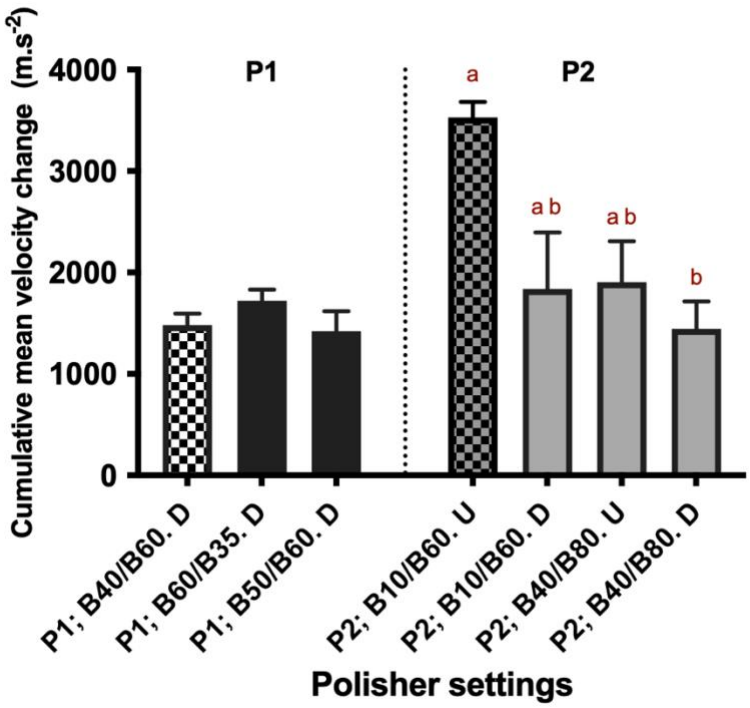


Figure 3.3.4.2.0) Cumulative mean velocity change ($m.s^{-2}$) experienced during polishing across polisher settings (*N*=4). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at $p<0.05$ according to Duncan's' multiple range test.

3.3.4.*3 Time (seconds) spent being polished across polisher settings*

No significant difference in the amount of time the device spent being polished was observed across P1 ($p=0.061$) whilst it was found to be extremely significant across P2 ($p=<0.001$). A Duncan's multiple range test was employed across polisher 2 results following the significant ANOVA *p* value ($p=<0.001$) that was calculated. Operating P1 on B60/B35 resulted in the device spending an average of 42.00 ± 1.53 seconds being polished, whilst B40/B60 took the lowest amount of time to complete polishing (Figure

3.3.4.3.0). The standard operating setting of B40/B60 ranked 2nd taking an average of 35.33 ± 5.03 seconds to polish roots.

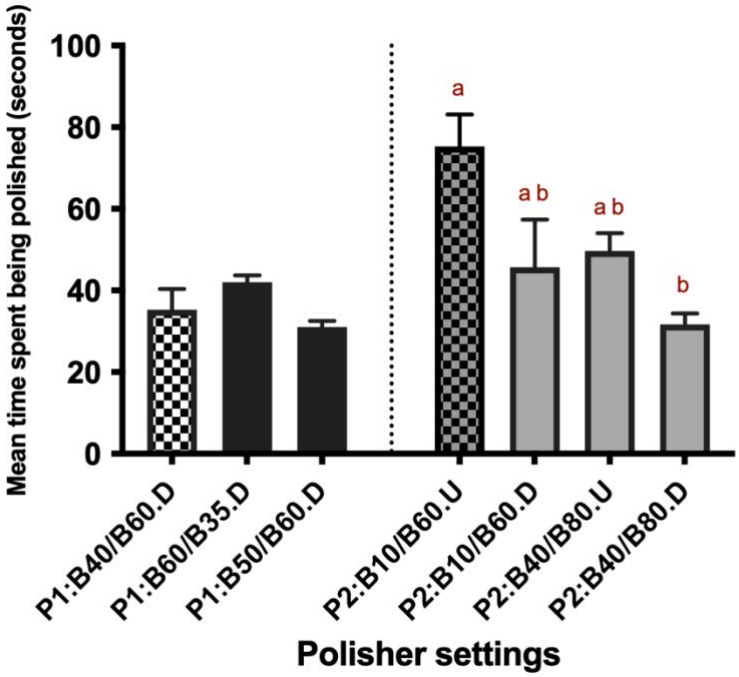


Figure 3.3.4.3.0) Time (seconds) spent being polished by 2 polishers across multiple settings. (*N*=4). Error bars show standard error of the mean (SEM). Means denoted by same letter did not differ significantly at $p<0.05$ according to Duncan's' multiple range test.

In contrast to P1, significant differences in time to complete polishing were observed across P2 settings ($p=<0.001$), with B10/B60 U taking an average of 75.33 ± 6.17 seconds to polish roots. This indicates that the standard operating settings for P2 result in roots spending significantly more time being polished as B10/B60 U took the longest to process roots. Running P2 on settings of B40/B80 with the door down resulted in the shortest polishing time (31.67 ± 2.73 seconds), for both B10/B60 and B40/B80

operating with the door down reduced the amount of time roots spent being polished, and therefore the impact and VC forces experienced.

3.3.4.4 Mean peak impact magnitudes (g) experienced by device across polisher settings

No significant differences in the mean peak impact (g) magnitude exerted onto the device were observed across P1 (p=0.183) or P2 (p=0.379). Operating P1 on B50/B60 exerted a peak impact of 23.10 ± 1.51 g the greatest across P1 settings, whilst B60/B35 exerted a mean peak impact of 19.57 ± 2.24 g (Figure 3.3.4.4.0). The highest peak impact magnitude witnessed across P2 settings was inflicted by B40/80 D (23.23 ± 2.97 g) whilst B40/B80 U ranked lowest exerting a mean peak impact of 19.93 ± 1.48 g.

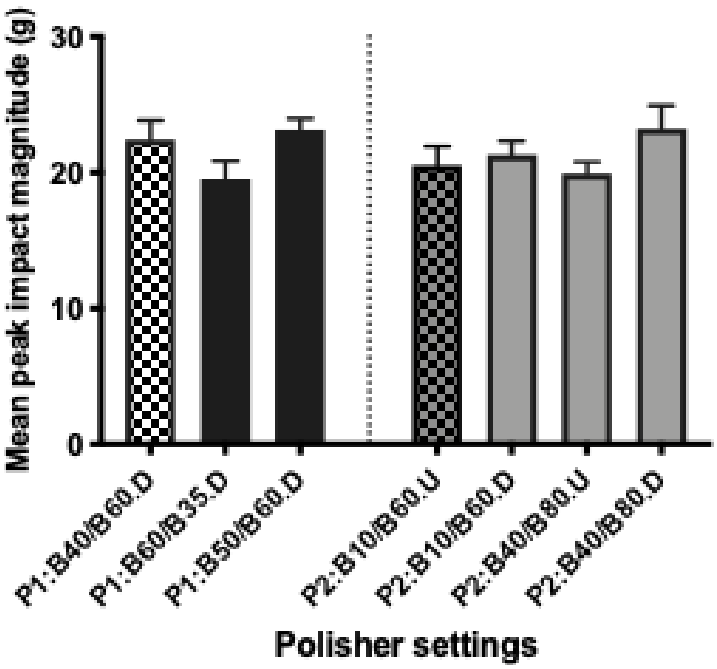


Figure 3.3.4.4.0) Mean peak impact magnitude (g) experienced by the device across polisher settings. (N=4). Error bars show standard error of the mean (SEM).

3.3.4.5 Size of impacts (g) witnessed during polishing across polisher settings

Across P1 no significant difference in the distribution of the impact forces experienced by the device was observed (p=0.25 for all 4 impact groups). For impacts sized between 0-5 g B40/B60 inflicted the greatest number of impacts (18.67 ± 4.17) whilst B50/B60 exerted on average 13.67 ± 3.28. B60/B35 ranked first inflicting an average of 17.66 ± 6.28 impacts sized between 5-10 g (Figure 3.3.4.5.0), in contrast B50/B60 inflicted an average of 8.33 ± 4.08 impacts in that range. For impacts sized between 10-20 g B40/B80 ranked top inflicting an average of 8.33 ± 3.45 impacts whilst B50/B60 inflicted the lowest number (7.33 ± 5.17). Finally, for impacts that exceeded 20 g, B50/B60 inflicted the greatest number of impacts (1.67 ± 0.87), the other two settings inflicted an average of 0.67 impacts that exceeded 20 g.

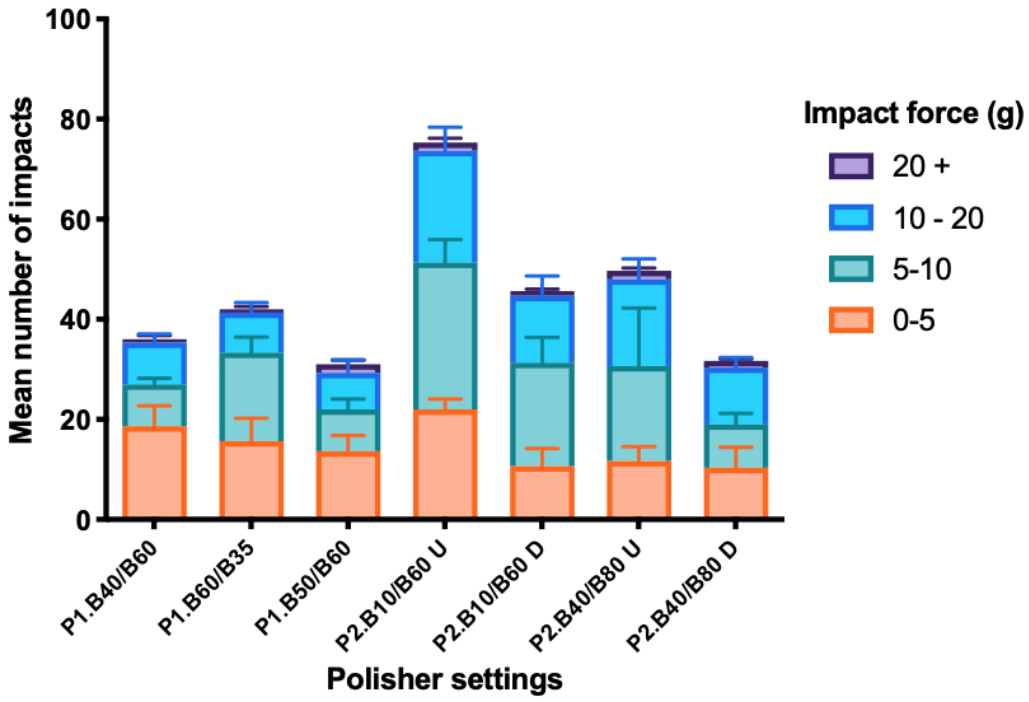


Figure 3.3.4.5.0) Size and number of impacts experienced by the device across polishers and settings. (N=4). Error bars show standard error of the mean (SEM).

No significant difference in the distribution of impact forces was observed across P2 setting across any of the 4 impact groups (*p*=0.125). For impacts that sized between 0-5 g, B10/B70 U exerted the greatest mean number (22.20 ± 4.17), whilst B40/B80 D inflicted the lowest mean number of impacts in that range (10.33 ± 8.19). B10/B60 U also inflicted the greatest number (29.33 ± 4.45) of impacts between 5-10 g, as B40/B80 again inflicted the fewest (8.67 ± 3.35). For impacts that ranged between 10-20 g B10B60 U again ranked 1st exerting 22.33 ± 6.98 impacts, in a similar vein to the previous ranges, B40/B80 D inflicted the fewest number (11.33 ± 4.08).Finally, both B10B60 U (1.67 ± 0.67) and B40/80 U (1.67 ± 1.12) inflicted the greatest number of impacts that exceeded 20 g. B10/B60 D was the setting that exerted the fewest number of impacts that exceeded 20g (1.00 ± 0.67).

3.4 Discussion

3.4.1. Identification of post-harvest processing procedures that cause mechanical damage in parsnips across Packhouse A

This study indicates that physical damage to parsnip roots is a result of mechanical impacts with machinery, stones or other roots; identifying processes which exert significant impact forces facilitates modifications to equipment that may reduce impact intensity that roots experience during processing (Opara & Panthere, 2014). However, it should be noted that a degree of mechanical damage is unavoidable due to the heavy mechanisation employed during harvesting, processing and transportation (Fernando *et al.,* 2019), which is especially true for root crops.

Throughout harvesting and processing in packhouse A, the mean number of bruises witnessed per parsnip increased from the first processing stage to the final stage from 0.55 ± 0.17 bruises exerted following harvesting, to 1.25 ± 0.30 bruises exerted following packing. A significant bias toward crown end bruising was observed (p=<0.001) indicating that parsnip shape (bulbous crown with a tapered tail end) causes the widest point (crown) to be more susceptible to bruising damage (Figure 3.4.1.0). Mechanical impacts to roots during harvesting and processing additionally cause scuffing, which also presented a slight bias towards crown end damage.

These results suggest that parsnip bruising accumulates throughout harvesting and processing, and due to the bruise latency period, this leads to a greater number of

bruises being present for consumers. This concurs with studies in bananas (Maia *et al.,* 2015; Fernando *et al.,* 2019) where skin abrasions (scuffs) and bruises accumulate along the supply chain.

Figure 3.4.1.0) Authors own image illustrating externally visible crown bruising on processed parsnip roots.

3.4.2 Impact damage acquisition and quantification via use of the electronic parsnip

3.4.2.1 Investigating the ability of the electronic parsnip to find significant differences in impact parameters between 8 different processing stages across Packhouse B

Quantifying impact forces and VC experienced by produce across whole packhouse operations has been achieved in citrus fruits (Roa *et al.,* 2015) where modifications to processing equipment reduced impact forces experienced by produce (Manetto *et al.,* 2017). Peters, (1996) noted that harvesting caused 70% of damage to potato tubers in comparison to processing, with transport and storage contributing 30% towards total mechanical damage. Bentini *et al.,* (2006) found that potato harvester settings significantly influenced tuber damage with the intensity of impacts being greatest in dry soil during one of the first contacts between tuber and harvester. Determining bruising thresholds for fresh produce, and the post-harvest factors influencing bruise formation, can assist operations reduce the size and number of impacts inflicted (Öztekin,& Güngör, 2020).

Whilst harvesting exerted significant mechanical forces onto roots, it was not the most destructive process across packhouse B. Polishing (1 & 2) and grading (1 & 2) were the four processes that exerted the greatest total mean impact force (g) and total mean VC ($m.s^{-2}$) onto roots. This is in contrast to previous work in potato's (Peters, 1996), blueberries (Yu *et al.,* 2014) and the personal belief amongst

parsnip producers, that the majority of damage is inflicted during harvesting. The 2 polishers employed in packhouse B inflicted the greatest number of impacts for all impact ranges (0-5, 5-10, 10-20 & 20+ g), exerting a significant number of mechanical impacts that exceeded 10 g. Other processes such as intake and harvesting exerted few impacts that exceeded 10 g, indicating that priority should be on modifying current polishers to reduce the mechanical stress roots experience during polishing. However, previous work in parsnips (chapter 2) has shown that even small impacts (<10 g) are sufficient to cause severe bruise formation; producers should investigate employing padded surfaces or modifying operating settings to reduce the impact forces experienced by roots, across all processing stages.

Previous work focussing on apple packhouses (García–Ramos *et al.,* 2002), demonstrated that the use of a instrumented impact device can successfully test padding materials at key points through processing; to reduce the impact and velocity forces exerted onto produce. Reducing the drop height and applying foam padding to cushion the fall at key points during blueberry harvesting reduced the observed bruising across all cultivars (Takeda *et al.,* 2013). Whilst Xu *et al.,* (2015) found padding blueberry packing lines effectively reduced both impact and velocity forces, and observed bruising damage.

Comparing total impact force and VC values exerted on roots between whole packhouse operations (e.g packhouse B vs packhouse C) is an area recommended for future study. Combining packhouse losses data and impact data observed (e.g

% loss due to mechanical damage from each stage and the impact force experienced) and comparing across packhouse operations may aid producers implement alterations aimed to reduce losses.

3.4.2.2 Investigating the ability of the electronic parsnip to find significant differences in impact parameters within specific processing stages across industry

Comparing the total impact force (g), total VC ($m.s^{-2}$), time taken to process (secs), peak impact magnitudes (g) and the distribution of impact force sizes within specific processes across industry, provides valuable information to producers on how their operation compares against competition. Providing anonymity to producers, as has been done here, encourages a more collaborative sharing of data which has facilitated this unprecedented industrial comparison of parsnip processing stages. However, confidentiality regarding equipment models and standard operating settings produces a more general comparison of industrial processes. It still provides producers with information on how their processes compare to their competitors, but no information on how modifying their operating settings may result in lower observed mechanical damage.

Identifying particularly destructive processes (such as Polisher G & Polisher I) that regularly exert impacts that could cause bruising, compared to their industrial counterparts, may motivate producers to address it directly, by upgrading or making alterations to existing machinery or operating procedures.

Significant variation in harvester performance regarding total impact force (g) was observed (p=<0.001), which may have been a result of the significant variation in time taken to harvest roots (p=<0.001) that was also witnessed. Dropping roots into an empty lorry did not increase the impact or velocity forces experienced by the device in comparison to full transport lorries. No significant differences in parameters were observed between intake methods, however employing a greater amount of water to dislodge roots from transport trailers caused an increase in the number of impacts sized 5-10 g that roots experienced (p=0.042).

Across the 10 industrial polishers significant differences in mean total impact force (g) (p=<0.001), mean total VC ($m.s^{-2}$) (p=0.027), mean time (secs) (p=<0.001) and mean peak impact magnitude (g) (p=0.002) were observed, indicating significant industrial variance in destructive potential. Polishing inflicted a significant number of impacts that exceeded 10 g across all polishers. Polisher G inflicted 25.33 ± 4.87 impacts sized between 10-20 g, and 7.33 ± 3.05 impacts that exceeded 20 g, signifying that polishing is a major source of mechanical impacts during parsnip processing. Reducing the number of large (10 g+) impacts experienced by roots during polishing may be achieved by updating models; a number of polishers (A-E) were not especially destructive compared to other post-harvest processes. Modifying current operating settings may reduce the impact forces experienced by roots, thus reduce mechanical damage and bruise formation. However, the impact of modifications is limited by polisher model; older models that are rotating metal drums and do not have internal brushes or an exit door have a limited range of operating settings. Newer models that have independently moving brushes allow

both the barrel and brush speed to be manipulated; along with the operation of the exit door, thus controlling the flow of roots through the polisher and dictating the degree to which roots are polished.

Graders were observed to inflict significant impacts onto roots during processing with significant variation in total mean impact force (g) across industry graders being witnessed (p=0.022). Grader F was the most destructive, inflicting an average of 5.00 ± 2.02 impacts sized between 10 - 20 g and 0.33 ± 0.67 impacts that exceeded 20 g. Previous studies have demonstrated quantifiable reductions in observed impact forces and bruising following padding of key transfer points and drops (Xu *et al.,* 2015). The peak impact witnessed during grading was a result of roots dropping through the grading rollers onto conveyors situated below; typically this drop is 50-90 cm in parsnip packhouses. The rubber conveyor belts that roots drop onto after being graded have a number of horizontal metal poles running underneath at approximately 50cm intervals reinforcing its shape; this often has the consequence the rubber belt not “giving” as it should. This results in roots contacting the rubber belt with the metal pole underneath, causing more of the impact force to be absorbed by the root tissue, increasing the likelihood of damage. Introducing a foam layer between the metal poles and the underside of the rubber belts may reduce impact forces exerted onto roots based on previous work (Takeda *et al*., 2013). However certain industrial graders were observed to not be that destructive in comparison to other industrial processes (graders A, B, E & G). Understanding why these graders perform better and how they can be improved is an area of future study that is recommended.

3.4.2.3 Investigating the ability of the electronic parsnip to analyse modifications to destructive processes

Kumar & Azad, (2020) studied the effect of industrially washing carrots on microbial infection and bruising damage, bruise quantification was measured as the bruising %, and they observed significant variance between washer settings. Fadiji *et al.,* (2016) found that employing modifications to apple packaging significantly reduced the impact forces exerted onto fruit, thus reduced bruising. Minimizing impact force reduces post-harvest damage thus increasing the quality and shelf life of produce (Ahmadi *et al.,* 2010).

After identifying polishers as the processing stage that exerted the greatest impact and VC forces onto roots, modifications to a destructive (P2) and an average performing polisher (P1) were applied to provide detailed information on how the performance of their specific polisher can be improved. The two polishers (P1 & P2) employed in this particular packhouse facilitated the testing of multiple barrel speed, brush speed and door position settings to investigate the least destructive operating settings. Not all packhouses employ such advanced polishers, thus older models of polishers provide less scope for modification limiting the effectiveness of this work for some producers. Running P2 on B10/B60 with the exit door down reduced the total impact force (g) experienced by roots from 644.60 ± 38.18 to 382.97 ± 97.90. This resulted in P2 becoming an average polisher in terms of destructive potential, in comparison to operating with the door up when this polisher exerted the greatest mean total impact force (g) and was second highest for total

VC ($m.s^{-2}$) across all 10 industry polishers. Furthermore, running P2 on B40/B80 U reduced total impact force (g) exerted on roots compared to B10/B60 U; putting the door down also reduced the witnessed impact force (g) for B40/B80. Significant improvements regarding destructive potential for P2 can be achieved by changing the barrel and brush speed, and by running the polisher with the door up, reducing the amount of time roots spend being polished. This analysis found that the standard operating setting of P1 (B40/B60 D) could not be significantly improved by modifying the barrel and brush speed.

There seems to exist a trade-off between amount of polish a root experiences and impact force (g) exerted; excessive polishing may cause bruising and also exacerbates any scuffs. Future studies are recommended to take the approach we did for P1 & P2 for all processing stages for a comprehensive analysis of the best possible operating procedures, for all processes in parsnip harvesting and processing. An approach which combines bruising data caused by mechanical processes and the impact data from those processes would provide the most reliable information regarding best working practices.

3.4.3. Conclusions

- Physical damage to roots (scuffs and bruises) are the result of mechanical impacts inflicted during harvesting and processing.
- Bruises accumulate throughout processing due to the formation latency period, however the majority of scuffs are removed during manual inspection.
- Significant differences in impact forces were observed across processes within packhouse B, suggesting that packhouse processing may be more destructive than harvesting.
- There is significant variation in equipment performance across the parsnip industry.
- Significant improvements to current destructive processes, such as polishing, can be achieved through modifications to operating procedures.

Chapter 4: Post harvest factors affecting bruising susceptibility in parsnip roots

4.1. Introduction

The population of Earth is estimated to exceed 9 billion people by 2050, thus, agricultural capacity must increase in order to match future demand (Godfray *et al.,* 2010). Mechanical damage to fresh produce remains a major cause of food waste and affects the ability to increase agricultural capacity. Projections predicting a +2°C global warming suggest the UK will experience longer and more severe droughts (Roudier *et al.,* 2016), but also more severe floods and extreme weather events. Chaves & Oliveira, (2004) reported that drought is currently a major limiting factor to expanding crop production, thus any future droughts will affect the UK's ability to increase capacity (Burns, 2017). Understanding how parsnip root bruising is affected by postharvest factors such as harvest date, irrigation scheme and variety may aid producers reduce packhouse losses and increase the sustainability and profitability of the industry.

Factors such as size and number of impacts, contact surface, temperature and maturity influence the bruising susceptibility of fresh produce to mechanical impacts (Zarifneshat *et al.,* 2010). Bruising damage can be exacerbated by physiological characteristics that affect bruising such as solute leakage, water content, cell wall strength, cellular organisation, phenolic content, temperature and cultivar (Toivonen, 1992, van Linden *et al.,* 2006; Zarifneshat *et al.,* 2010).

In the UK, parsnips are harvested from July through to March with the majority of roots destined for the Christmas market. Figure 4.1.0 illustrates how variable the average rainfall (mm) can be throughout the growing season is in the UK, especially so during 2019 and 2020. During dry periods (Figure 4.1.1), producers may be forced to irrigate parsnip fields to maintain plant water content and to make harvesting using heavy equipment possible.

Osmoregulation allows plants to maintain water uptake in soils with an irrigation deficit, solutes accumulate in the cytoplasm of plant cells resulting in a negative osmotic potential (Grove & Monaghan 2018). A cytoplasmic negative osmotic potential causes water to enter plant cells maintaining their turgidity in droughted soils. Potato tuber blackspot susceptibility was observed by Corsini *et al.,* (1999) to be lowest in fields with lower levels of soil water, furthermore a higher specific gravity of harvested tubers was found to result in greater discolouration susceptibility. Mwanamwenge, (1989) also observed reduced bruising susceptibility in potato tubers harvested from soils with a lower moisture content, in comparison to wetter fields.

During periods of heavy rainfall wet soils cause the harvesting of parsnips to become challenging as machinery has difficulty manoeuvring and soil is harder to dislodge from roots. Producers therefore struggle with harvesting in very dry or very wet fields (Figure 4.1.2); it is currently unclear how bruising susceptibility in parsnips is affected by soil water content and concurrently, relative root water content. Anecdotally, parsnip producers witness greater bruising and losses in roots from wet fields and attribute this to less control of harvesting equipment, more soil being stuck to roots thus more

agitation is required to remove, and finally roots weighing more due to a higher water content; thus impacts contain more energy (Personal communication with multiple parsnip producers, 2018-2019).

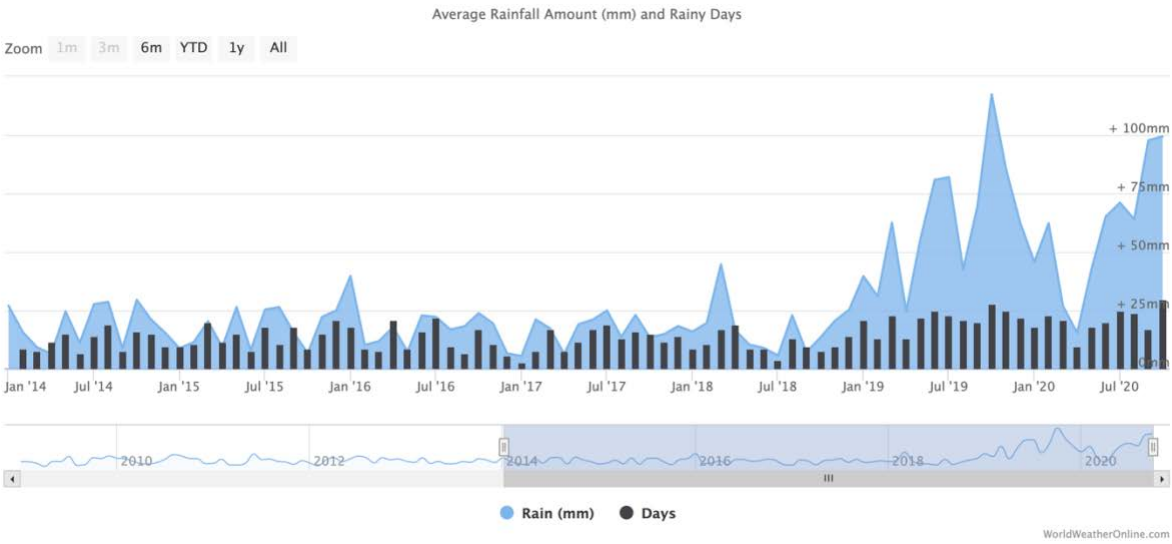


Figure 4.1.0) Graph illustrating average rainfall (mm) and rainy days for Norwich, East Anglia, a major parsnip growing region between January 2014 and October 2020. Produced on and taken from Worldweatheronline.com

Figure 4.1.1) Image by Frederick Hiam Ltd demonstrating parsnip harvesting during dry periods of weather. Taken from Twitter @FrederickHiam 25/03/2020.

Figure 4.1.2) Image taken from Twitter @davidbowe76, illustrating difficulty in harvesting root crops during wet weather 20/11/2019.

The maturity of produce at the time of harvest has been observed to influence bruising susceptibility by a number of other researchers. Mowatt, (1997) found that early harvested apples of 2 varieties displayed lower bruising susceptibility than fruits harvested at the end of the season. Corsini *et al.,* (1999) observed an increase in blackspot susceptibility in potato tubers as the growing season progressed in 2 varieties. Mondy and Munshi, (1993) found that phenolic compounds concentration, such as tyrosine, increased with maturing tuber age thus more substrate for PPO activity existed in older potato tubers.

There is currently a lack of information regarding how harvest and irrigation affect bruising in parsnip roots. Given that the majority of roots are destined for the winter market, typically a period of heavy rainfall in the UK, it is important to provide producers with data on how their pack out losses may be affected. Previously, harvest and irrigation management in apple orchards was found to significantly reduce bruising susceptibility (Opara, 2007). In addition to post-harvest factors such as impact force, storage duration and temperature, pre-harvest factors such as harvest date must be investigated to identify the best working practises. Furthermore, whilst varietal differences in parsnip tissue browning has previously been described (Toivonen, 1992), there is a need for more up to date studies on varietal differences in parsnip overall bruising susceptibility rather than just tissue browning.

4.1.1 Aim

The aim of this section of study was to investigate the effect of irrigation scheme, harvest date and variety on bruising susceptibility in parsnips to provide producers with mitigation strategies.

4.1.2 Objective

1) To assess whether harvest date, irrigation scheme and variety are significant factors affecting bruise severity, percentage likelihood to bruise, and relative root water content following employment of the falling bolt protocol, developed in chapter 2.

4.1.3 Hypotheses

H_0= Harvest date exerted on roots has no significant effect on bruising susceptibility

H_1= Irrigation scheme has no significant effect on bruising susceptibility

H_2= Variety has no significant effect on bruising susceptibility

4.2. Materials and methods

4.2.1 General methods

The trial site for this experiment was located at the Crop and Environment Research centre (CERC) at Harper Adams University, Shropshire UK. Parsnip plants were grown in a large polytunnel which had open aired but netted ends to prevent wildlife from entering. A total of 81 mesocosms were employed in this experiment; each mesocosm is a submerged wheelie bin (Figure 4.2.1.0) with 6 holes drilled in the bottom to facilitate drainage and is buried to a depth of 50 cm. The model of bin employed had an average height of 76.45 cm with a total capacity of 136 L (Grove & Monaghan 2018). The soil for each mesocosm was a mixture of the excavated sandy soil, and peat.

Figure 4.2.1.0) Authors own image illustrating mesocosm and polytunnel set up at CERC at experiment initiation.

Each mesocosm had a bulbous ended plastic tube running down through its central point to allow measurement of soil moisture measurements up to a depth of 70 cm, through the mesocosm at 10 cm intervals, via employment of a diviner 2000 probe (Sentek Technologies, Australia). Each mesocosm had approximately 10L of compost deposited onto the sandy soil/peat mixture to improve seed germination and propagation.

Figure 4.2.1.1) Authors own image illustrating CERC polytunnel and mesocosm set up in June, 2018. Author is seen tending to drip irrigation feeders.

Seeds were sourced from Elsoms Seeds Ltd and were spaced to the industry recommended standard (>2 inches) allowing a maximum 9 seeds per mesocosm. Before parsnip seeds were planted, the soil water content to 70 cm at 10 cm intervals

was recorded after a winter of bins being exposed to rainfall, to determine the starting capacity of each mesocosm; as previously done by Hall *et al.,* 1977 and Grove & Monaghan 2018. Once the starting soil water content was established, the plastic polytunnel covers were fitted to prevent rainfall from reaching mesocosms; thus the only source of water to the mesocosms was a drip irrigation setup (approximately 4 L a day) for the remainder of the experiment. A total of 3 irrigation scenarios were tested; firstly the “control” groups where plants were irrigated up to the day of harvest. Secondly the “mild“ scenario where mesocosms were deprived of irrigation for 7 days preceding the harvest date, and finally mesocosms were not irrigated for 2 weeks in the ”moderate” groups. The soil water content of each mesocosm was measured immediately prior to the harvesting of roots that respective replicate.

To analyse any varietal differences in bruising susceptibility 3 parsnip varieties were employed (V1, V2 and V3) with each variety undergoing each of the 3 irrigation scenarios for each harvest date. Roots were harvested monthly between September-November 2018 (H1 - H3 respectively) with 3 mesocosms from each of the 9 experimental groups per month being harvested and the roots from those bins undergoing analysis. The experiment contained 3 factors which all contained 3 levels leading to 27 experimental groups in total, with a *n* number of roots of between 18-24 with a mean root count of approximately 22.

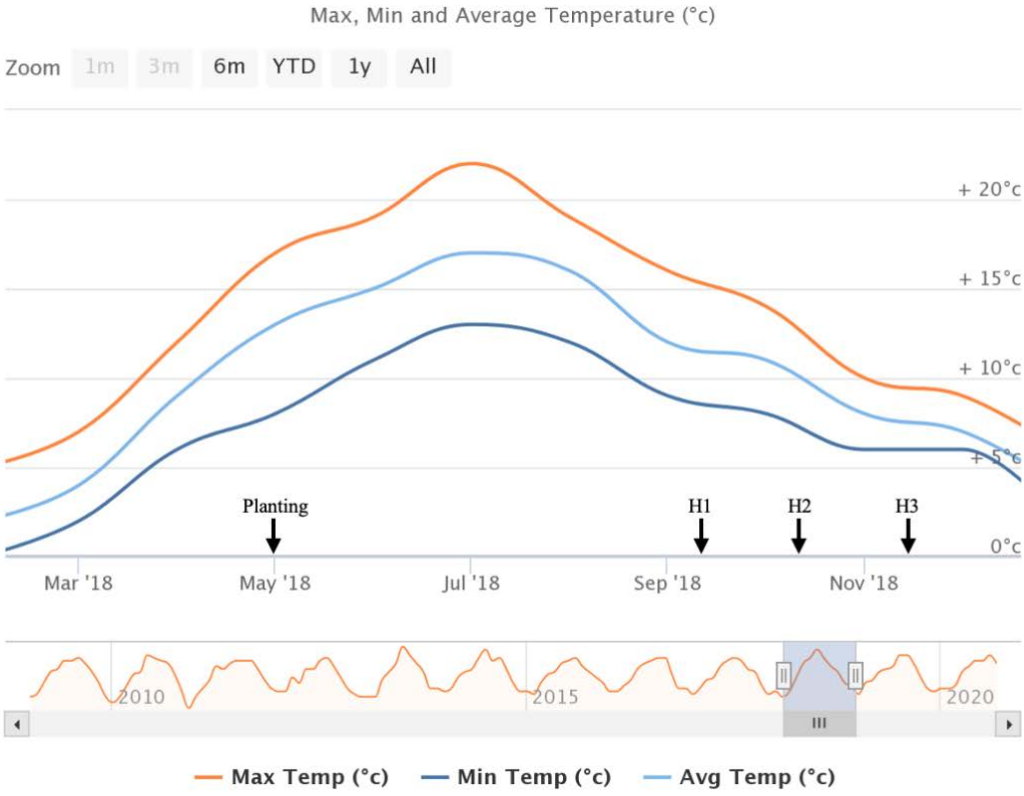


Figure 4.2.1.2) Historic climatic graph illustrating Max, Min and average temperatures (ºC) for Telford, Shropshire UK for the experimental period in 2018. Shown are planting and harvesting (H1, H2 & H3) dates. Data and graph from WorldWeatherOnline.com (2020).

4.2.2 Bruising replication and assessment

Once harvested from the mesocosms, parsnip plants were manually topped, briefly washed and roots were analysed for signs of mechanical damage and pathogens. Only commercially relevant roots with a crown diameter of between 35-70 mm, with no damage or infection passed inspection. Roots were then acclimatised at room temperature for 1 hour before undergoing the falling bolt test to replicate mechanical processing damage and bruise formation. The widest point of each root (crown) was positioned underneath a 1.25 metre tall PVC pipe held vertically above the root, a steel bolt weighing 200 grams was dropped through the pipe making contact with the parsnip crown below inflicting an impact force of 4.49 J.

Following mechanical impacts, roots were stored for 48 hours at 20 ^{o}C to facilitate bruise formation; the quantification of bruise severity was achieved through employment of callipers, potato peelers and the bruise colouration score chart (chapter 2). Roots that did not bruise were not included in the bruise severity analysis; however the number of roots that did not bruise was recorded and is presented as % likelihood to bruise following mechanical impact. Bruising susceptibility is therefore a combination of bruising severity and % likelihood to bruise.

Bruise severity was calculated using the following formula:

$$BS = ((BD*BW*BI)/CD)$$

where *BD*: Bruise depth (mm)

BW: Bruise Width (mm)

BI: Bruise intensity (0 – 3 colorimetric scale)

CD: Crown diameter (mm)

4.2.3 Relative water content %

A cork borer (15mm diameter) was used on 5 randomly selected roots from each group to extract 3 cores from each root and were pooled; each of the 3 replicates contained a core from each of the 5 roots selected for each experimental group. Each core was weighed to obtain sample weight (W), immediately following this cores were fully hydrated by suspension in de-ionized water in 50 mL bottles for 2 hours. Samples were then taken from the suspension and dried by blotting with filter paper before being weighed to measure full turgid weight (TW). Samples were then oven dried at 80 ^{o}C overnight and weighed again after cooling to measure the dry weight (DW). Weighing machines were accurate to 0.01 grams. The formula employed to calculate relative water content of root tissue (Barrs & Weatherly, 1962).

Root relative water content % was calculated using the following formula:

Relative water content % = ((W-DW)/(TW-DW))* 100

Where *W*: sample weight (grams)

DW: Dry weight (grams)

TW: Turgid weight (grams)

Statistical analysis and figure production was performed using R Studio Version 0.99.903 (© 2009-2016 RStudio, Inc.) and Prism 8 Version 8.4.3 (471) (June 2020, GraphPad Software, LLC.).

4.3 Results

4.3.1 Initial soil water content

At experiment initiation there was no significant differences in mean soil water capacity observed across the 9 experimental groups (Figure 4.3.1.0), to a depth of either 0 - 40 cm (p=0.548), or for 0 - 70 cm (p=0.837) across the 81 experimental mesocosms, (split into 9 experimental groups) following a Kruskal Wallis test. This suggests that no differential group responses are a result of the initial amount of water available to plants. Summed to 40cm the greatest initial soil water capacity was observed in mesocosms within the V3:Mild combination (69.23 ± 7.05 mm) whilst the lowest (49.11 ± 5.80 mm) was found in V3:Control. Similarly, summed to 70cm V3:Mild again exhibited the greatest initial soil water capacity (158.8 ± 17.26 mm) whilst V3:Control again exhibited the lowest value (122.3 ± 13.85 mm) across experimental groups. The mean across all 81 bins to a depth of 70 cm was 141.88 ± 5.13 mm whilst to a depth of 40 cm the mean soil water capacity was 57.43 ± 2.38 mm.

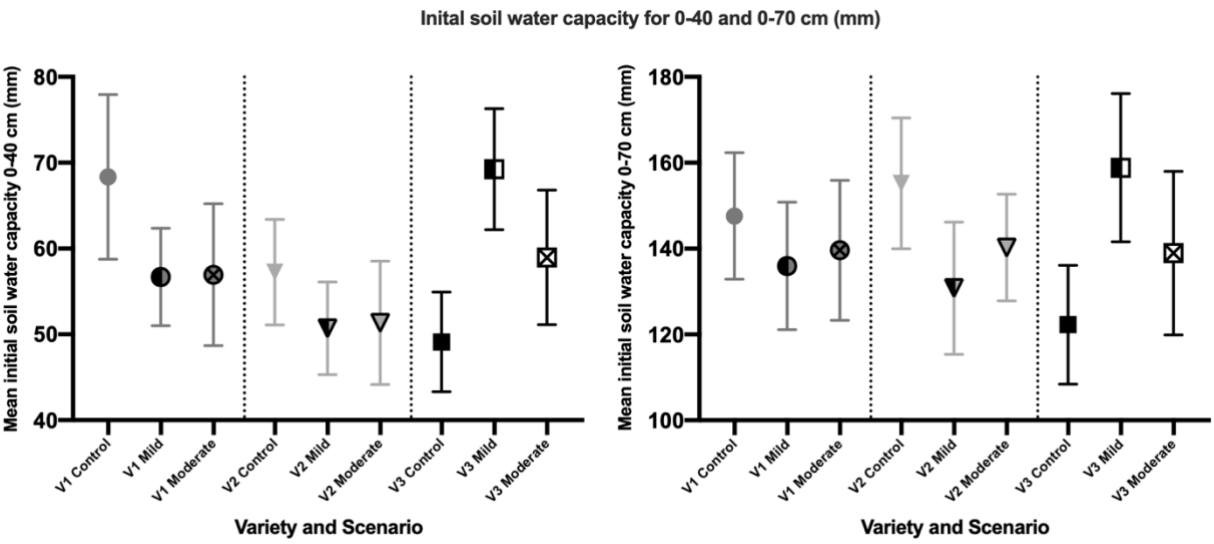


Figure 4.3.1.0) Graphs illustrating mean soil water capacity (mm) of mesocosms at experimental initiation to a depth of 40 cm (left) and 70 cm (right) respectively. (*N*=9) (*p*=0.548) and (*p*=0.837) respectively. Error bars show standard error of the mean (SEM).

4.3.2 Soil water deficit at time of harvest

4.3.2.1 SWD to a depth of 40 cm

The soil water deficit (mm) for each individual mesocosm was calculated by subtracting the soil water content value (mm) at time of harvest from the initial soil water capacity (mm) at experimental initiation.

No significant differences in soil water deficit to a depth of 40 cm were observed across groups during H1 following a Kruskal Wallis test (*p*=0.957) (Figure 4.3.2.1.0). During H2, again no significant differences across the 9 experimental combinations of

irrigation scheme and variety were found (p=0.920) to a depth of 40 cm. Finally, during the third harvest, no significant differences were found to a depth of 40 cm (p=0.719) across the experimental groups following a Kruskal Wallis test. Irrigation scheme did not elicit a significant response in SWD to a depth of 40 across any harvest date, this may indicate that 2 weeks is not enough time for significant soil water deficits to arise. The smallest SWD to 40 cm observed was elicited by V3:Control during H2 (-0.96 ± 11.56 mm) whilst the greatest SWD witnessed was 49.86 ± 21.43 mm by V3:Mild during H3.

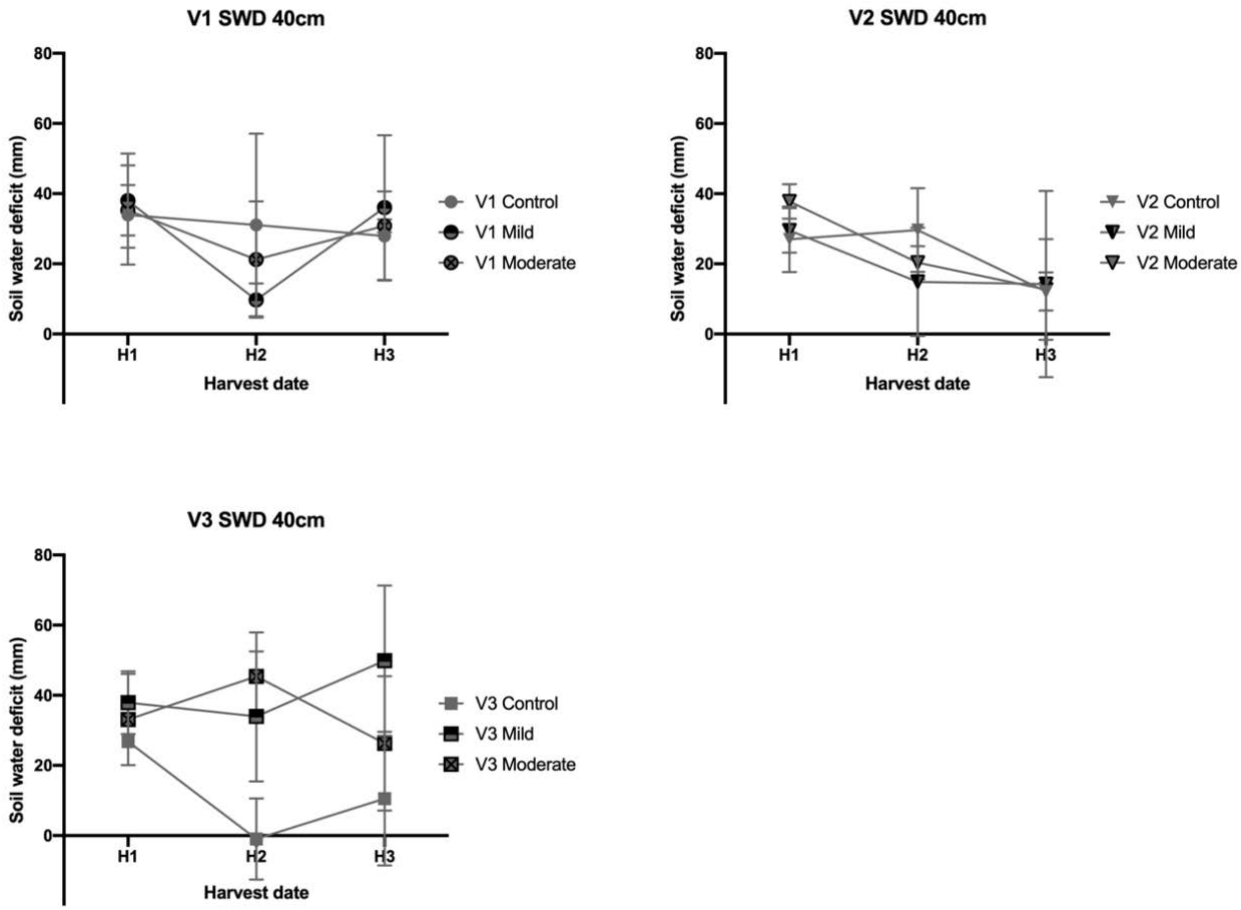


Figure 4.3.2.1.0) Set of graphs illustrating soil water deficits (SWD) (mm) across 3 varieties (V1, V2 & V3), 3 harvest dates (H1, H2 & H3) and 3 irrigation scenarios

(control, mild & moderate) to a depth of 40 cm. (*N*=3). Error bars show standard error of the mean (SEM).

4.3.2.2 SWD to a depth of 70 cm

The soil water deficit (mm) to 70cm for each group is presented in Figure 4.3.2.2.0 and was calculated by subtracting the individual mesocosm's soil water content (mm) to 70cm immediately prior to harvest from the starting soil water content (mm) established at the experiment initiation. Following employment of a Kruskal Wallis test, no significant differences in SWD to 70 cm were found across the 9 experimental groups during H1, H2 or H3 (p=0.904, p=0.917, and p=0.895 respectively). The greatest SWD observed across the experiment was elicited during H1 by V1:Moderate (122.42 ± 36.48 mm) whilst the smallest SWD, 43.47 ± 12.95 mm was found in V1:Control during H3. Irrigation scheme did not significantly affect SWD to a depth of 40 cm or 70 cm across any experimental group. These results indicate that no significant differences in soil water deficit were observed across varieties, harvest date or irrigation scenario.

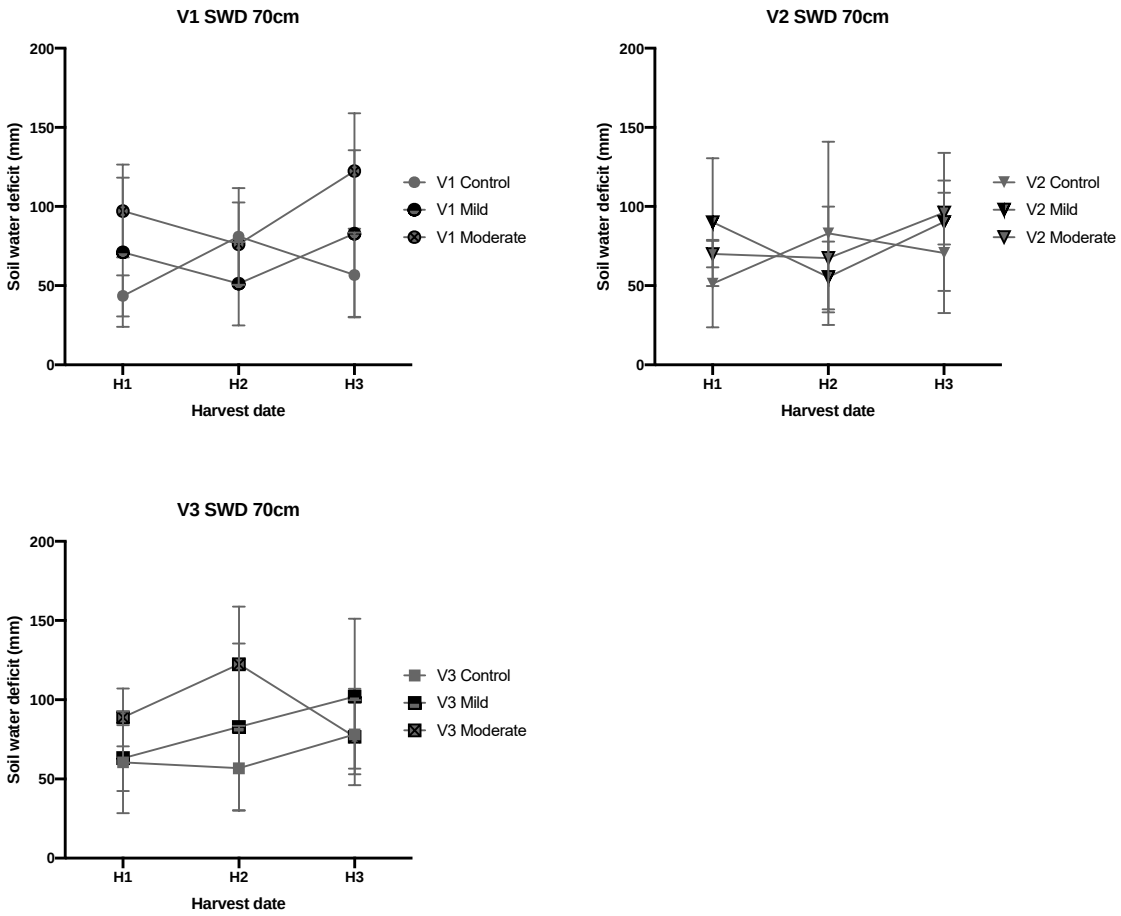


Figure 4.3.2.2.0) Set of graphs illustrating soil water deficits (SWD) (mm) across 3 varieties (V1, V2 & V3), 3 harvest dates (H1, H2 & H3) and 3 irrigation scenarios (Control, Mild & Moderate) to a depth of 70 cm. (*N*=3). Error bars show standard error of the mean (SEM).

4.3.3 Bruising susceptibility

4.3.3.1 H1

Significant differences in bruise severity were observed across groups in H1 (p=0.010) following a Kruskal Wallis test, however no significant differences between specific groups were observed following a Dunn's multiple comparison test (Figure 4.3.3.1.0). V3:Control was the group eliciting the greatest bruising severity across H1 (2.59 ± 0.71) and possessed the greatest % likelihood to bruise (85.71 %).

The variety: scenario combination that expressed the lowest bruising severity (0.82 ± 0.21) was V2:Moderate which bruised at a frequency of 64%, the lowest likelihood observed across H1. V1 and V2's bruising response were similar as irrigation scenario affected bruising likelihood (%) but not bruising severity to the same degree as was observed in V3. The % likelihood to bruise was lower in Moderate roots in comparison to Control roots across all varieties (Figure 4.3.3.1.1); the reduction achieved in V1 was 81.18 % to 70.00 %, V2 roots % bruising was reduced from 81.18 % to 64.00 % whilst V3 root bruise likelihood was reduced from 85.71 to 73.00 %. During H1 a consistent pattern of bruising % likelihood across irrigation scenarios is present, with time since irrigation reducing the likelihood to bruise of all varieties.

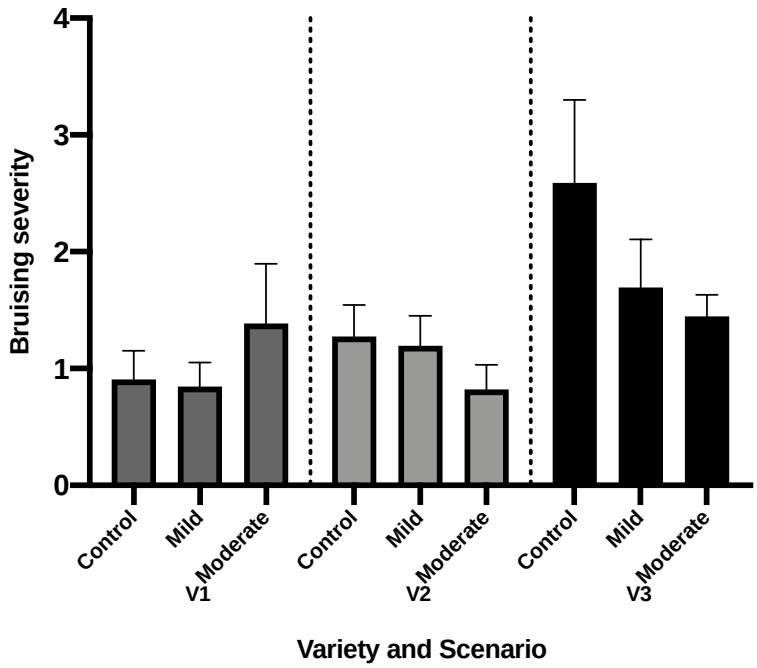


Figure 4.3.3.1.0) Graph illustrating bruising severity across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H1. (*N*=18-24). Error bars show standard error of the mean (SEM).

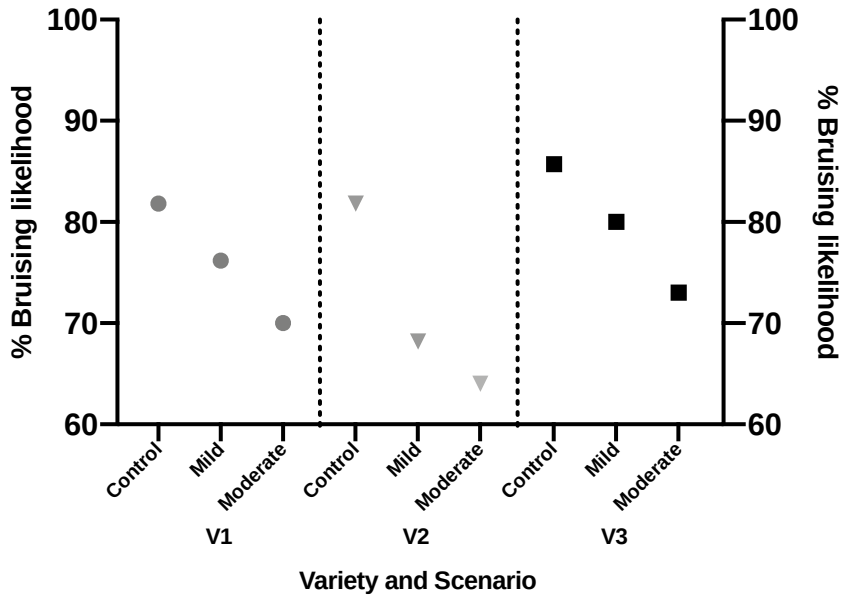


Figure 4.3.3.1.1) Graph illustrating % bruising likelihood across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H1. (*N*=18-24).

4.3.3.2 H2

Significant differences in bruise severity response was also observed across the 9 groups in H2 *(p*=0.0014) with 5 significant differences between groups highlighted by a Dunn's multiple comparison test. V3:Control was again the group eliciting the greatest bruise severity response (3.15 ± 0.41) (Figure 4.3.3.2.0) and bruising % likelihood (95.65 %) (Figure 4.3.3.2.1). V2:Moderate elicited the lowest likelihood to bruise at 65.00 % whilst V1:mild exhibited the lowest bruising severity response at 1.25 ± 0.33.

The bruising severity response of V3:Control was found to be significantly greater than V2:Control (*p*=0.035) and V1:Control (*p*=0.037) whilst the likelihood to bruise was also greater in V3:Control than V1:Control & V2:Control (90.00% & 85.00% respectively). This suggests that during H2, fully irrigated V3 roots are more susceptible to bruising than the other 2 varieties, as V3 roots are more likely to bruise, and when they do, the bruising is more severe. Significant differences in bruise severity were also observed between V3:Control and V1:Mild (*p*=0.0037), V3:Control and V2:Mild (*p*=0.045) and V3:Control compared to V2:Moderate.

Similarly to H1, roots of all 3 varieties displayed a reduction in the bruise % likelihood as the time since irrigation increased, however this was especially pronounced in V2. Whilst irrigation scheme did not significantly affect the severity of V2 bruises, droughting roots for 2 weeks reduced the likelihood to bruise from 90.00 % to 65.00 %.

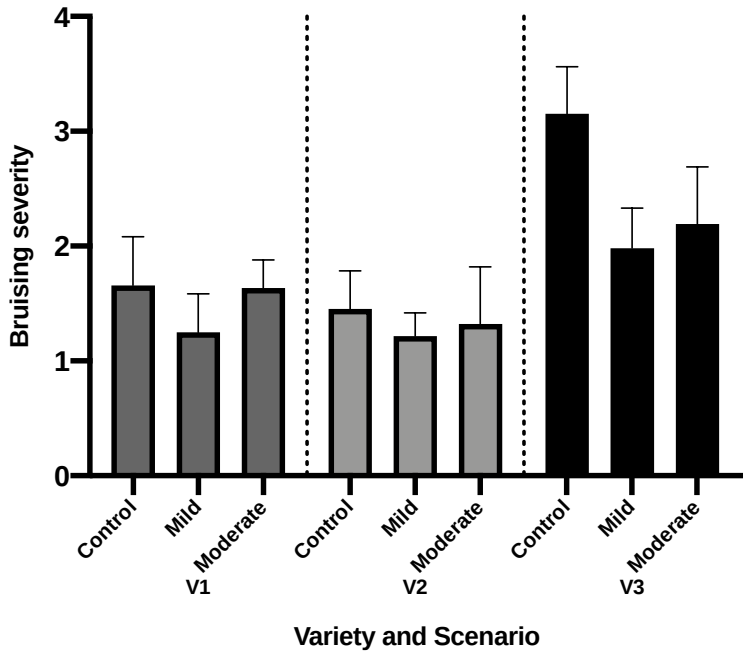


Figure 4.3.3.2.0) Graph illustrating bruising severity across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H2. (*N*=18-24). Error bars show standard error of the mean (SEM).

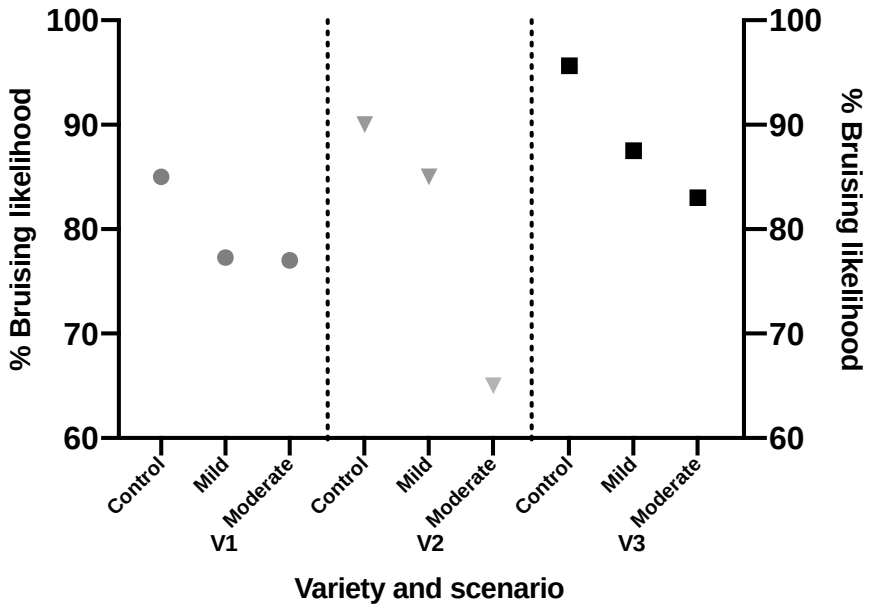


Figure 4.3.3.2.1) Graph illustrating % bruising likelihood across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H2. (*N*=18-24).

4.3.3.3 H3

Significant differences in bruise severity were observed across groups in H3 (p=0.037), however no significant differences in bruising severity between specific groups were found following a Dunn's comparison test (Figure 4.3.3.3.0). V3:Control again elicited the greatest bruising severity of any variety: scenario combination (3.09 ± 0.43). However, it did not elicit the greatest % likelihood to bruise (90.00%) in contrast to H1 and H2 where it was the group that bruised greatest and was most likely to bruise. V2:Control was the group which was the most likely to bruise (95.00%) (Figure 4.3.3.3.1) whilst V2:Moderate and V2:Mild were the least likely (85.00% respectively). V2:Moderate also exhibited the lowest bruising severity of any group (1.62 ± 0.37) during H3 but this was not observed to be significantly different from V2:Control (p=0.708).

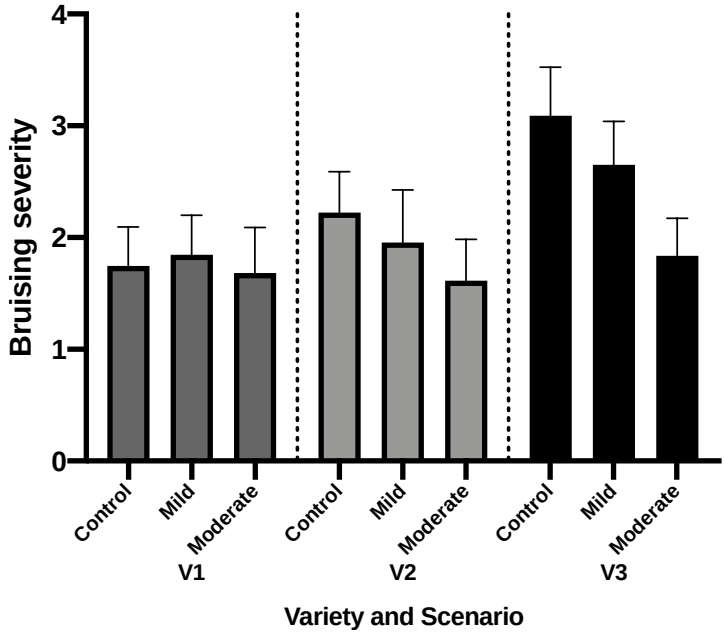


Figure 4.3.3.3.0) Graph illustrating bruising severity across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H3. (N=18-24). Error bars show standard error of the mean (SEM).

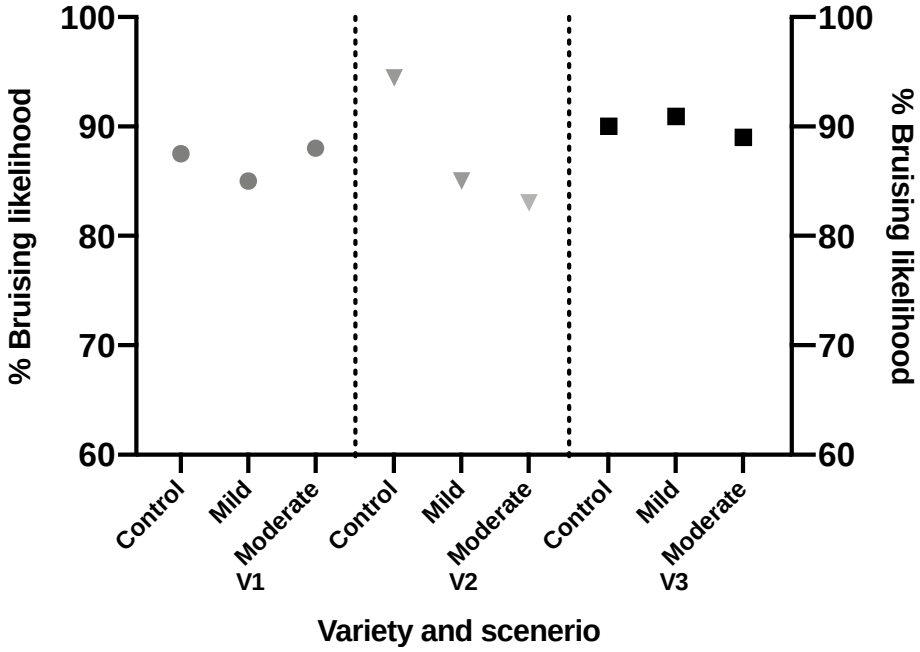


Figure 4.3.3.3.1) Graph illustrating % bruising likelihood across 3 varieties (V1, V2 & V3), and 3 irrigation scenarios (Control, Mild & Moderate) during H3. (*N*=18-24).

Throughout the 3 harvests, the % likelihood to bruise was variable across the varieties and there seems to be an overall reduction in the frequency of bruised roots as drought time increases. The overall highest frequency of bruising across varieties was witnessed during H3, and irrigation protocol did not reduce the number of roots as effectively as during H1 and H2.

4.3.4. Relative root water content (%)

To analyse differences in relative root water content (%) across varieties, scenarios and harvests, a two way repeated measures ANOVA was employed (Figure 4.3.4.0). Harvest date (p=<0.001) and variety (p=<0.001) were found to significantly affect root

relative water content (%) whilst irrigation scenario was not found to significantly affect water content (p=0.074). No significant interaction between factors was observed (p=0.789). A Tukey's HSD test was performed to investigate significant differences between the 27 experimental groups. The lowest relative water content observed during H1 was V1:Moderate (78.05 ± 0.24 %) whilst V3:Control elicited the greatest (81.94 ± 0.37 %). V3:Control relative water content % was observed to be significantly greater than V1:Control (p=0.044) and V2:Control (p=0.032) suggesting that V3 roots possess an higher % water than the other two control groups during the first harvest.

During the second harvest the highest % relative water content was again exhibited by V3:Control (80.87 ± 0.56 %) whilst V2:Moderate had the lowest water content observed with 77.98 ± 0.47 % (difference between (p=0.003). In contrast to H1, no significant difference in relative water content was found between V1:Control and V3:Control (p=0.6288) or between V2:Control and V3:Control (p=0.923). The relative water content (%) of V3:Control roots was significantly greater than V3:Moderate roots (p=0.013) whilst no significant difference between V3:Control and V3:Mild roots was observed (p=0.567). This indicates that irrigation scenario may affect relative root water content in V3 roots, whilst no significant differences were found between scenarios in V1 and V2 roots.

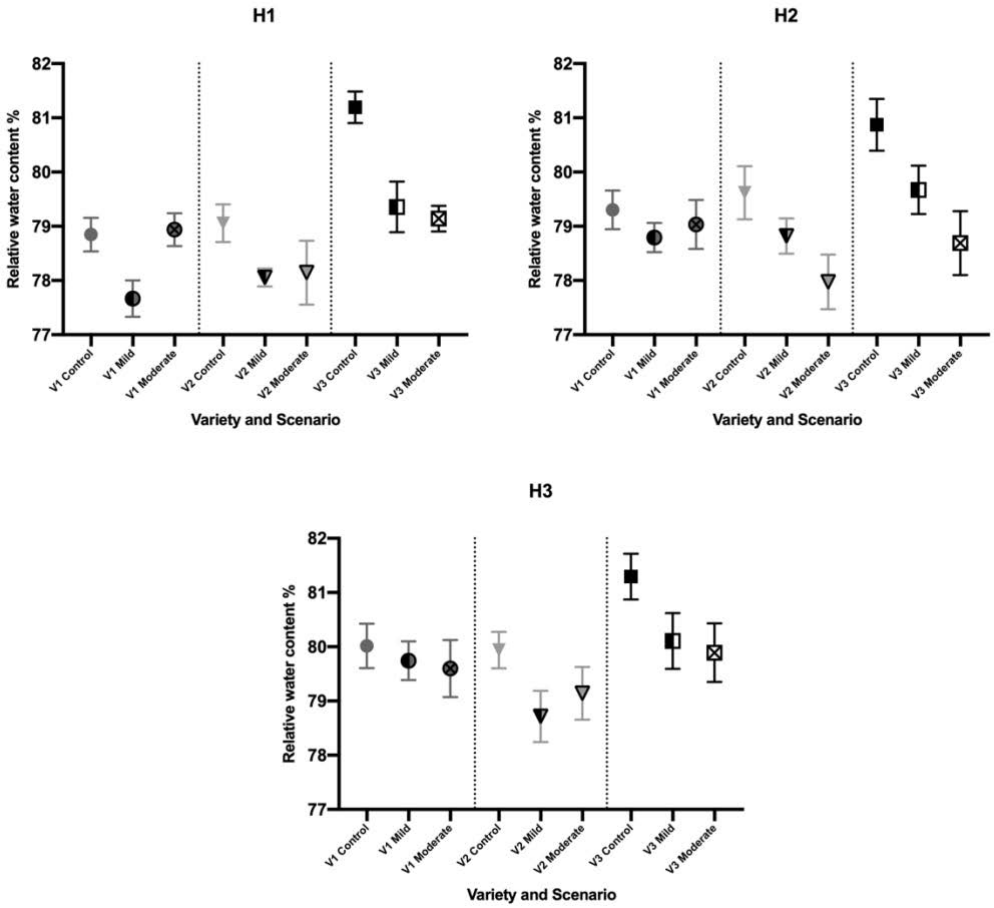


Figure 4.3.4.0) Graphs displaying % relative water content of 3 varieties of parsnip root (V1, V2 & V3) across 3 harvest dates (H1, H2 & H3) and 3 irrigation scenarios (control, mild & moderate). (*N*=3). Error bars show standard error of the mean (SEM).

V3:Control again possessed the highest relative water content (81.28 ± 0.42 %) during the third harvest however it was not found to be significantly greater than V3:Mild (p=0.812) or V3:Moderate (p=0.955). No significant difference between V3:Control and V2:Control (p=0.782) or between V3:Control and V1:Control (p=0.658) was observed. The only significant difference between any of the 9 experimental groups in H3 was between V2:Moderate and V3:Control (p=0.015).

Throughout the 3 harvests no significant differences in relative water content (%) were observed across irrigation scenarios in V1 and V2 roots. In comparison, irrigation scenario did significantly affect relative water content % in V3 roots; V3:Control roots had significantly greater relative water content than V3:Moderate roots (p=0.023) during H1, and during H2 (p=0.013) however not during the final harvest (p=0.093). V3:Mild did not significantly differ from V3:Control or V3:Moderate across any of the 3 harvest dates. The only harvest date where V3:Control roots had a significantly greater relative water content % than V1:Control and V2:Control was during H1. Whilst V3:Control relative water content % was greater than the other 2 varieties for the remaining 2 harvests, no significant differences were observed between them following a Tukey's test.

Bruising severity was greatest in V3:Control groups across all 3 harvest dates and it was the group that exhibited the highest % likelihood to bruise across 2 of the 3 harvests (H1 & H2). No significant differences in soil water deficit (mm) were observed to depths of 40cm or 70cm, yet significant differences in relative water content (%) were found across V3 irrigation scenarios, and between control groups across the 3 varieties.

4.4. Discussion

Significant differences in bruise severity were observed across the 9 variety and irrigation groups during H1, H2 and H3 (p=0.010, p=0.0014 & p=0.037 respectively). Whilst V3:Control exhibited the greatest bruising severity in H1 & H3, it was only observed to exhibit significantly greater bruising severity than the other varieties during H2. It should be noted that no significant difference in SWD to 40 or 70 cm was observed across any harvest as harvest date and variety were not observed to be factors affecting SWD. It is likely that 2 weeks was not sufficient time to elicit a significant droughting effect that could be measured by the mesocosm set up. During late September to November, the temperature in the UK falls, thus transpiration and evaporation rates would be lower during H1, H2 and H3 than earlier during the summer. Furthermore, no significant interaction between irrigation regime and relative water content % was observed (p=0.074), whilst harvest date (p=<0.001) and variety (p=<0.001) were found to be significant factors affecting relative water content % of parsnip roots.

Across harvests it appears that V3 fully irrigated roots bruise at a greater % likelihood and bruises are more severe than in V1 and V2 roots, however these differences in bruise severity were not always found to be significant. Irrigation scheme was not found to significantly affect either bruising severity, or relative water content, but in a number of cases, roots that were withheld irrigation, bruised at a lower frequency than fully irrigated roots. V3 possessed a consistently greater relative root water content % than the other varieties across all 3 harvest dates. More work is required to assess whether

irrigation can significantly affect relative water content and bruising severity in parsnips. The results of this study suggest that the irrigation droughting employed here did not translate into changes in soil water availability, relative root water content % or bruising severity. However, the fact that fully irrigated parsnips generally bruised at a greater frequency is of interest. V2 exhibited a consistent pattern throughout the 3 harvests where the mild irrigation treatment caused a 17.81 %, 25.00 % and 11.44 % decrease in bruising frequency, during H1, H2 and H3 respectively, compared to control V2 roots. V1 and V3 also exhibited a similar pattern of falling % likelihood to bruise as droughting time increased.

Future studies employing a similar mesocosm setup to facilitate manipulation of the SWD are recommended to employ fewer experimental groups and more replicates, to reduce the noisiness and variability of the diviner data. In addition, this study suggest that a more severe droughting method is required in order to elicit significant changes in soil water availability, and concurrently root relative water content.

This novel study represented a pilot attempt to grow parsnips in a mesocosm set up, typically employed for wheat and other cereal crops, in order to manipulate and measure the water availability of the environment surrounding parsnip roots. The droughting method employed here was not sufficient to prompt significant differences in SWD between irrigation schemes as was intended, as such no significant differences were observed in relative root water content between irrigation schemes across varieties. But V3 displayed a greater relative water content % across all harvests. Future studies are recommended to investigate how SWD affect parsnip

physiology and bruising in field trial set ups, rather than the mesocosms employed here, to gain a more representative treatment on how roots are in industry.

Chapter 5 *Physical and Transcriptional changes in parsnip tissue across 3 varieties as a response to mechanical processing damage.*

5.1 Introduction

Mechanical impacts experienced by parsnip roots during harvesting and post-harvest processing often result in the formation of bruises (Chapter 2), with bruising susceptibility being affected by post-harvest factors such as impact magnitude, storage temperature and storage duration. Parsnip packhouses often experience pack-out losses that exceed 50 %; with approximately 20 % of roots being rejected as a result of mechanical damage and bruising (Chapter 3). Bruising remains a limiting factor to the quality of fresh produce: impacts that exceed the mechanical limits of plant cells cause membrane leakage, rupturing, and a subsequent visible discolouration of subcutaneous tissue (Opara & Panthare, 2014).

It has previously been hypothesised that enzymatic browning of tissue is a direct result of membrane disintegration and leakage following mechanical impacts (Franck *et al.,* 2007). In parsnips, extreme variation in enzymatic browning between varieties has previously been observed (Toivonen, 1992). Cytoplasmic enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) (Figure 5.1.0) come into contact with phenolic compounds originating from vacuoles following cell and organelle rupture (Tomás-Barberán & Espín, 2001). Cantos *et al.,* (2002) suggested that membrane integrity is a major factor controlling the rate of tissue browning in potatoes, whilst browning was not rate-limited by either enzyme or polyphenol concentration. Lærke *et*

al., (2002) demonstrated intracellular compartmentalisation as the determining factor in how susceptible to bruising potato tubers were, with no correlation observed between the discolouration potential of potato tissue and the phenolic content.

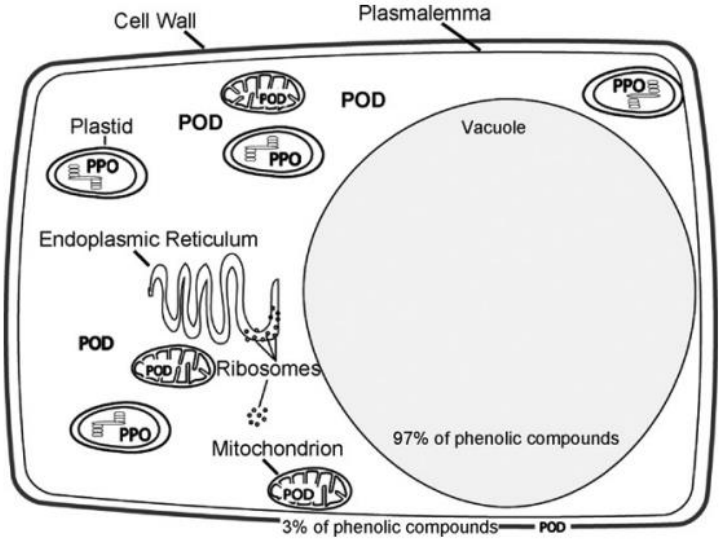


Figure 5.1.0) Diagram illustrating location of phenolic compounds and associated oxidizing enzymes (PPO and POD) in plant cells. Taken from Toivonen & Brummell, (2008).

Goyer & Pelle, (2018) also found that sensitivity of membrane leakage and loss of cell compartmentalisation was the key determining factor in susceptibility to bruising, rather than phenol or substrate concentration in potato tubers. The varying bruising susceptibility of individual varieties within crop species is therefore hypothesised to be a result of factors such as membrane strength, cell wall integrity, turgidity, phenolic content and enzymatic activity (Hussein *et al.,* 2018). Toivonen, (1992) found that solute leakage following wounding in parsnips was the determinant factor regarding varietal differences in bruising susceptibility, rather than enzyme or phenolic content.

Close association between tissue discolouration and the activity of enzymes that facilitate phenolic oxidation has also been observed in potatoes (Scharf, 2014), walnuts (Zhang *et al.,* 2019), pomegranates (Hussein *et al.,* 2019; Hussein *et al.,* 2020) and apples (Rocha *et al.,* 2002). Bruising is often spatially heterogeneous (Li & Thomas, 2014) as phenolic and enzymatic content varies across tissue type; this causes some produce to exhibit greater external bruising whilst others suffer greater internal bruising (Hussein *et al.,* 2018). In closely related carrots, phenolic content has been reported to range from 150 to 750 milligrams per kilogram (Geoffriau & Simon, 2020). Chubey & Nylund, (1970) found that phenolics are concentrated in vascular or cortical tissue, thus mechanical damage induces spatially variable phenolic biosynthesis in carrots depending on tissue type (Alegria *et al.,* 2016).

The production of lignin and antioxidants is a result of the activation of the phenylpropanoid pathway where PPO (polyphenol oxidase), POD (peroxidase) and PAL (phenylalanine ammonia lyase) (Cantos *et al.,* 2002) facilitate tissue discolouration and enzymatic bruising. PPO, POD and PAL have been highlighted as the main 3 enzymes that cause phenolic degradation in fresh produce, resulting in quality losses for consumers.

PAL is a crucially important enzyme (Figure 5.1.1), acting as the first step in the phenylpropanoid pathway, and working in close co-ordination with 4CL and C4H (Cheniany & Ganjeali, 2016). This pathway ultimately produces the 3 monolignol subunits (*p*-coumaryl, coniferyl and sinapyl) from amino acids phenylalanine and tyrosine. PAL catalyses the conversion of the essential amino acid L-phenylalanine,

itself produced by the upstream Shikimate pathway, to trans-cinnamic acid (Zhang *et al.,* 2019).

A 200 fold increase in PAL activity was observed following mechanical impact in potato tubers, with PAL activity peaking 48 hours after injury induction (Belknap *et al.,* 1990). Increases in PAL activity have also been correlated with bruise formation in *Lactuca sativa (*lettuce) (Couture *et al.,* 1993). In carrots it has previously been reported that steam treatments effectively inhibited PAL activity (Howard *et al.,* 1994); correlating the observed decline in PAL activity with a reduced accumulation of downstream soluble phenolics and lignins.

The phenolic compounds synthesised by the phenylpropanoid pathway are subject to oxidation via PPO (Figure 5.1.2) or naturally, causing the conversion of mono-phenolic compounds to diphenols, which are then converted to quinones as part of the plant response to wounding. Tyrosine, and phenylpropanoids such as chlorogenic acid and caffeic acid are substrates for PPO; the stress induced overexpression of *PAL* increases phenolic concentration and leads to enzymatic browning of the substrate (Goyer & Pelle, 2018).

Tyrosine is typically converted to tyramine via tyrosine decarboxylase (TYDC) and is an tetrahydroisoquinoline alkaloid compound and is a suitable substrate for PPO activity. Concentrations of tyrosine and phenylalanine have been found to account for 80% of the variation in biochemical potential observed between potato varieties (Goyer & Pelle, 2018). However, this did not translate into significant varietal variation in

bruising susceptibility. Tyrosine was found to be the limiting substrate for melanic pigment formation via PPO as it was found in much greater concentrations than phenylpropanoids, such as chlorogenic acid and had a greater discolouration potential.

Lærke *et al.,* (2002) found that during the growth period the abundance of dark intermediate compounds and black final products increased in potato tubers. However, no correlation between bruising susceptibility and total oxidative potential was observed. Thus intracellular compartmentalisation is proposed as a key determinant as the potential for oxidation of phenolic compounds is always present, whilst the resistance to membrane leakage and rupture is vastly different between *in vivo* and homogenised tissue (Goyer & Pelle, 2018).

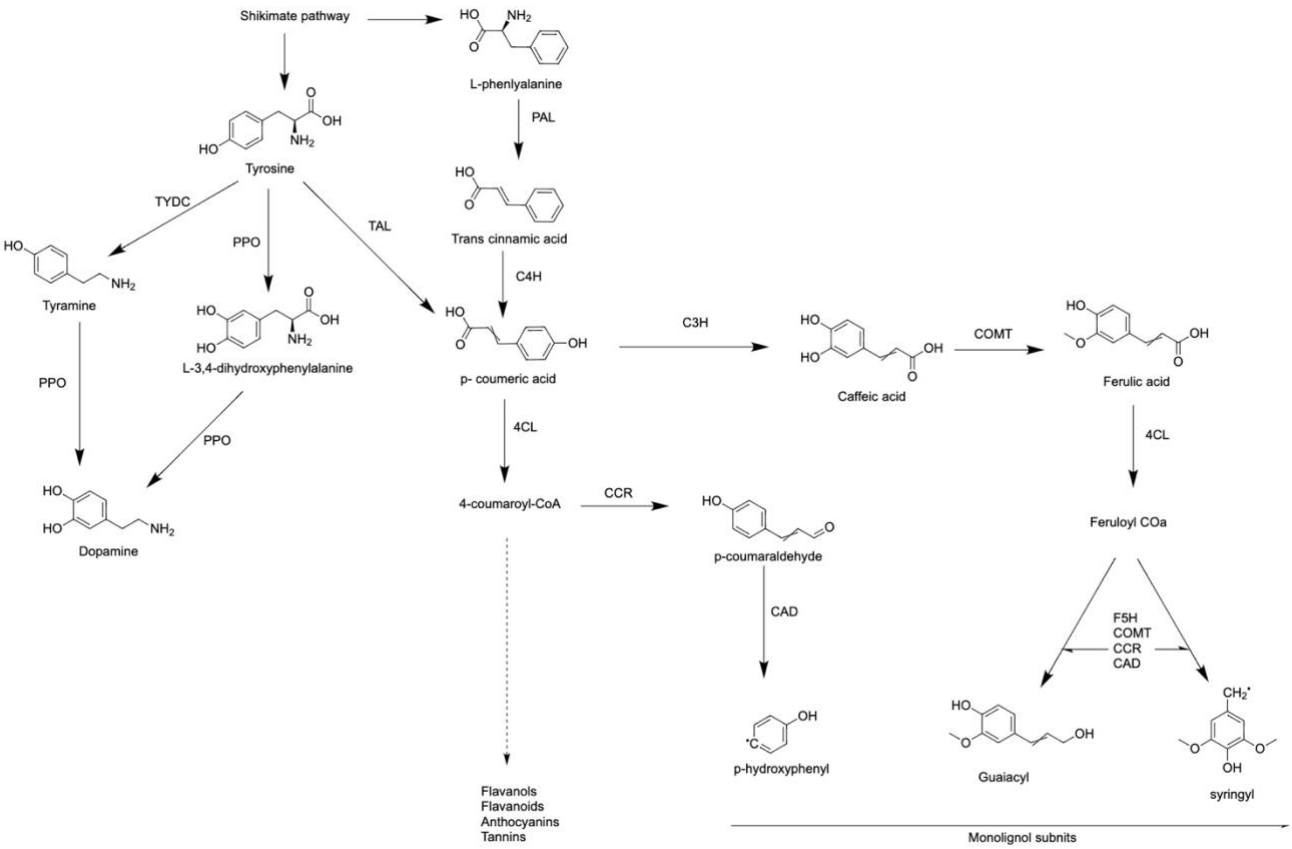


Figure 5.1.1) Schematic representation of phenylpropanoid metabolism in plants; adapted from Emiliani *et al.,* (2009) & Araji *et al., (*2014), displaying conversion of L-Phenylalanine and L- Tyrosine into lignin sub-units and flavonoids as part of the defence response. Solid arrows represent a single enzyme reaction whilst dashed lines indicate multiple enzymatic reactions. Enzyme names are abbreviated as follows; PAL- Phenylalanine ammonia lyase: TYDC- Tyrosine decarboxylase: TAL- Tyrosine aminotransferase: PPO- Polyphenol oxidase: C4H- Cinnamate 4-hydroxylase: 4CL- 4-coumarate-CoA ligase: C3H- 4-coumarate-3-hydroxylase: CCR- Cinnamyl-CoA reductase: CAD- Cinnamyl alcohol dehydrogenase: F5H- Ferulate 5-hydroxylase: COMT- Caffeic acid O-methyltransferase. Authors own diagram, created using ChemDraw 19.1.1 (PerkinElmer, 2020).

In most plant species PPO enzymes are encoded by multiple gene families indicating a variety of functions (González *et al.,* 2020): potatoes have had 5 *PPO* genes described (Thygesen *et al.,* 1995); 7 genes encode PPO in tomatoes (Pinto *et al.,*

2008); earthmoss contains 13 *PPO* genes and a number of angiosperms have 1 *PPO* gene (Tran *et al.,* 2012). This may indicate that the *PPO* gene family is variable in size and structure; some species (such as Arabidopsis) report no *PPO* encoding genes (Tran *et al.,* 2012).

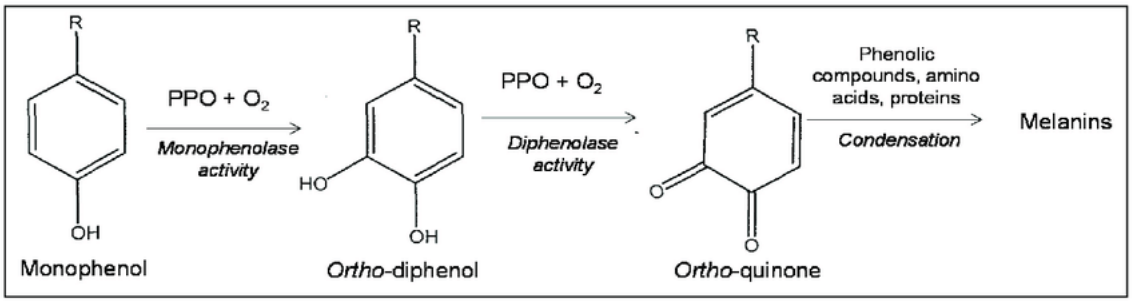


Figure 5.1.2) Simplified schematic diagram illustrating Polyphenol oxidase (PPO) activity converting monophenols to quinones. Taken from Taranto *et al.,* (2017).

In potato tubers, Chi *et al.,* (2014) found that different *PPO* genes did not equally contribute to total PPO content in damaged tissue, with a single version contributing 55% of total PPO protein content. Silencing of multiple *PPO* genes in combination resulted in reduced PPO activity and consequently reduced bruising susceptibility of tubers. Genetically engineered potato lines with silenced *PPO* genes have been shown to exhibit significantly lower bruising susceptibility, and may express “unintended metabolic modifications” (Llorente *et al.,* 2010, page 9) in comparison to wild type tubers. Thus targeted modifications of *PAL* and *PPO* genes should be done with care as it may seriously affect downstream metabolite production (Wagner *et al.,* 2009) with unintended consequences. Editing via CRISPR of a single *StPPO* gene in potato tubers reduced PPO activity by 69% and enzymatic browning by 73% (Gonzalez *et al.,*

2020); thus understanding the degree to which *PPO* genes contribute significantly to total enzymatic activity may support plant breeders in producing bruise resistant varieties. Llorente *et al.,* (2014) found that *PPO* silenced potato lines exhibited a shift from producing phenylpropanoid precursors to phenolic compounds associated with an increased pathogen defence. *PPO* silenced tubers exhibited enhanced resistance to *Phytophthora infestans* due to an increase in phenolic compound production (such as chlorogenate) that creates an unfavourable environment for the pathogen (Llorente *et al.,* 2014).

POD enzymes are typically upregulated by plants as a response to mechanical damage as they are involved in the formation of bridges between cell walls and the oxidation of quinones during the final stage of lignification (Whetten *et al.,* 1998). Lignin supports the cell wall, and is used to reinforce regions of tissue that are damaged, monolignols are formed via the phenylpropanoid pathway. A main substrate of POD is hydrogen peroxide (H_2O_2), which regulates its activity as H_2O_2 is found in very low concentration in typical plant cells (Toivonen & Brummell, 2008). The generation of quinones from phenols via PPO can lead to the accumulation of H_2O_2, thus enabling significant tissue discolouration via increased POD activity (Jiang & Miles, 1993). POD enzymes catalyse the oxidative polymerisation of quinones to form lignin; POD can thus enhance phenolic degradation, when simultaneously working with PPO (Li *et al.,* 2007). Lignin and its intermediaries (such as melanin), formed via oxidation of the 3 monolignols, are red-brown in colour and cause the browning observed in damaged tissue (Gondwe *et al.,* 2019). Previously, POD activity has been observed to be a major

contributor to tissue discolouration in other fresh produce (Aquino-Bolanos *et al.,* 2000; Aquino-Bolanos & Mercado-Sila, 2004).

It is of crucial importance to identify and study genes involved in the bruising response such as those encoding PAL, POD, TYDC and PPO to understand the molecular mechanism of bruising to provide breeders information to improve parsnip varieties.

5.1.1 Aim

The aim of this chapter was to study the physiological and transcriptional response to mechanical damage in parsnip tissue across 3 varieties. This study aims to identify how physiological differences between varieties translates into bruising susceptibility. Furthermore, the identification of differentially expressed genes that are involved in the bruising response may provide information regarding the molecular mechanism of bruising in parsnip roots.

5.1.2 Objectives

1) To investigate physiological responses to mechanical damage in parsnip roots across 3 varieties and 3 harvest dates
2) To identify differentially expressed genes involved with the bruising response across 3 varieties on 1 harvest date

5.1.3 Hypotheses

H_0= There are no significant differential physiological responses to bruising across the 3 varieties

H_1= There is no significant difference in the transcriptional response to bruising across the 3 varieties

5.2. Materials and methods

5.2.1 Bruise replication and assessment

Parsnip roots of three varieties (V1, V2 and V3) were harvested by hand from an Elsoms Seeds Ltd. trial site in Brancaster, Norfolk in November 2019 (H1), December 2019 (H2) and January 2020 (H3). A total of 10 or 11 replicates were employed per experimental group. Parsnips were manually topped, washed and transported to the University of Birmingham for testing; roots had to possess a diameter of between 40 - 70mm and not exhibit signs of mechanical damage or pathogens following inspection. Roots were acclimatised at room temperature for an hour before bruise replication where the falling bolt method (fully described in section 2.2.1) was used to replicate mechanical impacts during processing. Briefly, a steel bolt weighing 200 grams was dropping through a perforated PVC pipe from a height of 1.25 m inflicting an impact of 4.49 J onto the widest part of the parsnip crown.

Roots were then stored for 48 hours at 20 ^{o}C to facilitate bruise formation before analysis, and quantification of bruise size and severity was performed using a potato peeler and callipers to produce a bruising susceptibility score for each experimental group. Roots that did not bruise were not included in bruise severity calculations however the number of roots that did not bruise was recorded and is presented as % likelihood to bruise following mechanical impact.

Bruising severity was calculated using the following formula:

*BS= ((BD*BW*BI)/CD)*

where *BD*: Bruise depth (mm)

BW: Bruise Width (mm)

BI: Bruise intensity (0 – 3 colorimetric scale)

CD: Crown diameter (mm)

5.2.2 Root tissue solute leakage % (TSL)

Tissue solute leakage (% TSL) was analysed employing a modified methodology described by Hussein *et al.,* (2018) in order to investigate how variety, harvest date and tissue status (control vs bruised) affected solute leakage in parsnip tissue. Using a 25mm diameter cork borer, 4 peel discs from healthy and 4 discs from bruised tissue (10mm thick) were taken from each root (5 per treatment) and pooled leading to 4 replicates per group each containing 20 discs.

Discs were weighed, then rinsed in deionized water before being added to a 100 mL. Duran bottle containing 35 mL of 0.4 M mannitol and incubated at 20 ^{o}C whilst being constantly shaken. The conductivity of the mannitol solution (initial conductivity) was measured using a conductivity meter (Vernier instruments) for each of the three experimental groups per harvest date, and tissue status, after 4H and 48H of incubation. Two time points were analysed to provide information on membrane leakage over the latency period (48H) for bruise formation. Following removal from incubation, bottles were then autoclaved at 121 ^{o}C for 25 minutes and cooled to room

temperature before the conductivity was again measured, to provide a measure of total conductivity following the total leakage of ions from the parsnip tissue.

% Tissue solute leakage was calculated using the following formula after both 4H and 48H of incubation:

*% TSL = ((IC/ FC-IC) * 100)*

Where: *IC: Initial conductivity of solution*

FC: Final conductivity of solution

5.2.3 Total oxidative potential of root tissue

The methodology was adapted from McNabnay *et al.,* (1999) and Scharf, (2014). The total oxidative potential of parsnip root tissue was determined by proxy via measuring the degree to which homogenised tissue discoloured a buffer solution, under controlled laboratory conditions. A cork borer (diameter 25mm) was used to extract cores of collenchyma and parenchyma tissue, which were then cut into discs with a 10mm thickness. Discs from three replicate roots pooled to produce a single replicate, with three replicates in total per group. Tissue was then lyophilized with liquid nitrogen and ground using a pestle and mortar to produce a fine powder; tissue was kept constantly frozen during this homogenization to prevent thawing. 0.5 g of lyophilized tissue was suspended in 6 mL of 0.05 M phosphate buffer (pH 6.5) and vortexed until complete homogenization of the tissue was achieved. The suspended homogenate was stored

in a controlled temperature cabinet set at 30 ^{o}C and left for 48 hours. Samples were filtered (Whatman filter paper) and discolouration of the buffer solution was measured at 475 nm with a spectrophotometer.

5.2.4 Relative water content %

A corker borer (15mm diameter) was used 4 times on each root, and cores with a length of 50mm were cut to size to produce 4 discs (one core replicate). A total of four replicates (each comprised of discs from different roots) were used for each experimental group. Each core was weighed to obtain sample weight (W), immediately following this cores were fully hydrated by suspension in de-ionized water in 50 mL bottles for 3 hours. Samples were then taken from the suspension and dried by blotting with filter paper before being weighed to measure full turgid weight (TW). Samples were oven dried at 80 ^{o}C overnight and weighed again after cooling to measure the dry weight (DW). Weighing machines were accurate to 0.001g. The formula employed to calculate relative water content of root tissue (Barrs & Weatherly, 1962)

$$\textit{Relative water content \%} = ((W-DW)/(TW-DW))* 100$$

Where *W*: sample weight (grams)

DW: Dry weight (grams)

TW: Turgid weight (grams)

5.2.2.5 Scanning electron microscopy

To investigate physical changes in parsnip tissue following mechanical damage and to analyse varietal differences, a scanning electron microscope (Harper Adams University, 2020) was employed to analyse microstructural changes in control and bruised tissue (Hussein *et al.,* 2019). Cubes (1cm^3) of healthy and bruised tissue were removed from parenchyma tissue immediately below the collenchyma layer, from the 3 varieties of parsnip. Samples were histologically fixed (University of Glasgow, 2020) in glutaraldehyde solution in phosphate buffer (pH 6.8) and stored overnight to ensure total fixation.

Slices of healthy and bruised from each variety were sectioned using a rotary microtome to a thickness of approximately 15 µm before being mounted onto a metal stub. Samples were then washed with a phosphate buffer to remove the fixative. The metal stubs then underwent a dehydration series where the buffer was replaced by ethanol in increasing concentrations (25, 50, 75 & 100 %) in plastic test tubes. Dehydration in ethanol was repeated twice, samples were left in the final 100% ethanol stage for 3 hours.

The samples, mounted on the metal stubs, were then dehydrated using Hexamethyldisilane (HMDS); stubs were taken from the 100% ethanol solution and transferred into a 2:1 100% ethanol : HDMS solution and left for 15 minutes. Stubs were then transferred into a 1:2 100% ethanol : HDMS solution and left for another 15 minutes. Finally the stubs were transferred into a 100% HDMS solution and left

overnight in a fume hood to allow evaporation. Once dry, stubs were sputter coated with gold via a sputter coater (Edwards S100) and examined using an scanning electron microscope.

5.2.2.6 Sample collection and RNA extraction

Parsnip roots from the December 2019 harvest (H2) were randomly selected following bruise replication and storage, and 4 roots from each of the 3 varieties (V1, V2 & V3) were removed for RNA extraction. Physical responses were measured during H1 & H3 but due to the seasonal surge in demand for parsnips over the Christmas period (H2), it was decided that transcriptional analysis should focus on roots harvested from a trial site in December (H2). Roots were stored in the same manner as those in section 2.1. From all 12 individual roots, tissue was taken from a) healthy areas and b) bruised areas to provide 2 replicates per root (24 samples from 12 roots). Tissue was removed and flash-frozen in liquid nitrogen and stored at $^{-}80$ ^{o}C before being sent to Novogene Ltd (Cambridge, (2020)); the total RNA of each sample was extracted using the RNeasy Mini Kit by Qiagen, Ltd following the instructions recommended by the manufacturer. Agarose gel (1%) was employed to analyse RNA contamination and degradation. A NanoPhotometer spectrophotometer (IMPLEN, CA, USA) was employed to analyse RNA purity. The integrity of RNA within each sample was tested by employing a RNA Nano 6000 assay kit of the Bioanalyzer 2100 system (Table 5.2.2.7.0) (Agilent Technologies, CA, USA) (Novogene, 2020).

5.2.2.7 Library construction, UniGene annotation and functional classification

A total of 1 µg RNA per sample was inputted to produce sequenced libraries by Novogene, Ltd (2020), using NEBNext® Ultra TM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations. Clustering of index coded samples was achieved via the employment of a PE Cluster Kit cBot-HS (Illumina). This was done to the specifications recommended by the manufacturer (Novogene Ltd, 2020). An Illumina platform was employed after the preceding cluster production to prepare libraries and produce paired-end reads. Reads containing poly N sequences or adapters were trimmed to produce clean reads. Concurrently, Q20, Q30 and GC content of the clean data was analysed and is presented in table 5.2.2.7.1.

Due to the absence of a well annoted parsnip genome, a *denovo* transcriptome reconstruction was performed by Trinity employing default options (Grabherr *et al.,* 2011). CORSET eliminated redundancy from the Trinity derived results and was also employed to recover full length transcripts (Novogene, 2020). BUSCO was employed to analyse the anticipated gene content of the generated parsnip transcriptome. Unigenes are therefore defined as the longest transcript found in each hierarchical cluster (Zhang *et al.,* 2019). Assembled parsnip Unigenes were then annotated by 7 databases (NT, NCBI, BLAST, NR, SwissProt, KOG: Diamond) using a threshold E value < 0.00001 (Buchfink *et al,.* 2015) to assign functional annotation to Unigenes.

V1		V2		V3	
Control	**Bruised**	**Control**	**Bruised**	**Control**	**Bruised**
V1Con1	V1Br1	**X**	V2Br1	V3Con1	V3Br1
V1Con2	V1Br2	V2Con2	V2Br2	V3Con2	V3Br2
V1Con3	V1Br3	V2Con3	V2Br3	V3Con3	V3Br3
V1Con4	V1Br4	V2Con4	V2Br4	V3Con4	V3Br4

Table 5.2.2.7.0) Table displaying samples that passed (23/24) during quality control. V2:Control Replicate 1 failed quality control due to excessive degradation of RNA.

Sample	Raw reads	Clean reads	Raw bases	Clean bases	Error(%)	Q20(%)	Q30(%)	GC(%)
V3Br1	25258113	24724993	7.6	7.4	0.03	97.98	93.84	42.89
V3Br2	22095642	21933772	6.6	6.6	0.02	98.10	94.19	43.22
V3Br3	20485007	19965831	6.1	6.0	0.03	98.04	93.95	43.00
V3Br4	22268333	21758917	6.7	6.5	0.03	97.94	93.76	43.11
V3Con1	20898821	20705992	6.3	6.2	0.02	98.30	94.58	43.59
V3Con2	21438378	21258495	6.4	6.4	0.02	98.20	94.23	43.26
V3Con3	21766304	21598892	6.5	6.5	0.02	98.42	94.83	43.63
V3Con4	21236231	20625988	6.4	6.2	0.02	98.20	94.37	43.06
V2Br1	21908567	21514047	6.6	6.5	0.02	98.23	94.42	43.39
V2Br2	23154683	22970859	6.9	6.9	0.02	98.31	94.56	43.65
V2Br3	20417223	19924078	6.1	6.0	0.03	97.95	93.77	43.01
V2Br4	23794836	23169083	7.1	7.0	0.03	97.87	93.56	43.26
V2Con2	23194340	22470952	7.0	6.7	0.03	98.07	94.09	43.27
V2Con3	22453120	21846401	6.7	6.6	0.02	98.15	94.25	42.99
V2Con4	22326100	22089252	6.7	6.6	0.03	98.02	93.84	43.34
V1Br1	23893961	23031702	7.2	6.9	0.03	97.75	93.28	43.09
V1Br2	21539057	21327022	6.5	6.4	0.02	98.18	94.27	43.05
V1Br3	22441965	21775946	6.7	6.5	0.03	97.97	93.80	43.02
V1Br4	22807694	21971322	6.8	6.6	0.03	97.97	93.79	42.44
V1Con1	21787191	21342431	6.5	6.4	0.03	97.88	93.67	42.68
V1Con2	21744603	21183083	6.5	6.4	0.03	98.08	94.07	43.15
V1Con3	22086019	21799824	6.6	6.5	0.02	98.23	94.42	43.20
V1Con4	24194259	23741312	7.3	7.1	0.02	98.36	94.66	43.09

Table 5.2.2.7.1) Table displaying 23 samples with accompanying quality control parameter values.

5.2.8 Differential gene expression analysis

For each variety, differential gene expression between the healthy and bruised regions of parsnip tissue was investigated using the R package DESeq2 due to the presence of at least 3 biological replicates for each of the 6 experimental groups. The Benjamini and Hochbergs approach for controlling the false discovery rate was employed for the *P* values created by DESeq2, producing a *Padj* value where values of <0.05 are considered differential expression. ClusterProfiler was used for GO enrichment of DEGs and to apply term classification and enrichment, where GO terms with *Padj* values of <0.05 were considered significant. KEGG pathway enrichment was performed by KOBAS software (Novogene, 2020) to assess the significant enrichment of difference expression genes in KEGG pathways, where an adjusted *Padj* values of < 0.05 was considered significant enrichment.

5.3 Results

5.3.1 Bruising susceptibility of parsnip roots

Significant differences in bruise severity were observed across all groups (p=0.0354) following a Kruskal Wallis test, however no significant differences between specific groups were observed following a Dunn's multiple comparison test.

During H1 no significant differences in bruising severity were observed across the 3 varieties (Figure 5.3.1.0). V3 elicited the greatest bruising severity (1.84 ± 0.47) and

the greatest % likelihood to bruise (92.00 %) during the first harvest, whilst V1 exhibited the lowest bruise severity and % likelihood to bruise (1.11 ± 0.26 and 69.00 % respectively). Whilst the bruising severity of V3 was not significantly greater than the other 2 varieties, its % propensity to bruise was as 23.00 % & 18.00 % higher compared to V1 & V2 roots respectively during the first harvest.

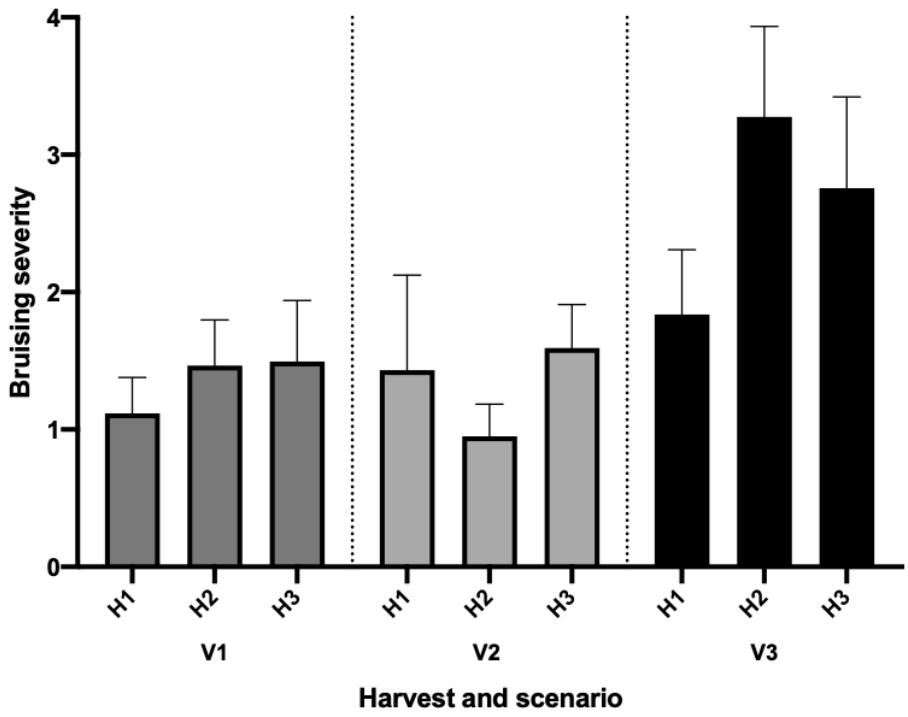


Figure 5.3.1.0) Graph illustrating bruise severity of parsnip roots across 3 varieties (V1, V2 & V3) and 3 harvest dates (H1, H2 & H3). (*N*=10-11). Error bars show standard error of the mean (SEM).

V3 exhibited the greatest bruising severity (3.28 ± 0.66) during H2, the highest value observed across all harvest dates whilst V2's bruising severity for H2 was 0.95 ± 0.23, the lowest observed across all harvest dates. V2 also exhibited the lowest % likelihood to bruise (78.00) whilst V3 was the most likely to bruise (85.00 %) during

H2. A total of 80% of V1 roots bruised during H2 exhibiting a bruising severity of 1.47 ± 0.33.

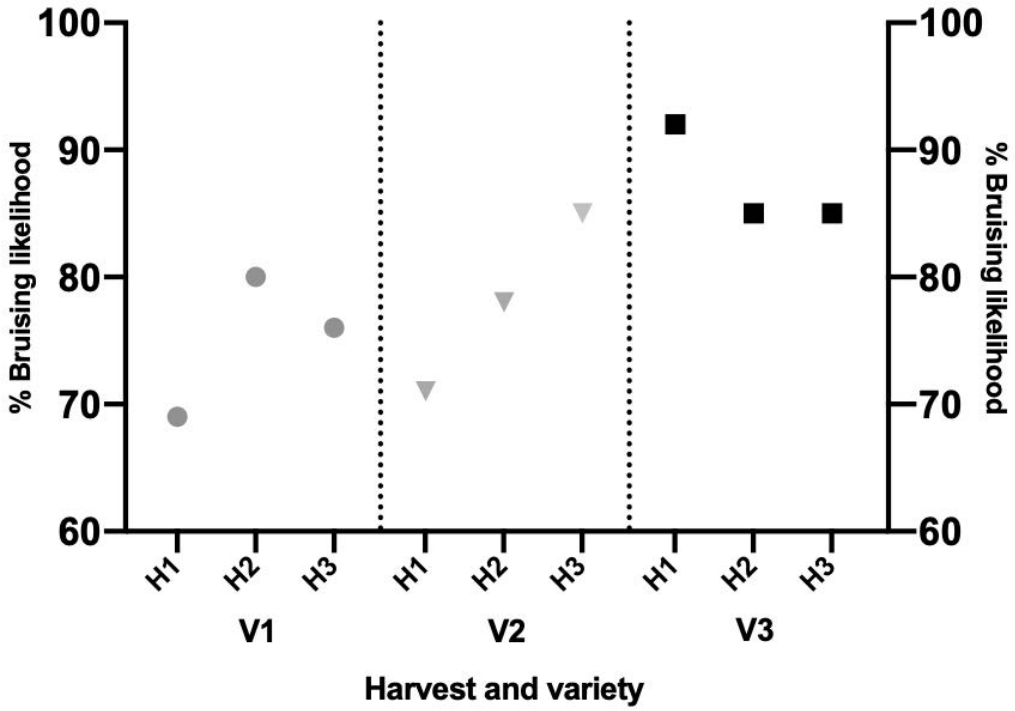


Figure 5.3.1.1) Graph illustrating % bruising likelihood of parsnip roots across 3 varieties (V1, V2 & V3) and 3 harvest dates (H1, H2 & H3). (*N*=10-11).

During the final harvest (H3) V3 was again the variety exhibiting the greatest bruising severity (2.76 ± 0.68) and % likelihood to bruise (85.00%), 85.00% of V2 roots also bruised but the bruising severity was 1.59 ± 0.32 and no significant difference between V2 and V3 was observed (*p*=0.406). V1 roots exhibited the lowest bruising severity (1.49 ± 0.44) and % likelihood to bruise (78.00 %) during the final harvest.

5.3.2 Tissue solute leakage (% TSL)

4 Hours incubation

Across all groups removed from incubation after 4 hours, a 2 way ANOVA found that harvest date (p=<0.0001) and variety (p=<0.0001) significantly affected % tissue solute leakage (% TSL). No significant interaction between factors was observed. During H1, no significant difference was observed between V1:Con and V3:Con (p=0.198) or between V2:Con and V3:Con (p=0.937), despite V3:Con displaying a % TSL of 26.64 ± 0.65 whilst V1 and V2 exhibited % TSL of 22.54 ± 57 and 23.90 ± 0.34 respectively (Figure 5.3.2.0).

During H1, it was found that V3:Br tissue displayed significantly greater (p=0.0020) % TSL (27.62 ± 0.91) than V1:Br (22.74 ± 0.48), but not significantly greater than V2:Br (22.19 ± 0.24) (p=0.079). No significant difference was found between the % TSL of V3:Con and V3:Br tissue (p=0.848) or between treatments in the other two varieties.

The only significant difference witnessed in % TSL after 4 hours between groups from the second harvest was between V2:Br and V3:Br (p=0.035) (22.74 ±0.56 & 25.75 ± 0.66 respectively). No significant difference between V3:Con (23.55 ± 0.57 %) and V3:Br was found (p=0.818) or across the 3 control groups during H2. V3:Br elicited the greatest solute leakage during both H1 and H2 but witnessed a decline in % solute leakage, however this was not observed to be significant (p=0.891).

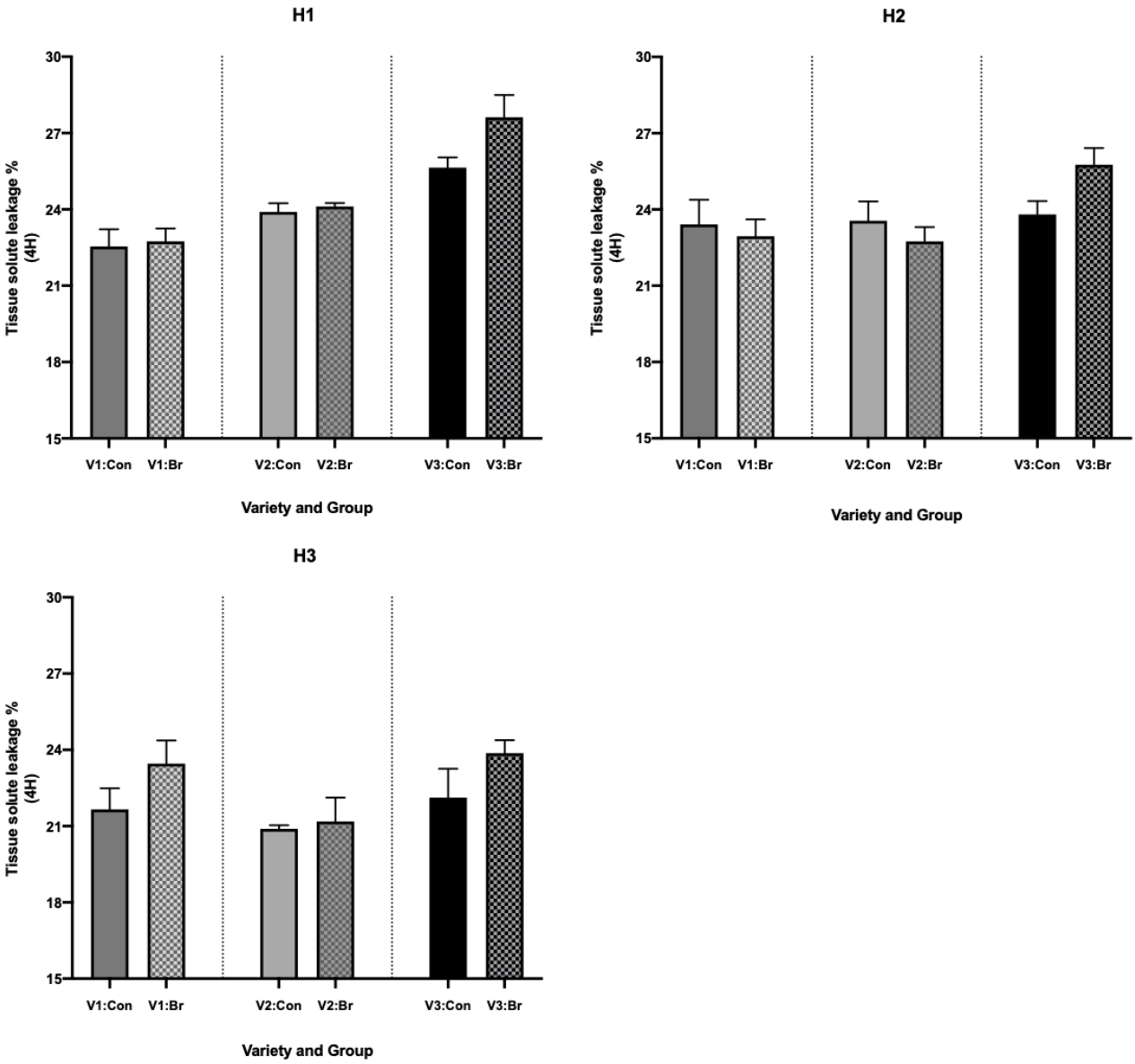


Figure 5.3.2.0) Graphs illustrating % tissue solute leakage (%TSL) after 4 hours of incubation in control (Con) and bruised (Br) tissue, across 3 varieties (V1, V2 & V3), split by harvest date (H1, H2 & H3). (*N*=4). Error bars show standard error of the mean (SEM).

In contrast, a significant decline in % TSL of V3:Br tissue was observed between H1 and H3 (p=0.044) as V3:Br again was the group eliciting the greatest % leakage (23.87

± 0.39) during H3, but to a lesser degree than during H1 and H2. No significant differences were otherwise witnessed across all groups from H3.

48 Hours incubation

The % TSL increased between 4 hours and 48 hours in all groups across varieties and harvest dates (Figure 5.3.2.1). After 48 hours of incubation it was found that harvest date (p=<0.0001) and variety (p=<0.0001) significantly affected TSL %, however no significant interaction between factors was observed (p=0.221). Similarly to 4H, during H1 after 48H, V3:Br elicited the greatest % TSL (39.05 ± 1.26) which was found to be significantly greater than V3:Control (p=0.006) which exhibited a leakage of 34.97 ± 0.54 %. No significant difference was observed between V3:Br and V1:Br (34.51 ± 0.38 %) (p=0.443) or V2:Br (p=0.618). Across the 3 control groups, no significant differences were observed (p=>0.9) during H1.

During H2, the % TSL of V3:Br tissue increased to 44.22 ± 1.26; this increase from H1 was not significant (p=0.240) but it was significantly greater (p=0.006) that the response of V3:Con during H2 (36.43 ± 1.03 %).

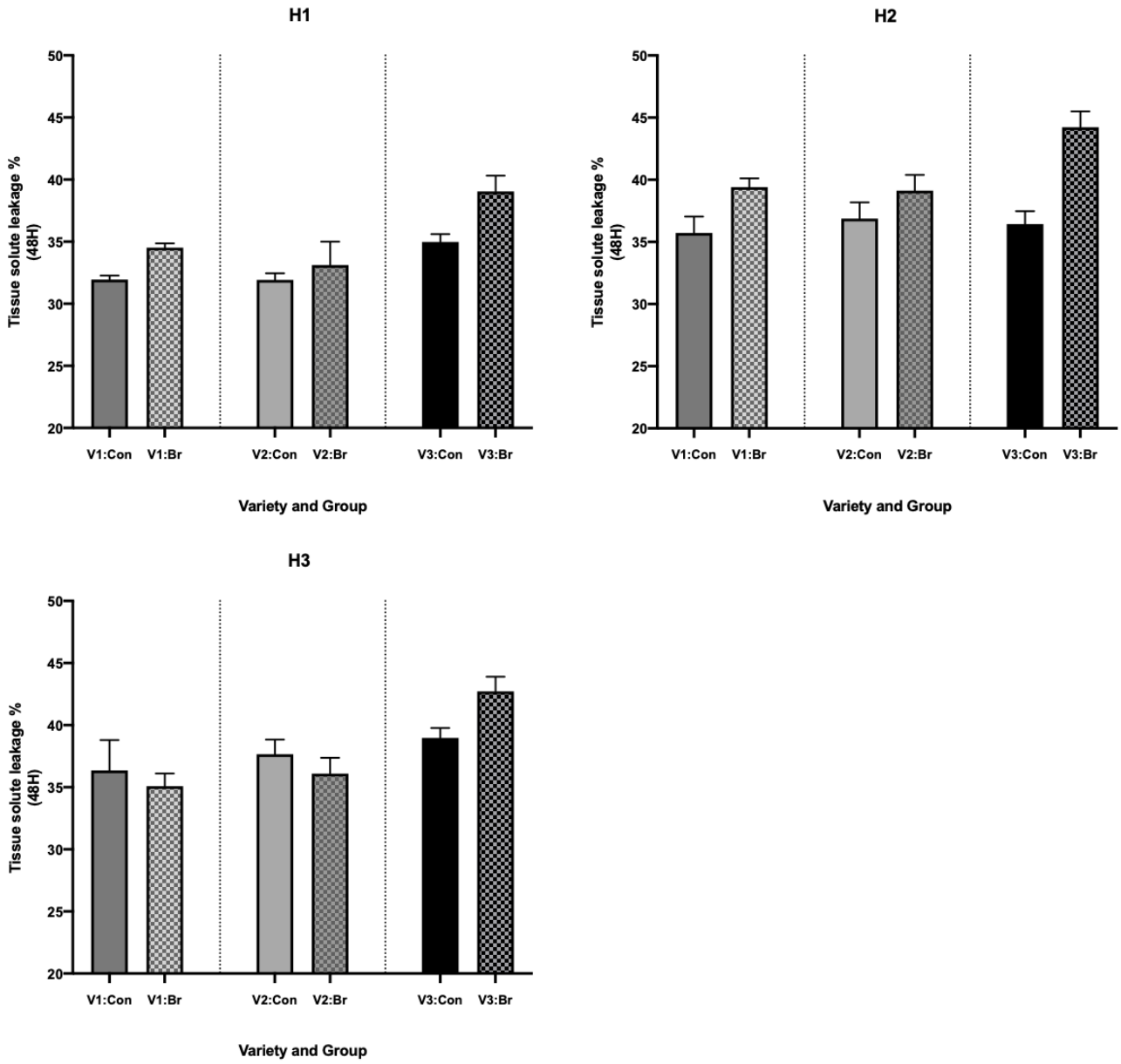


Figure 5.3.2.1) Graphs illustrating % tissue solute leakage (% TSL) after 48 hours of incubation in control (Con) and bruised (Br) tissue, across 3 varieties (V1, V2 & V3), split by harvest date (H1, H2 & H3). (*N*=4). Error bars show standard error of the mean (SEM).

No significant difference in % TSL was observed between control and bruised tissue in V1 (p=0.998) and V2 (p=0.930). Thus, during the second harvest bruised V3 tissue was the only variety to elicit a greater % TSL than control tissue suggesting that mechanical impacts may cause the contents of V3 cell vacuoles to leak at a greater rate.

The % TSL of V3:Br declined in H3 to 42.73 ± 1.17 however this decrease from H2 was not observed to be significant (p=0.989), and % TSL in bruised V3 tissue was not significantly different (p=0.741) from V3:Con tissue (36.09 ± 1.28). In contrast, %TSL in V3:Br was significantly greater (p=0.0076) than V1:Br (35.08 ± 1.10) and V2:Br (36.09 ± 1.29) (p=0.036). No significant differences were found between V1:Con and V1:Br (p=0.999) or between V2:Con and V2:Br (p=0.985), furthermore no significant variance between control groups was observed (p> =0.9).

H3 was the only harvest where % TSL in V3:Br tissue was found to be not significantly greater than V3:Con tissue, however the % TSL during the third harvest was significantly higher than the response in bruised V1 and V2 tissue. These results suggest that after 48H of incubation, damaged V3 tissue elicits greater solute leakage than bruised tissue in other varieties, and greater % TSL than control V3 tissue.

5.3.3 Total oxidative potential

The total oxidative potential of root tissue was significantly affected by harvest date (p=0.002) and variety (p=0.003) following a 2 way analysis of variance test (ANOVA),

no significant interaction between factors was observed (p=0.260). The highest total oxidative potential at 475 nm absorbance during H1 was exhibited by V3 (0.187 ± 0.004) whilst V2 elicited the lowest (0.174 ± 0.006) however no significant difference was observed between the two (p=0.428). V1 elicited the greatest total oxidative potential during H2 (0.195 ± 0.005) whilst in contrast to H1, V3 exhibited an absorbance of 0.191 ± 0.007 which was the lowest during the second harvest. No significant difference between V1 and V3 was observed during H2 (p=0.719) (Figure 5.3.3.0).

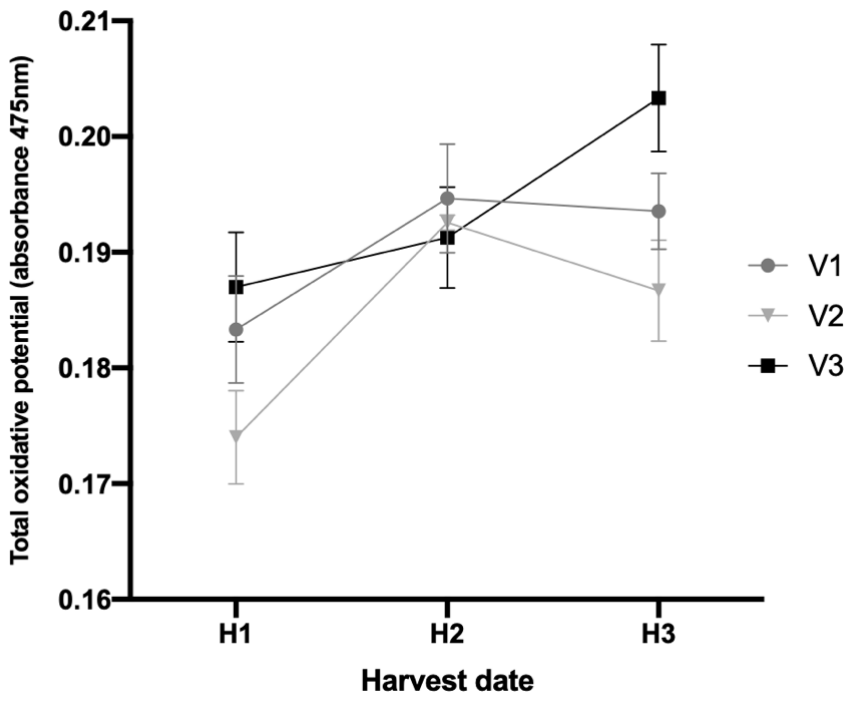


Figure 5.3.3.0) Graph illustrating total oxidative potential (absorbance 475 nm) of 3 varieties of parsnip (V1, V2 & V3) across 3 harvest dates (H1, H2 & H3). (N=3). Error bars show standard error of the mean (SEM).

During the final harvest (H3), V3 exhibited the greatest total oxidative potential (0.203 ± 0.008) however this was not found to be significantly greater than V1 (p=0.750) or V2 (p=0.165). The lowest absorbance during H3 was exhibited by V2 (0.187 ± 0.007), V2 elicited the lowest oxidative potential during H1 and H3, and was second lowest during H2. Whilst variety was observed to be a factor significantly affecting the inherent oxidative potential of parsnip tissue (p=0.003), no specific significant differences between varietal groups were found across any harvest date, following a Tukey's HSD test. The oxidative potential of all 3 varieties increased between the first and third harvest, as harvest date was observed to significantly affect oxidative potential (p=0.002). It would appear that the oxidative potential of parsnip tissue increases throughout the growing season, but these increases were not sufficient to elicit significant differences between groups.

5.3.4 Scanning electron micrographs

The production of scanning electron micrographs was a key objective in this section of study as previously, SEM micrographs have been used to analyse microstructural changes in tissue following mechanical impacts (Hussein *et al.,* 2019). Use of the scanning electron microscope, courtesy of Harper Adams University, was conducted in late February/early March of 2020. Due to the coronavirus outbreak and the subsequent UK wide lockdown from March 2020 to September 2020, this section of work was cut short. The SEM micrographs produced are useful as they illustrate tissue damage in bruised parsnip tissue following mechanical impact, and illustrate intact sections of tissue across all 3 varieties.

Figure 5.3.4.0 and Figure 5.3.4.1 illustrate cell rupture and loss of compartmentalisation in V1 parenchyma tissue following mechanical impact, in discoloured regions of tissue. There are regions of intact tissue present, however the falling bolt impact caused a change in the integrity and structure of tissue.

Figure 5.3.4.2 shows healthy, intact V2 tissue where cell walls are intact, there is clear differentiation and no evidence of membrane rupture or leakage. V2 tissue responds to mechanical impact by rupturing (Figure 5.3.4.3; Figure 5.3.4.4; Figure 5.3.4.5) which leads to large intracellular spaces to be present.

V3 parenchyma cell size and organisation appear different to the other varieties (Figure 5.3.4.6) with perhaps larger cells and air spaces being present in healthy tissue. From these limited number of micrographs, it appears as though V3 elicits a similar response to mechanical impacts as V1 and V2, with cells rupturing, a loss of compartmentalisation, a loss of membrane integrity and the presence of greater intracellular air spaces (Figure 5.3.4.7; Figure 5.3.4.8). Figure 5.3.4.9 shows a V3 parenchyma cell at 1650x magnification; this facilitates the viewing of the cell wall and membrane in greater detail, and clearly visible is the presence of a starch granule in the middle of the cell.

However, the desired number of micrographs to facilitate quantifiable analysis (I.e., cell size/number) was not achieved, thus the images are a snapshot into microstructural changes in tissue following impacts, and may not provide the full picture.

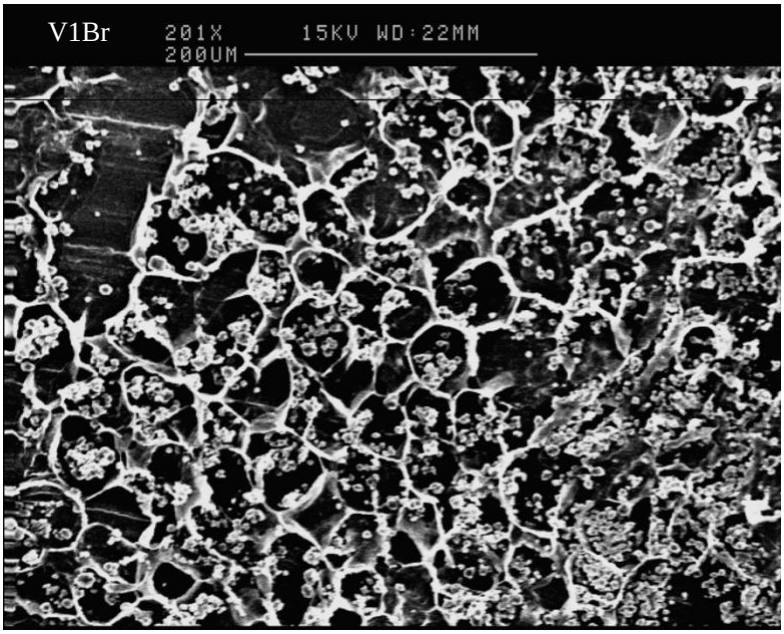


Figure 5.3.4.0) Authors own Scanning electron micrograph at 201x magnification of V1 parenchyma tissue following mechanical impact. Scale bar = 200 μM.

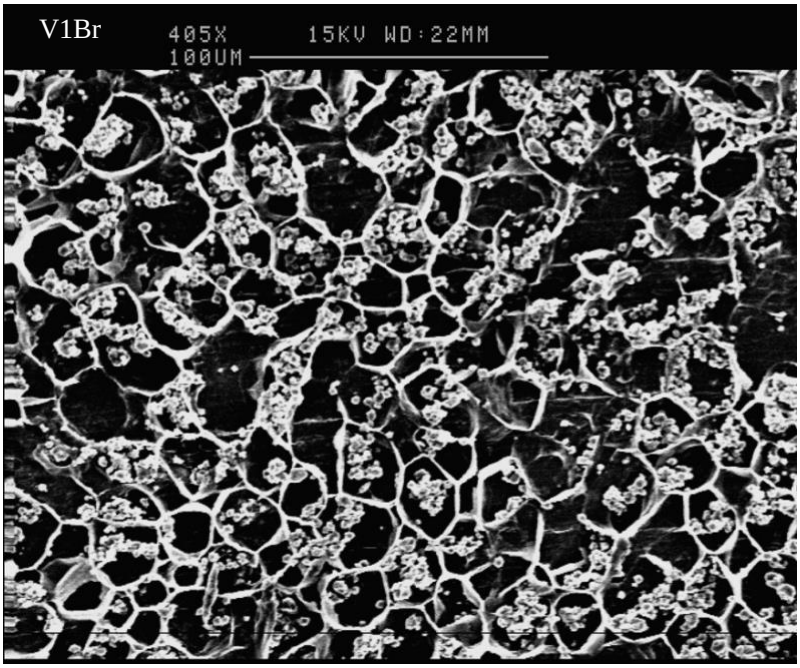


Figure 5.3.4.1) Authors own micrographs at 201x magnification of V1 parenchyma tissue following mechanical impact. Scale bar = 100 μM.

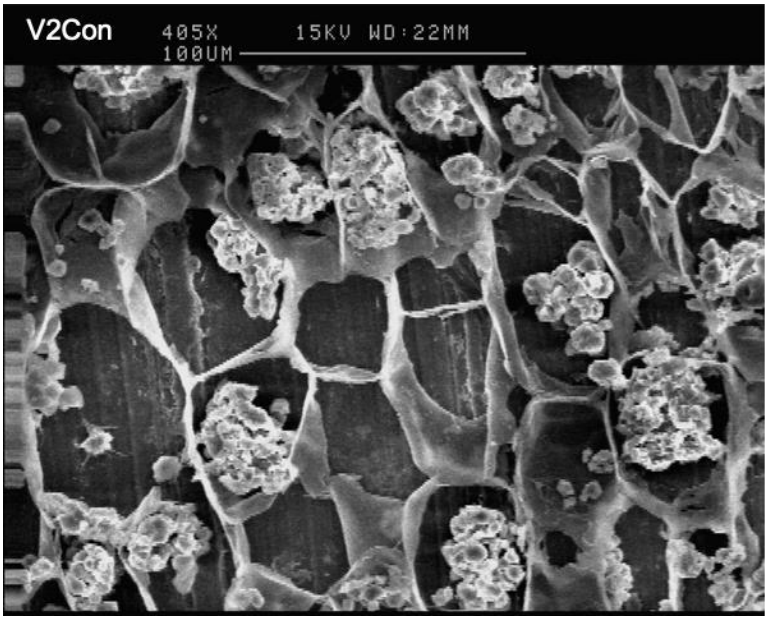


Figure 5.3.4.2) Authors own micrograph of control V2 parenchyma tissue at 405x magnification. Scale bar = 100 μM.

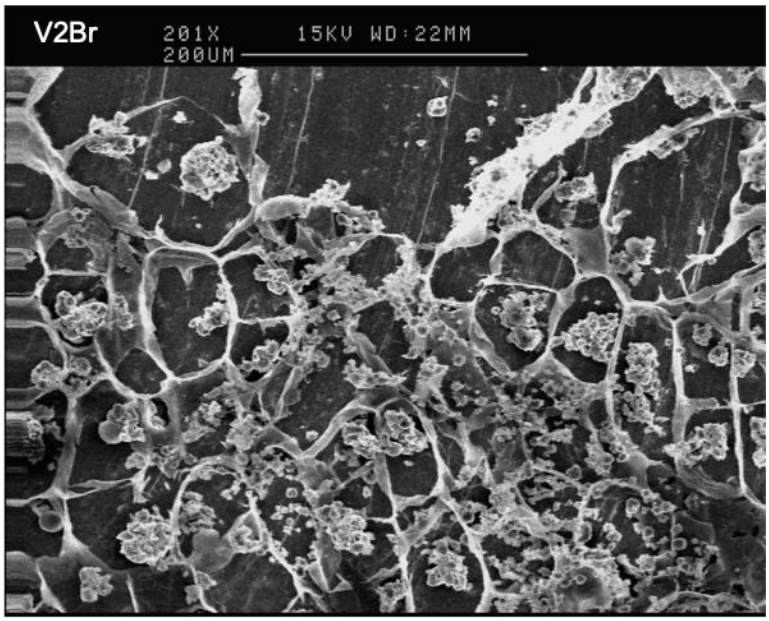


Figure 5.3.4.3) Authors own micrograph of V2 parenchyma tissue following mechanical impact at 201x magnification. Scale bar = 200 μM.

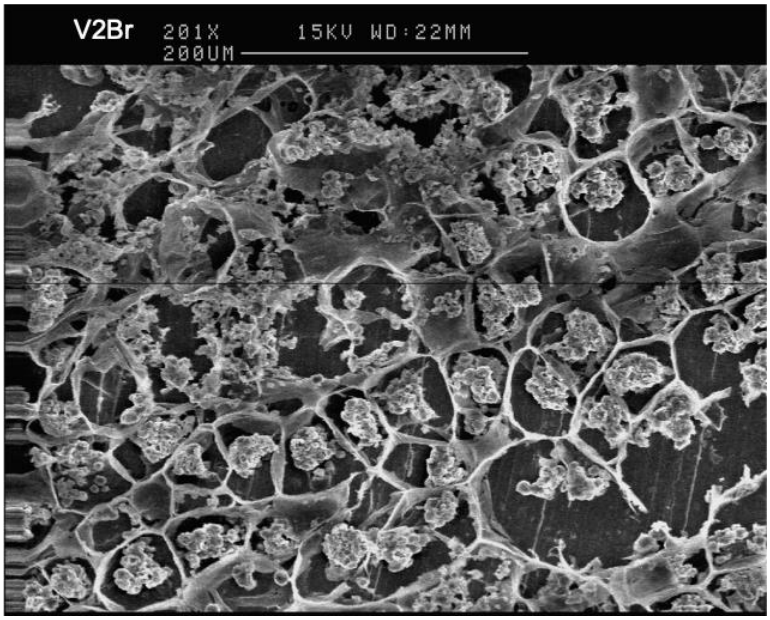


Figure 5.3.4.4) Scanning electron micrograph of V2 parenchyma tissue following mechanical impact and the onset of bruising at 201x magnification. Scale bar = 200 μM.

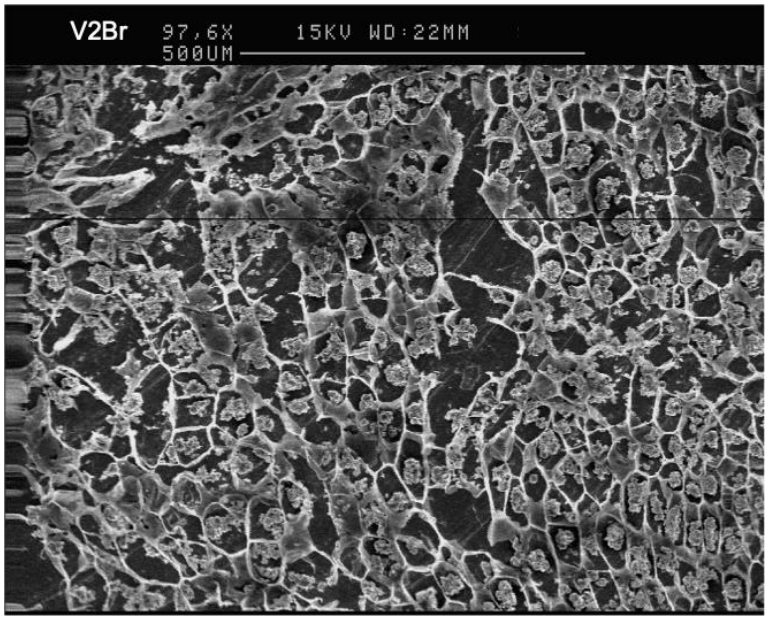


Figure 5.3.4.5) Scanning electron micrograph of bruised V2 parenchyma tissue following mechanical impact at 97.6x magnification. Scale bar = 500 μM.

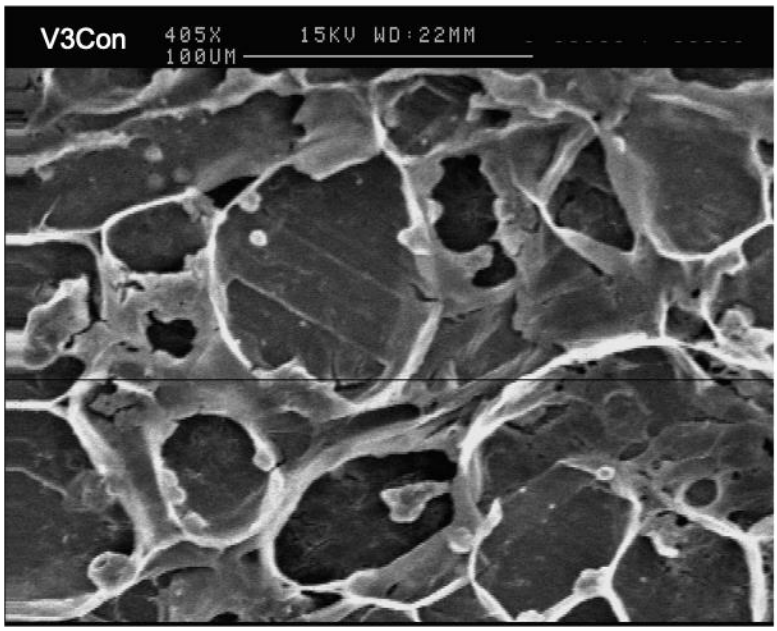


Figure 5.3.4.6) Scanning electron micrograph of control V3 parenchyma tissue under 405x magnification. Scale bar = 100 μM.

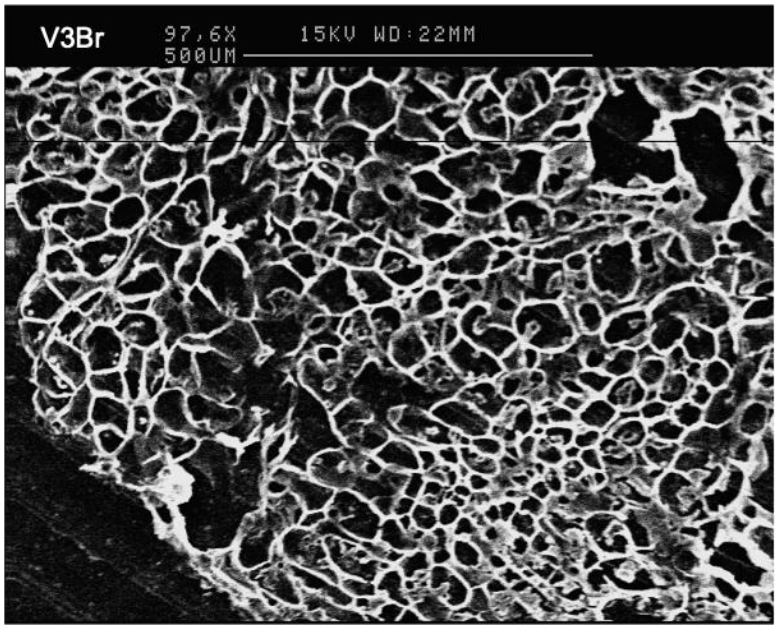


Figure 5.3.4.7) Scanning electron micrograph of bruised V3 tissue following mechanical impact at 97.6x magnification. Scale bar = 500 μM.

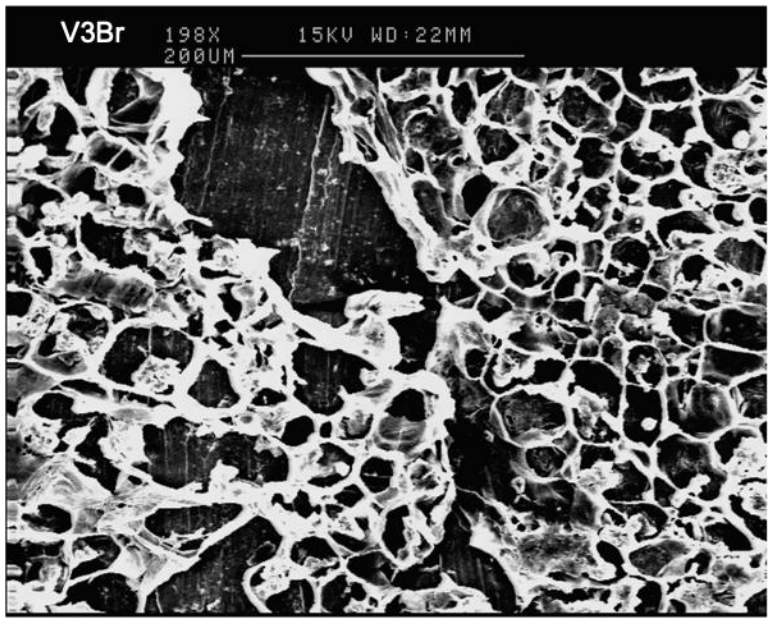


Figure 5.3.4.8) Scanning electron micrograph of bruised V3 tissue following mechanical impact at 198x magnification. Scale bar = 200 μM.

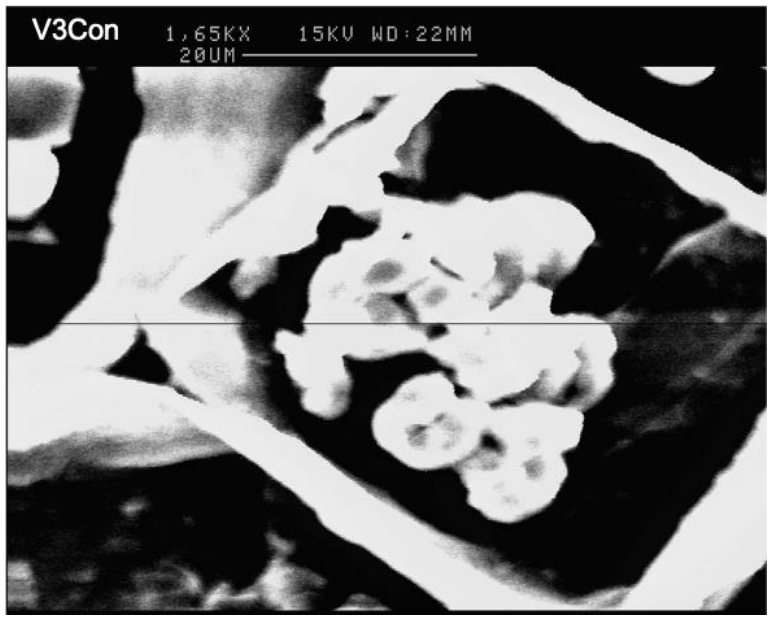


Figure 5.3.4.9) Scanning electron micrograph of V3 tissue at 1650x magnification. Scale bar = 20 μM.

5.3.5 Relative water content %

Following a 2 way ANOVA, harvest date (p=0.0017*)* and variety (p=<0.0001) were found to be factors significantly affecting the relative water content of parsnip roots. During H1 the highest relative water content observed was in V3 (81.28 ± 0.21 %) (Figure 5.3.5.0) which was found to be significantly greater than V2 (p=0.0131), the lowest % relative water content witnessed during H1 (79.58 ± 0.22). No significant difference was found between V1 and V3 (p=0.183) despite V1's relative water content being 80.07 ± 0.40 %.

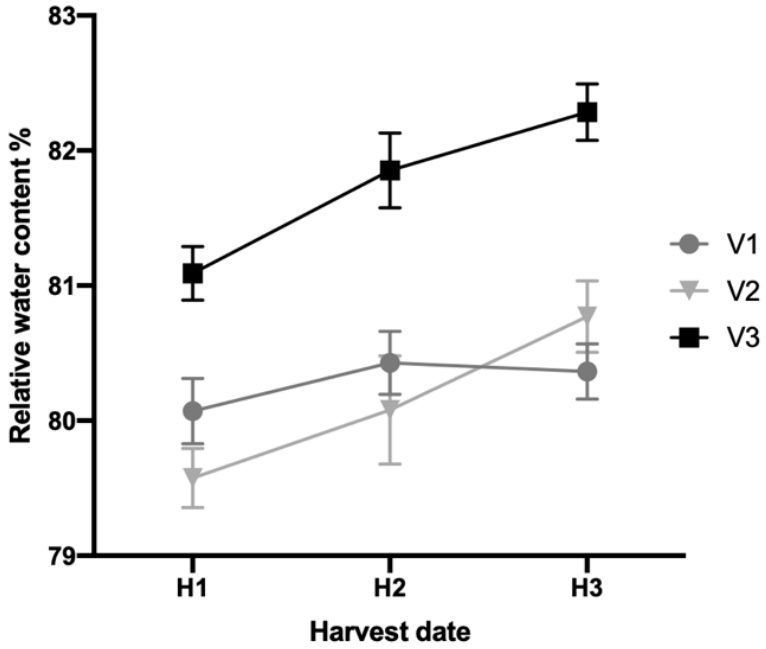


Figure 5.3.5.0) Graph illustrating root relative water content (%) of 3 parsnip varieties (V1, V2 & V3) across 3 harvest dates (H1, H2 & H3). (N=3). Error bars show standard error of the mean (SEM).

During H2, V3 again elicited the greatest % relative water content (81.85 ± 0.28), however this was not found to be significantly different to the response in H1 (p=0.502). V2 again elicited the lowest relative water content (80.07 ± 0.31 %) which was found to be significantly lower than V3 (p=0.022) but not significantly different from V1 (p=0.985).

During the final harvest, V3 again elicited the greatest relative water content % (82.25 ± 0.24) which was the highest observed across all harvests, and was significantly greater than H1 (p=0.048) but not H2 (p=0.917). The relative water content % of V3 during the third harvest was significantly greater than V1 (p=0.0031) and V2 (p=0.0113) whose % water content (80.37 ± 0.16 and 80.77 ± 0.28 respectively) was not observed to significantly differ during H3 (p=0.963).

5.3.6 RNA sequencing and de novo transcriptome assembly

A total of 71 GB of data was generated during Illumina sequencing by Novogene, Ltd. Following sequencing and the removal of adapter sequences and low quality reads, 23 of the original 24 samples passed quality control (Table 5.2.6.0). The number of clean reads per sample ranged from 19.98 to 24.19 million, the remaining percentage of clean reads with Q30 bases ranged from 93.67 to 94.83%. The mean GC content of the 23 samples was 41.15% (Table 5.2.6.1). The clean reads from the 23 samples of 6 groups were combined and a *denovo* construction of a parsnip transcriptome was performed using Trinity. A total of 179,103 clean reads comprising 308,757,681 base pairs (bp) were assembled into 58,551 parsnip Unigenes which possessed an average

length of 1460 bp (Table 5.3.6.0). The species classification of the constructed transcriptome can be viewed in Figure 5.3.6.0.

Nucleotide length	**Transcripts**	**Unigenes**
200-500 bp	30986	16085
500-1000 bp	39957	15955
1000-2000 bp	50264	12336
>2000 bp	57896	14175
Total	179103	58551
Min Length bp	301	301
Max Length bp	115203	115203
Mean Length bp	1724	1460
N50 bp	2505	2412
N90 bp	828	581
Total Nucleotides	308757681	85457222

Table 5.3.6.0) Table illustrating number of Transcripts and Unigenes per Nucleotide length obtained from sequencing of 23 parsnip samples and *denovo* transcriptome construction.

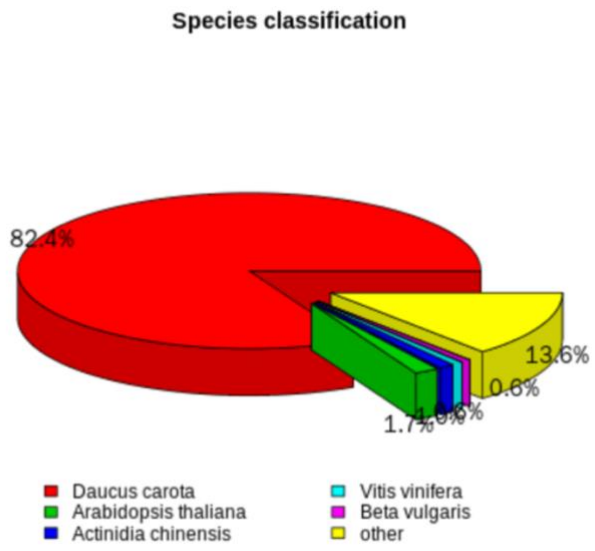


Figure 5.3.6.0) Pie chart showing species classification of Unigenes assembled for 23 parsnip samples. A total of 341 species were identified with *D. carota* comprising the majority (82.4%). To note, *P. Crispum,* a fellow member of the Apiaeae, ranked 23rd on the species similarity list with only 0.18% of hits.

5.3.7 Functional annotation and classification of Unigenes

The 58,551 Unigenes identified were searched against the NR, NT, KO, SwissProt, PFAM, GO and KOG databases (Table 5.3.7.0). Of the 58,551 total Unigenes identified, 32,813 (56.04%) were annoted by the NR database based upon sequence homologies, 28,596 (48.83%) were annoted in NT, 11,143 (19.03%) could be annoted in KO, 24,353 (41.59%) were annoted in SwissProt, 23,997 (40.98%) were annoted in both PFAM and GO, 6899 (11.78%) were annoted in KEGG, with 37,463 (63.98%) of

Unigenes being annoted by at least one database. A total of 36.02 % of genes were not annotated in any of the seven databases.

Statistical Items	**Number of Unigenes**	**%**
Annotated in NR	32813	56.04
Annotated in NT	28596	48.83
Annotated in KO	11143	19.03
Annotated in SwissProt	24353	41.59
Annotated in PFAM	23997	40.98
Annotated in GO	23997	40.98
Annotated in KOG	6899	11.78
Annotated in all databases	4036	6.89
Annotated in at least one database	37463	63.98
Total Unigenes	**58551**	**100**

Table 5.3.7.0) Table with number of and % of Parsnip Unigenes annotated by function via 7 databases.

To describe the functions of genes and gene products the GO database was employed assigning the 23,997 Unigenes functional terms to 1656 functional terms categorized into either biological process (BP), cellular component (CC) or molecular functions (MM) (Figure 5.3.7.1). Biological process assignments accounted for the majority (67.0 2%), with cellular component accounting for 22.04 % and molecular functions accounting for 10.92 % (Figure 5.3.7.1).

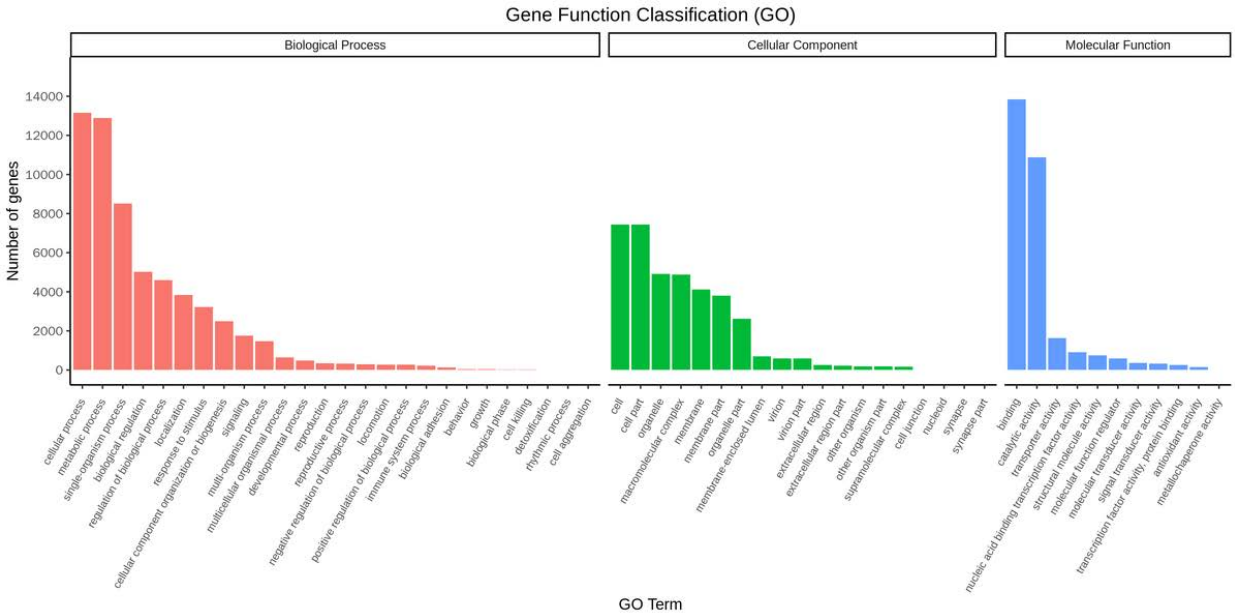


Figure 5.3.7.1) Gene function classification (GO) term annotation of Unigenes assigned to terms within 3 main categories (Biological Process (red), Cellular Component (green) and Molecular function (blue).

To explore significantly enriched pathways following mechanical damage to parsnip roots, the database of KEGG (Kyoto Encyclopaedia of Genes and Genomes) was used to link transcripts with higher order functions by using pathway maps of known and standardized cellular processes and gene annotations. A total of 11,948 Unigenes were assigned into five main categories; A= Cellular processes, B= Environmental information processing, C= Genetic information processing, D= Metabolism and E=Organismal system (Figure 5.3.7.2). The category Metabolism ranked highest accounting for 4854 Unigenes (40.63% of total), with Genetic Information Processing ranking second with 2490 (20.87%), then Organismal system (1813, 15.17%), Cellular processes (1435, 12.01%) and finally Environmental information processing ranked last with 1356 Unigenes (11.34%).

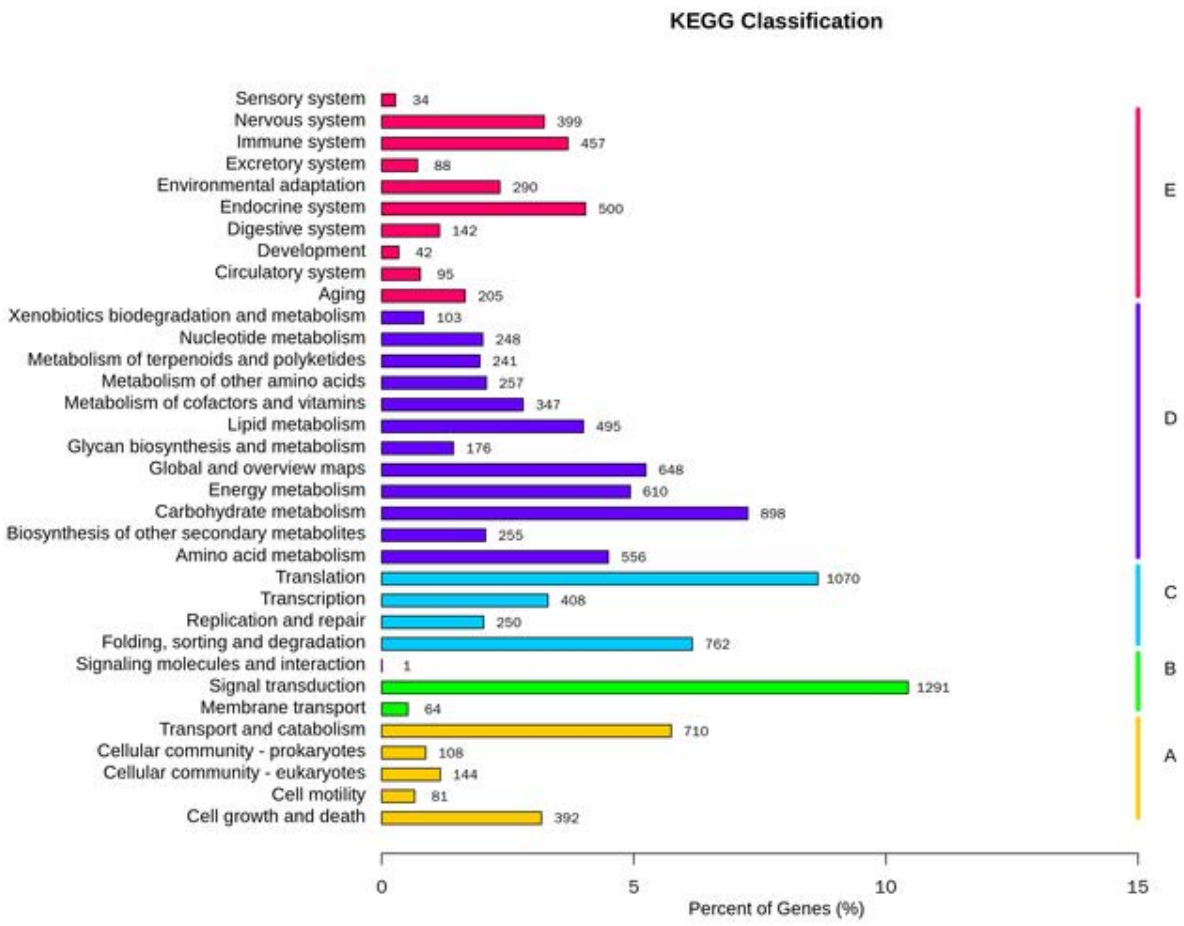


Figure 5.3.7.2) KEGG classification of 11,948 Unigenes into categories according to function.

5.3.8 Identification and classification of DEGs between bruised and control parsnip tissue

5.3.8.1 Differential gene expression between bruised vs control V1 tissue

After analysing the results of DESeq2 a total of 1635 Unigenes (2.77% of all Unigenes) were identified as being significantly differentially expressed between bruised and control tissue within V1 (Figure 5.3.8.1.0). Up-regulated genes accounted for 1337 (81.77%) of differentially expressed genes, with 296 (18.23%) genes being significantly down regulated following mechanical damage and bruise formation.

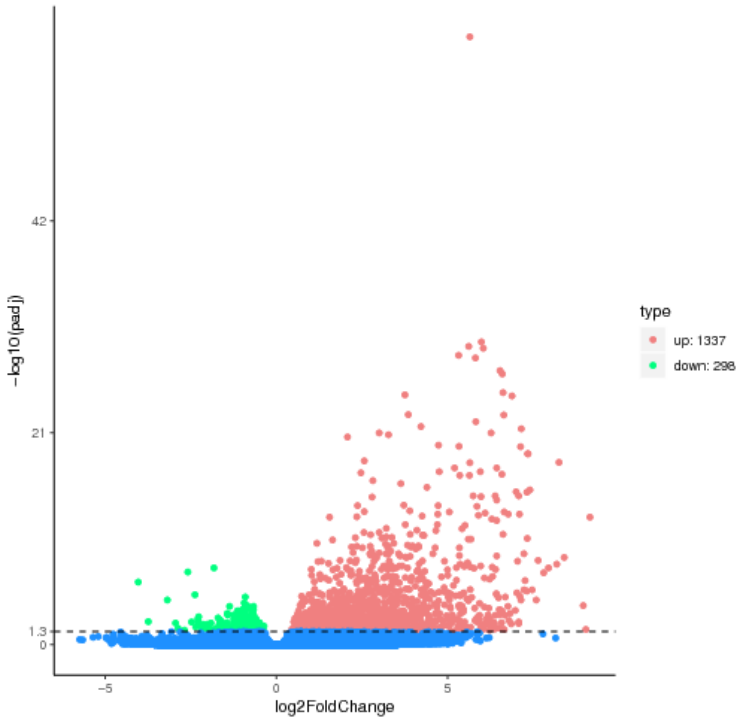


Figure 5.3.8.1.0) Volcano plot illustrating differential gene expression between healthy and bruised tissue in V1 roots. (*N*=4).

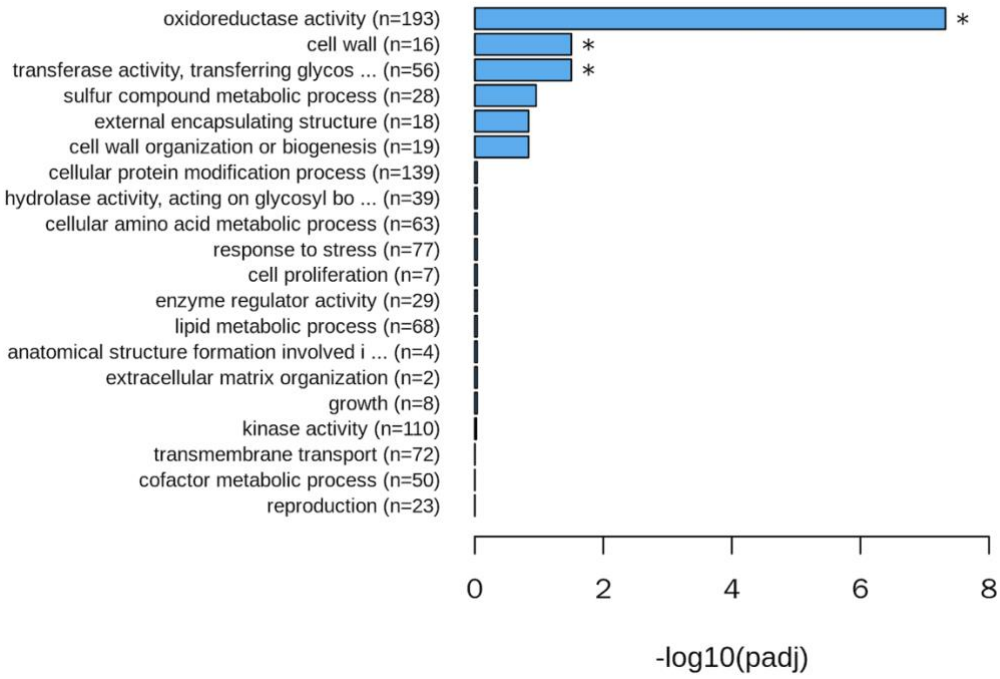


Figure 5.3.8.1.1) Bar graph illustrating significantly differentially expressed Unigenes between bruised and healthy V1 tissue annotated to GO terms. (*N*=4).

A total of 193 Unigenes were annotated as “oxidoreductase activity” (Figure 5.3.8.1.1), 16 Unigenes were annotated with “cell wall” and 56 Unigenes with “transferase activity”. No other annotations were observed to be significant. Significantly enriched GO terms were then split by function.

The annotated GO terms were split and assigned as either MF, CC or BP. Of the 63 terms with a MF identified, 5 significant GO ID’s were found to be significantly upregulated following mechanical damage to tissue (Padj< 0.05) (Figure 5.3.8.1.2); GO:0016684 (oxidoreductase activity, acting on peroxide as acceptor), GO:0046906 (tetrapyrrole binding), GO:0016667 (oxidoreductase activity, acting on a sulphur group of donors), GO:0016614 (oxidoreductase activity, acting on CH-OH group of donor)

and GO:0016229 (steroid dehydrogenase activity). CC functions accounted for 191 GO terms, with no significant downregulation, with two significantly upregulated GO ID's being GO:0030312 (external encapsulating structure) and GO:0031226 (Intrinsic component of plasma membrane).

No significant GO terms with BP functions were significantly downregulated following mechanical damage, but 5 significant upregulated GO ID's were identified. The most significantly upregulated was GO:0071555 (Cell wall organization), followed by GO:0045229 (external encapsulating structure, structure organization), GO:003474 (Cellular hormone metabolic process), GO:0010817 (Regulation of hormone levels) and GO:0071669 (Cell wall organization or biogenesis).

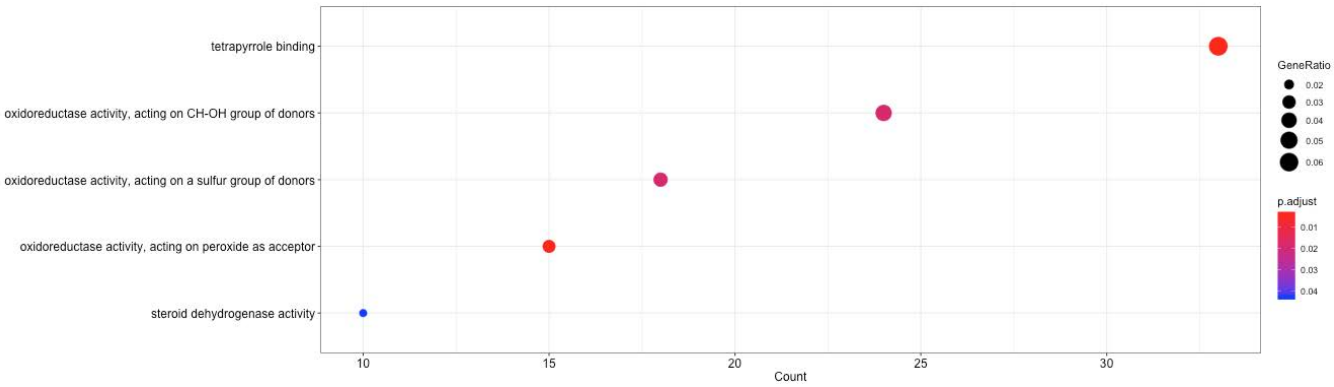


Figure 5.3.8.1.2) Significant GO term enrichment of DEG's in V1 tissue following mechanical impact. (*N*=4).

No KEGG pathways were significantly downregulated following mechanical damage to V1, however 18 pathways were found to be significantly upregulated following damage (Figure 5.3.8.1.3). The most 5 most significantly enriched pathways in order

were ko00940 (Phenylpropanoid biosynthesis) (Figure 5.3.8.1.4), ko00923 (Drug metabolism- cytochrome P450), ko00980 (Metabolism of xenobiotics by P450), ko00480 (Glutathione metabolism) and ko001360 (Phenylalanine metabolism) (Figure 5.3.8.1.5).

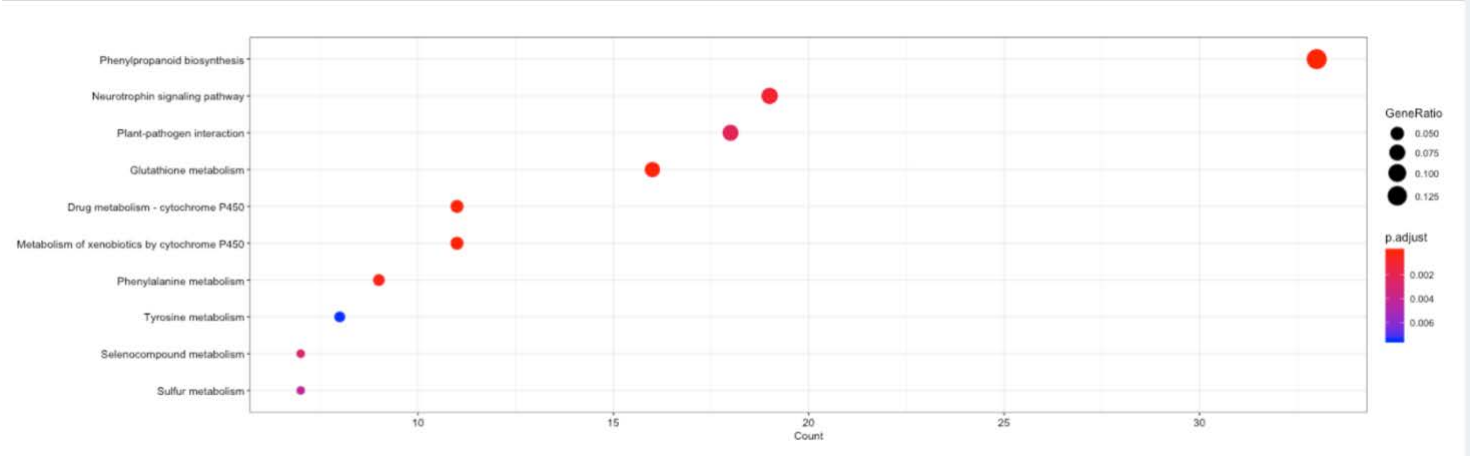


Figure 5.3.8.1.3) Significantly enriched KEGG pathways in bruised tissue in comparison to healthy V1 tissue. (*N*=4).

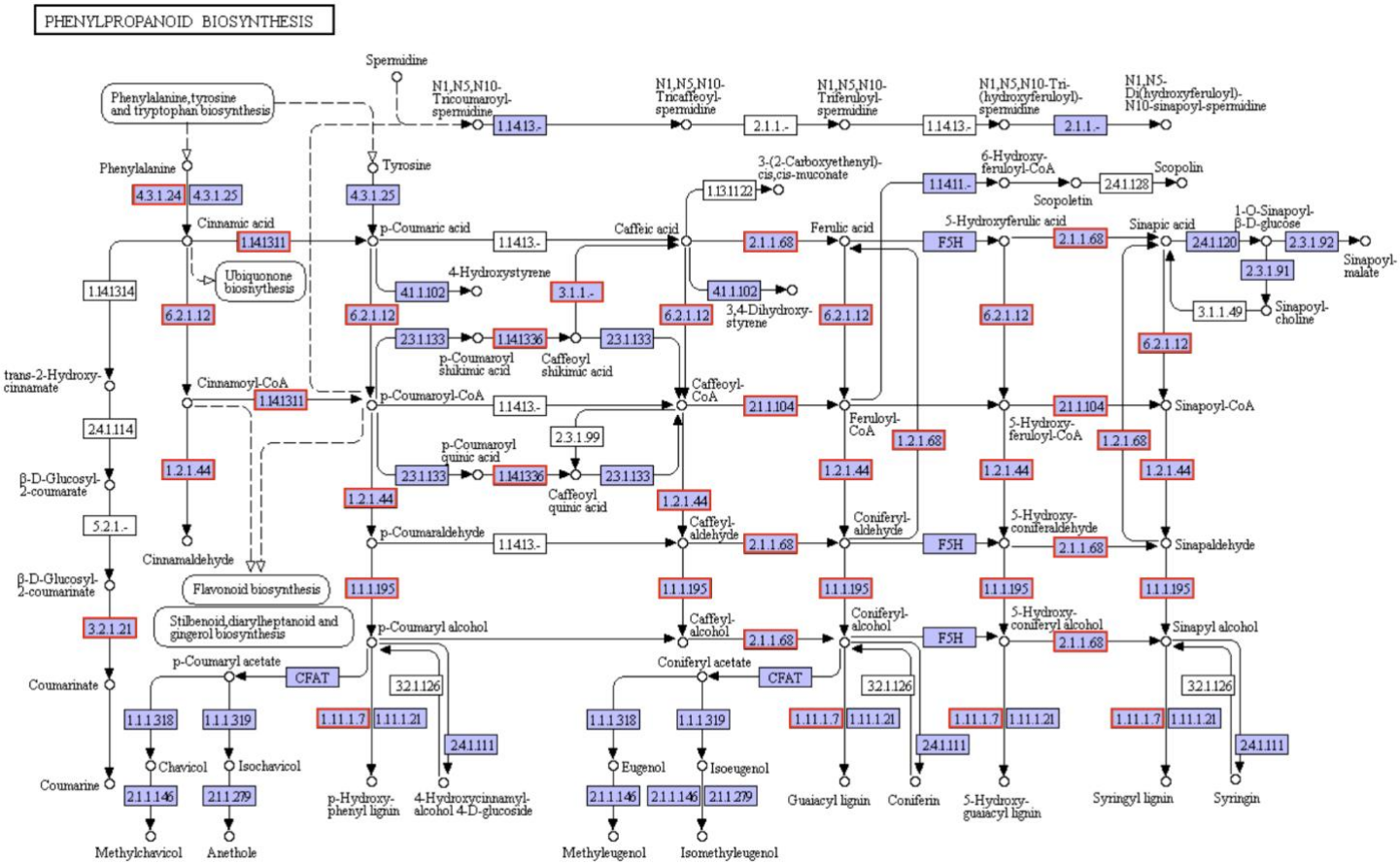


Figure 5.3.8.1.4) Phenylpropanoid biosynthesis pathway with overly expressed genes (highlighted in red) in bruised V1 tissue compared to control tissue (*Padj*= <0.05). (*N*=4).

Xu *et al.,* (2015) demonstrated that the activation of the phenylpropanoid pathway, and the resultant metabolites produced, were heavily involved in callus browning; bruised parsnip tissue of V1 also exhibits overexpression of this pathway following mechanical damage.

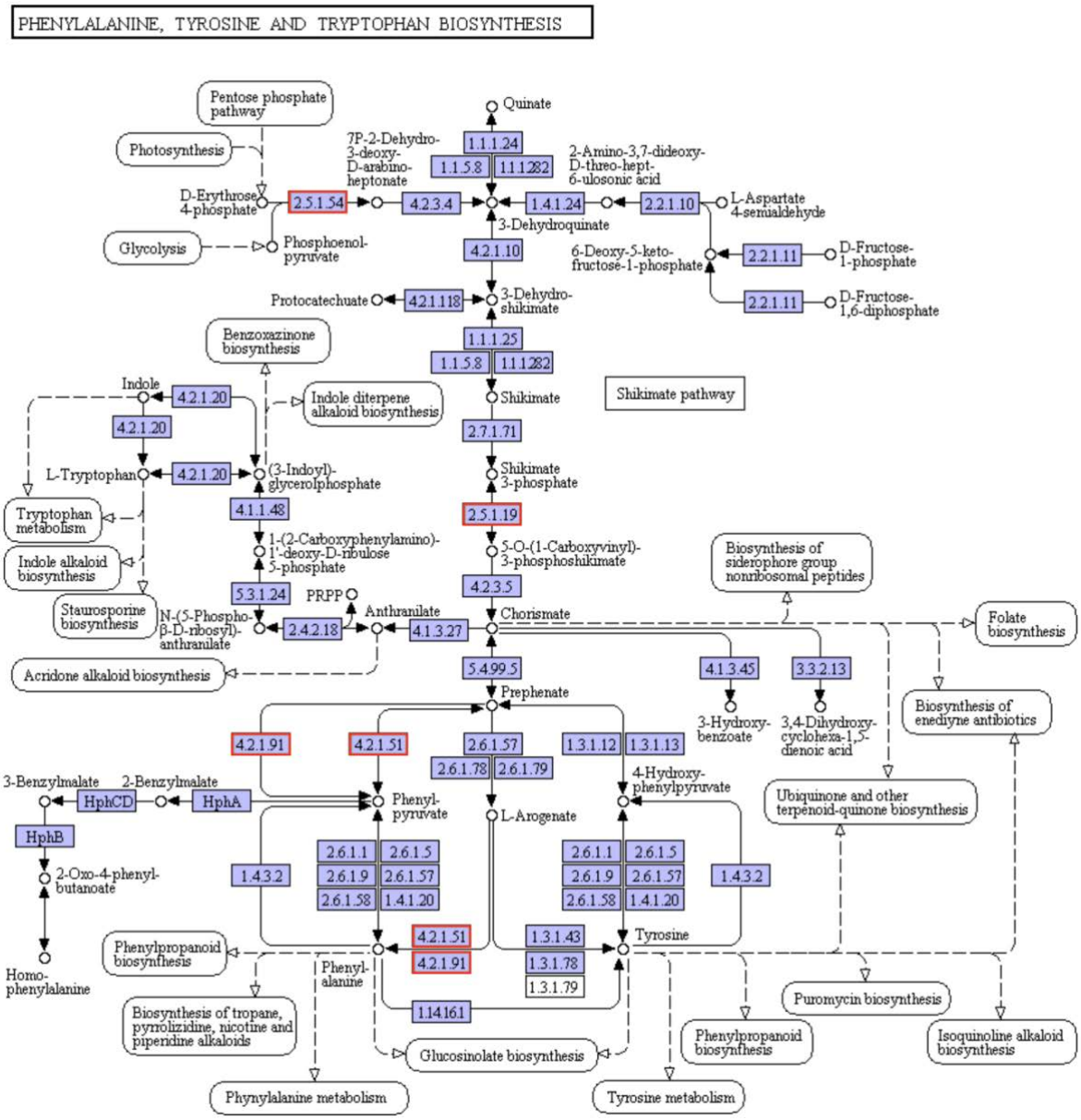


Figure 5.3.8.1.5) Phenylalanine, tyrosine and tryptophan biosynthesis pathway with overexpressed genes in bruised V1 tissue highlighted in red. (*N*=4).

5.3.8.2 Differential gene expression between bruised and control V2 tissue

After analysing the results of DESeq2 a total of 956 Unigenes (1.63 % of all Unigenes) were identified as being significantly differentially expressed between bruised and control tissue within V2 (Figure 5.3.8.2.0). Up-regulated genes accounted for 794 (83.05%) of differentially expressed genes, with 162 genes (16.95%) being significantly down regulated following mechanical damage. To determine the functions of DEG's between bruised and control parsnip tissue, annotated genes were mapped to the GO database. Of the 956 DEG's, functional groups were categorised into 3 categories (Figure 5.3.8.2.1). Molecular Function, Biological Process and Cellular Component.

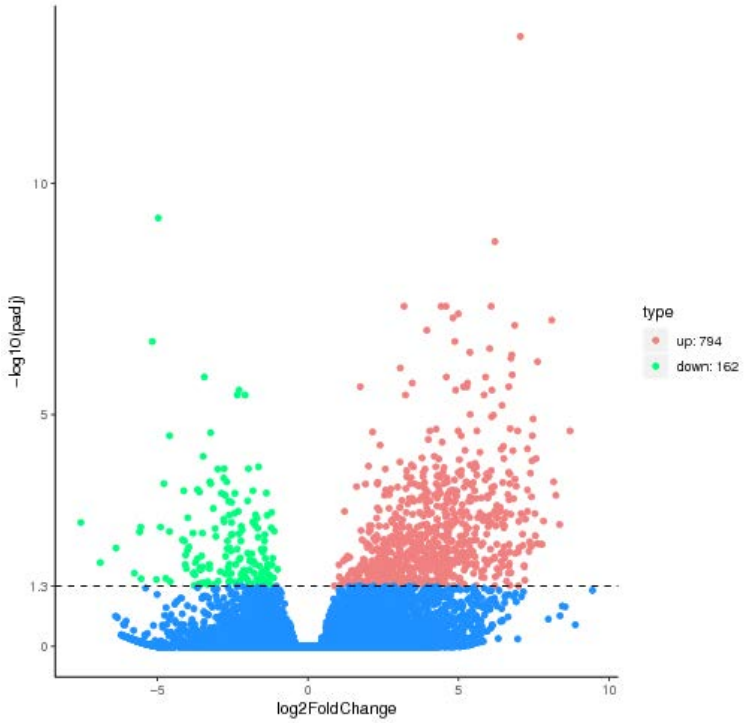


Figure 5.3.8.2.0) Volcano plot illustrating differential gene expression between control and bruised tissue in V2 roots . (*N*=3-4).

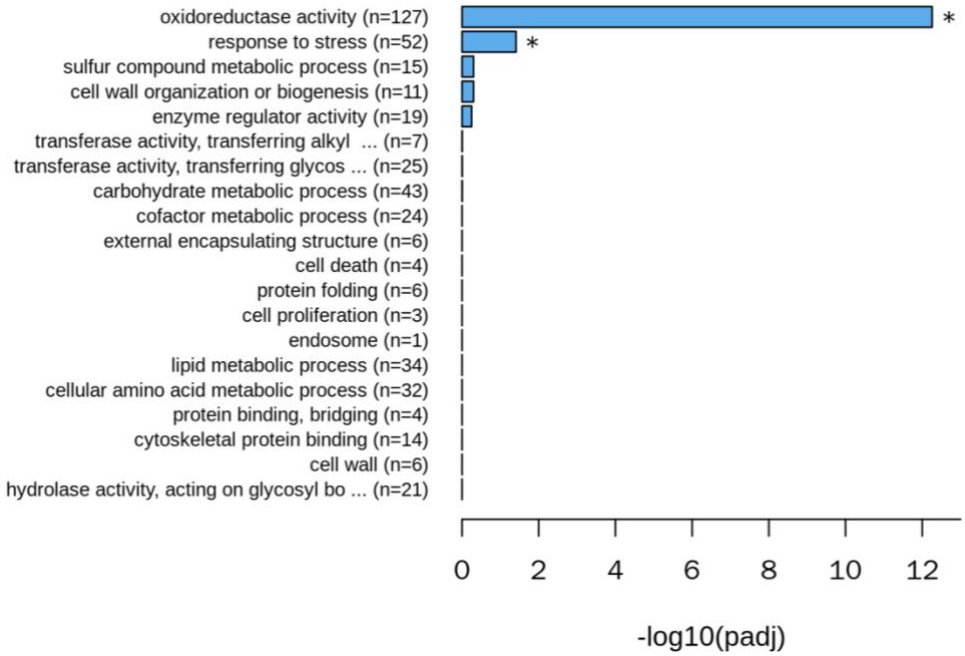


Figure 5.3.8.2.1) GO term annotation of DEG's between bruised and control V2 tissue. (*N*=3-4).

A total of 127 Unigenes were annotated with "oxidoreductase activity" whilst 52 were annoted with "response to stress", no other annotations were found to be significant. MF was the only main category with differently expressed functional GO terms; no terms were downregulated, but a total of 4 terms were found to be significantly enriched following mechanical damage (Figure 5.3.8.2.2). GO:0016684 (oxidoreductase activity, acting on peroxide as acceptor), GO:0046906 (tetrapyrrole binding), GO:0016667 (oxidoreductase activity, acting on a sulphur group of donors), GO:0016614 (oxidoreductase activity, acting on CH-OH group of donor). No GO ID's associated with BP or (CC functions were identified as significantly differentially expressed between bruised and control V2 tissue.

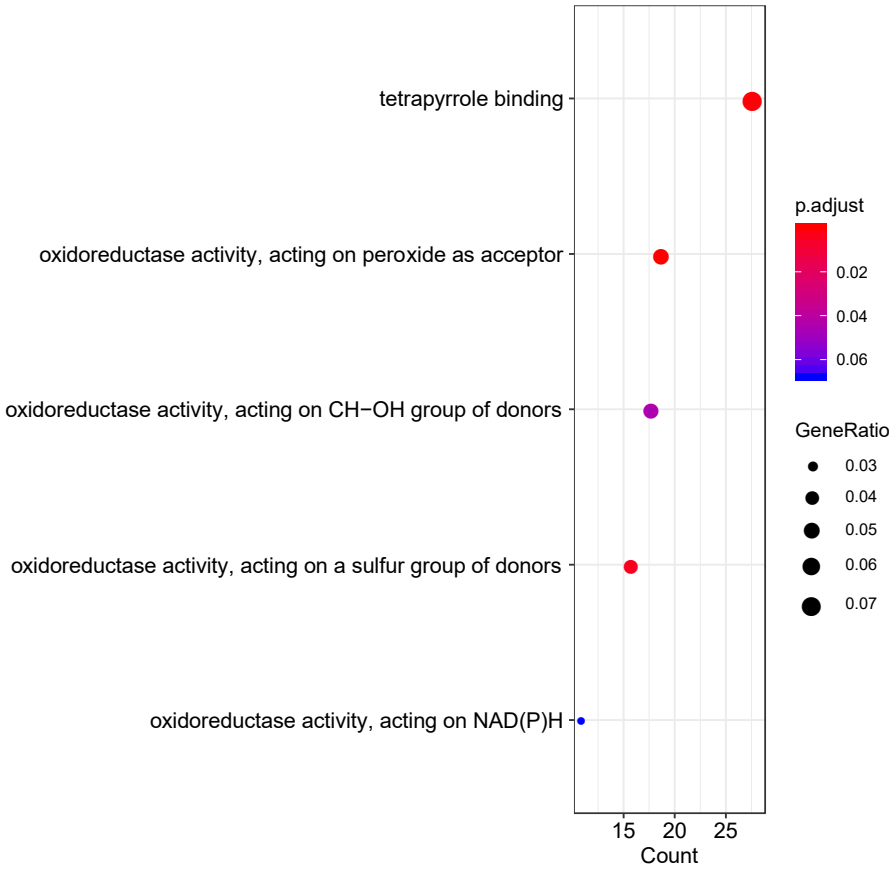


Figure 5.3.8.2.2) Significant GO term enrichment of DEG's in V2 tissue following mechanical impact). (*N*=3-4).

To investigate the specific biochemical pathways of the identified DEG's between bruised and control V2 tissue, DEG's were mapped to terms in the KEGG database to assess whether any significantly enriched pathways existed following mechanical damage (Figure 5.3.8.2.3). A total of 11 KEGG pathways were significantly enriched (*Padj*< 0.05) following mechanical damage, with 4 pathways being significantly upregulated and 7 downregulated. The most significantly upregulated pathway was ko00940 (Phenylpropanoid biosynthesis) followed by, ko00480 (Glutathione

metabolism), ko00982 (Drug metabolism- Cytochrome P450) and ko00980 (Metabolism of xenobiotics by P450).

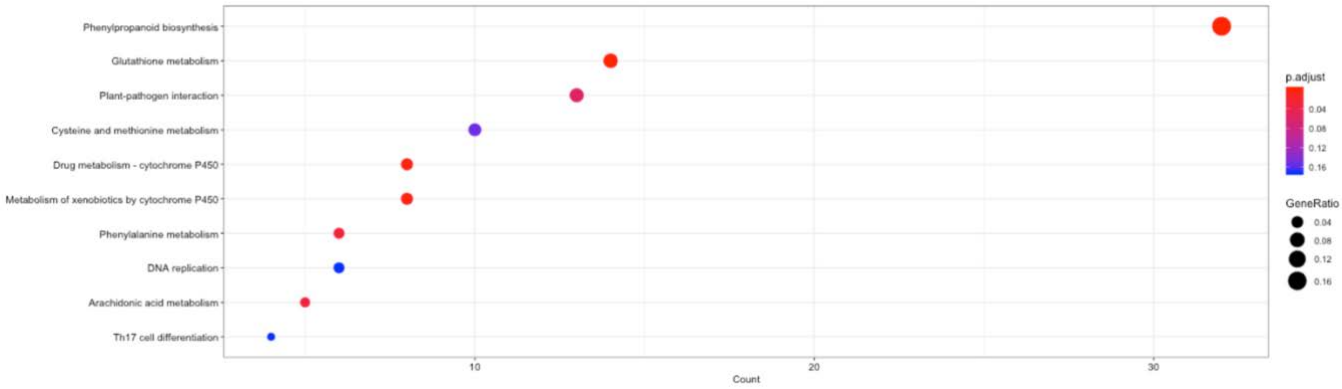


Figure 5.3.8.2.3) Significantly enriched KEGG pathways in bruised tissue in comparison to control V2 tissue, significance is determined by *Padj* <0.05. (*N*=3-4).

The pathway which experienced the most significant downregulation was ko04624 (Toll and Imd signalling pathway) followed by ko04620 (Toll-like receptor signalling pathway), ko04064 (Nf-kappa B signalling pathway), ko04722 (Neurotrophin signalling pathway), ko04075 (Plant hormone signal transduction) and ko00100 (Steroid biosynthesis).The Phenylpropanoid biosynthesis pathway (ko00940) (Figure 5.3.8.2.4) was the most significantly overexpressed pathway between bruised and control V2 tissue (*Padj*= 3.99 x10^{-20}). Furthermore, V2 significantly upregulated the closely related pathway ko00360 (*Padj*= 0.0137) involved in phenylalanine, tryptophan and tyrosine metabolism, a key step in the biosynthesis of primary and secondary metabolites such as lignin, involved in plant defence and cell repair.

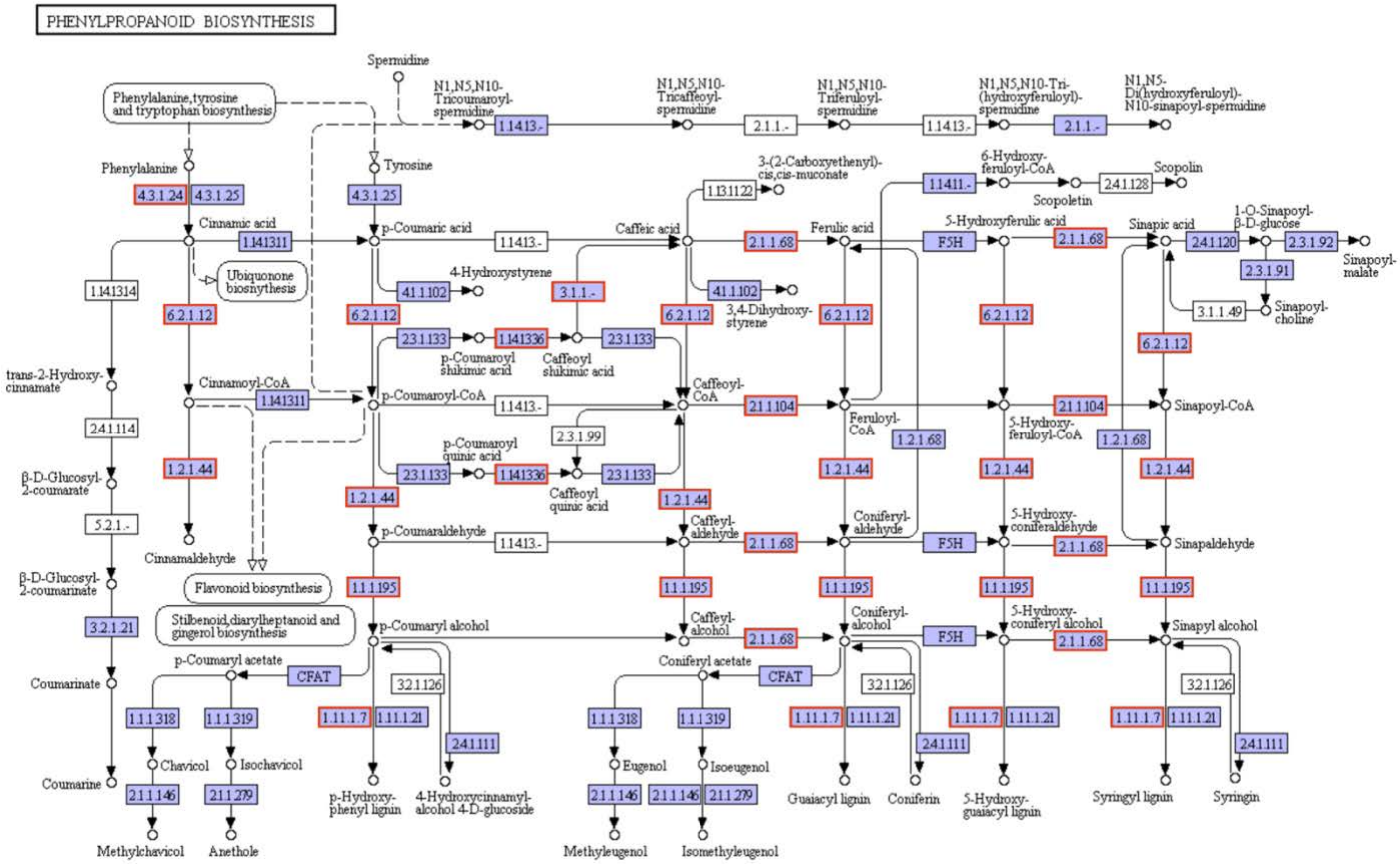


Figure 5.3.8.2.4) Phenylpropanoid biosynthesis pathway with overly expressed genes (highlighted in red) in bruised V2 tissue compared to control tissue (*Padj*= <0.05). (*N*=3-4).

5.3.8.3 Differential gene expression between bruised and control V3 tissue

After analysing the results of DESeq2 a total of 84 Unigenes (0.14 % of all Unigenes) were identified as being significantly differentially expressed between bruised and control tissue within V3 (Figure 5.3.8.3.0). Up-regulated genes accounted for 33 (39.29%) of differentially expressed genes, with 51 (60.71%) genes being significantly down regulated following mechanical damage. From the 84 DEG's identified following tissue damage, none were matched to terms within the 3 functional categories (*Padj* value ≤0.05) (Figure 5.3.8.3.1). No KEGG pathways were significantly enriched as a response to mechanical damage in V3 tissue (Figure 5.3.8.3.2) (*Padj* =≤0.05 to determine significance).

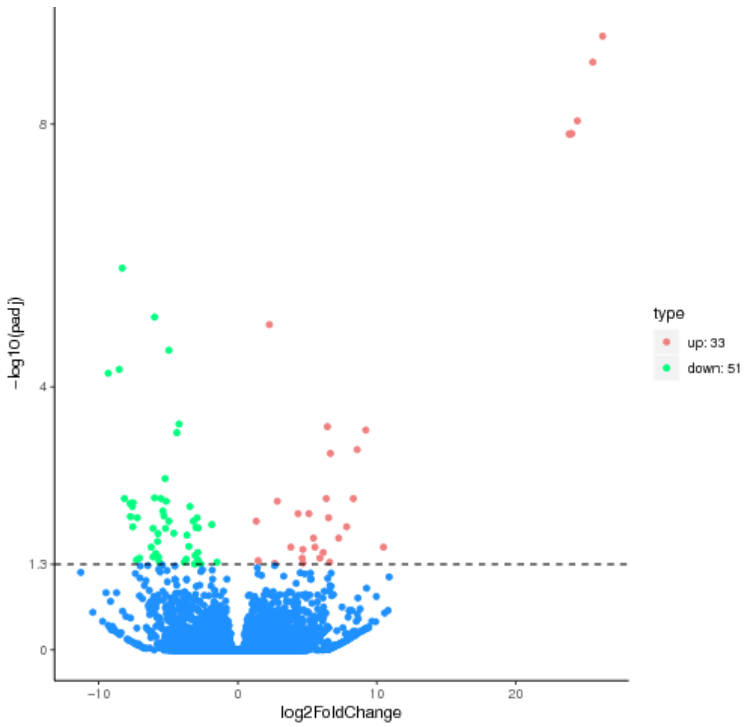


Figure 5.3.8.3.0) Volcano plot illustrating differential gene expression between control and bruised tissue in V2 roots. (*N*=4).

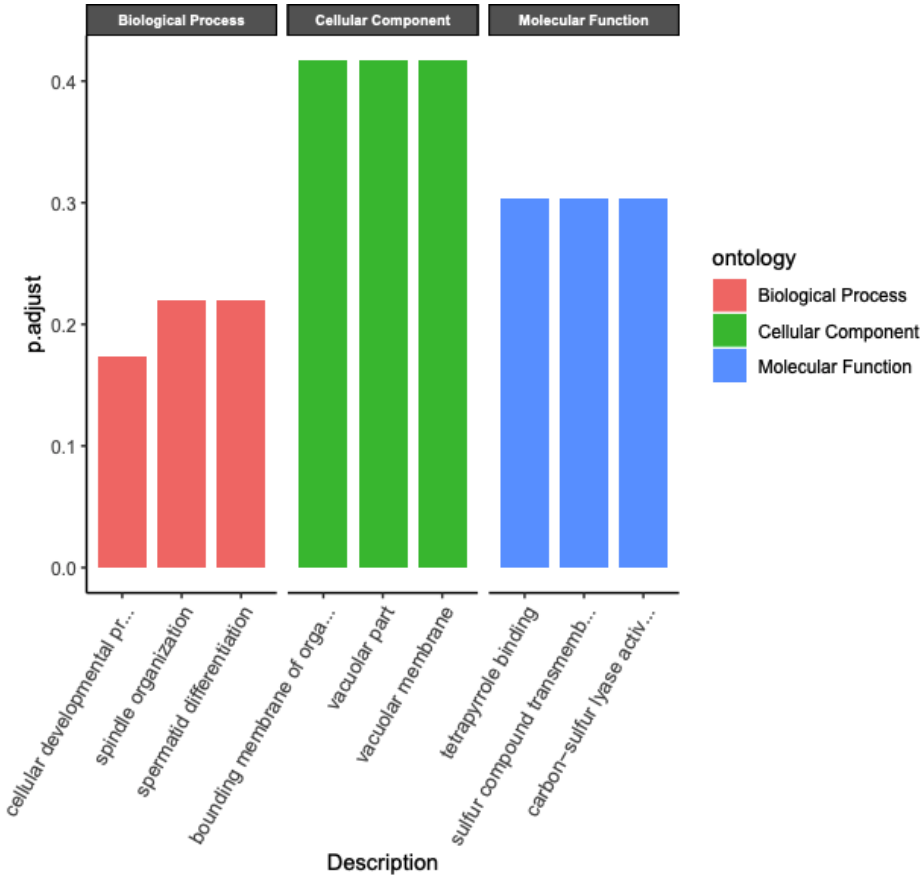


Figure 5.3.8.3.1) GO term annotation of DEG's between bruised V3 and control V3 tissue split by function (BP,CC or MF). (*N*=4).

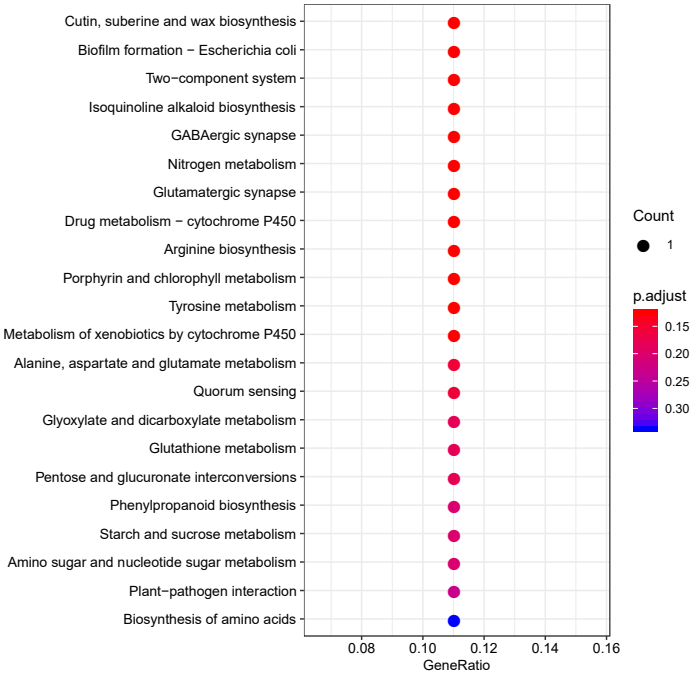


Figure 5.3.8.3.2) Significantly enriched KEGG pathways in bruised V3 tissue in comparison to control V3 tissue. (*N*=4).

Despite being the variety that displayed the greatest susceptibility to membrane leakage, greatest total oxidative potential and bruising susceptibility (severity and % bruising likelihood), we found no enriched GO terms or KEGG pathways that were significantly over or under expressed in V3 (*Padj*< 0.05), in contrast to the other two varieties, which also exhibited lower bruising susceptibility, total oxidative potential and tissue leakage during some harvest dates.

5.3.9 Identification of genes associated with the bruising response in parsnips

Following the significant enrichment of the phenylpropanoid metabolism pathway in bruised tissue in V1 and V2 (*Padj*= 2.42 x10^{-16} & *Padj*= 3.99 x10^{-20}, respectively) and enrichment of the phenylalanine, tyrosine and tryptophan biosynthesis pathway in V1 and V2 roots (*Padj*= 0.0001 & *Padj*= 0.0137 respectively)*;* differentially expressed genes associated with the biosynthesis and metabolism of PAL and closely associated genes are listed in Table 5.3.9.0. DEG's associated with PPO and tyrosine were identified and the log2FoldChange and *Padj* values across the three varieties are listed in Table 5.3.9.1. In addition, a number of DEG's associated with the production of POD enzymes that catalyse the oxidative polymerization of quinones are listed in Table 5.3.9.2.

V1 and V2 exhibited significant enrichment of the phenylpropanoid pathway where traditionally, enzymes such as PAL (phenylalanine ammonia lyase), 4CH (4-courmourate-CoA ligase), C4H (Trans-Cinnamate 4 monooxygenase), CCR (Cinnamoyl-CoA Reductase), F5H (Ferulate 5-hydroxylase) and CAD (Cinnamyl alcohol dehydrogenase) regulate the production of phenolic monolignol compounds (*p*-coumaryl, coniferyl and sinapyl). These monolignols when oxidized by PPO, or naturally, cause browning of the enzyme substrate (Zhang *et al.,* 2019). Further downstream, POD catalyses the oxidative polymerization of hydroxycinnamoyl alcohols to produce lignin (as part of the monolignol pathway), which is used to build bridges in cell walls and increase resistance to pathogens and repair wounded regions of tissue. Previously, increases in POD activity has been observed in correlation with

increased PAL and PPO activity as part of the plant defence response (Macheka *et al.*, 2013).

Identified DEGs involved with phenylpropanoid pathway				V1		V2		V3	
Gene name & UniGene ID	Gene length	Description	Homologous species	log2FoldChange	Padj	log2FoldChange	Padj	log2FoldChange	Padj
PAL 13487.0	1389	Phenylalanine ammonia-lyase	*P. taeda*	+2.882	5.69 $x10^{-5}$	NA	NA	NA	NA
PAL 13349.0	2993	Phenylalanine ammonia-lyase	*C sinensis*	+3.046	0.025	+7.001	0.002	NA	NA
PAL1 11454.22358	5497	Phenylalanine ammonia-lyase	*P. crispum*	+3.266	1.17 $x10^{-9}$	+4.434	0.009	NA	NA
PAL1 11454.21830	742	Phenylalanine ammonia-lyase	*P. crispum*	+2.887	0.008	+4.899	0.007	NA	NA
PAL4 11454.20394	1389	Phenylalanine ammonia lyase	*P. crispum*	+3.448	2.90 $x10^{-5}$	+4.233	0.006	NA	NA
PAL4 11454.21655	3348	Phenylalanine ammonia lyase	*D. carota*	+2.465	9.02 $x10^{-18}$	+3.032	4.15 $x10^{-5}$	NA	NA
C4H 11454.19871	3051	Trans-cinnamate 4-monooxygenase	*P. kitakamiensis*	+1.057	0.005	NA	NA	NA	NA
4CL1 11454.20081	3763	4-coumarate--CoA ligase	*P. crispum*	+3.261	1.82 $x10^{-10}$	+3.997	0.014	NA	NA
CCR1 11454.18141	1531	Cinnamoyl-CoA reductase 1	*A. thaliana*	+4.309	1.55 $x10^{-10}$	+3.941	0.0022	NA	NA
CCR2 13487.0	1389	Cinnamoyl-CoA reductase 2	*A. thaliana*	+2.882	5.69 $x10^{-5}$	NA	NA	NA	NA
CAD1 11454.20351	2135	Cinnamyl alcohol dehydrogenase	*A .cordata*	+1.095	0.00089	NA	NA	NA	NA

Table 5.3.9.0) Table detailing 11 key genes involved in the phenylpropanoid pathway. Values given for each UniGene (log2FoldChange and *Padj*) are direct comparisons of damaged and control tissue within varieties (e.g *V1:Br vs V1:Con)* thus facilitating comparison of expression levels for particular key genes across the 3 varieties following mechanical damage.

PAL catalyses the first step of phenylpropanoid biosynthesis converting the essential amino acid L-phenylalanine to trans-cinnamic acid (Peiser *et al.,* 1998), acting as a gatekeeper, regulating the production of phenolic compounds via the phenylpropanoid pathway (Figure 5.3.9.0). A total of 6 separate *PAL* genes were significantly upregulated following mechanical damage in V1, 5 were overexpressed in V2 whilst

V3 did not significantly differentially express any of the 6 *PAL* genes identified in parsnips.

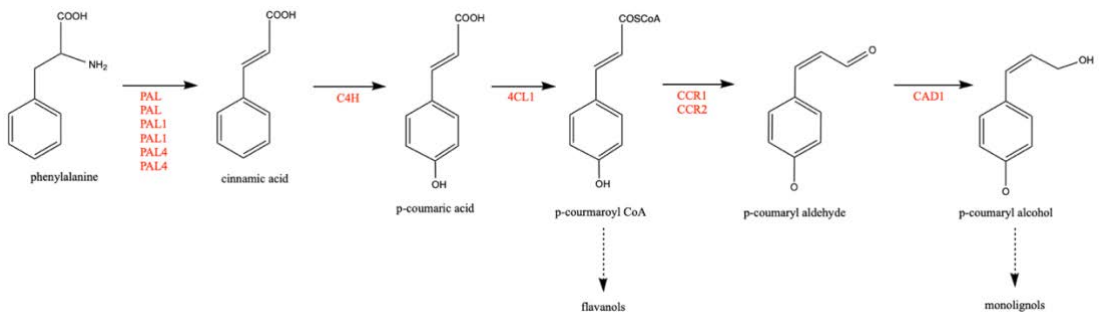


Figure 5.3.9.0) Proposed pathway for metabolism of phenylalanine in parsnip tissue following mechanical damage with specific differentially expressed genes (*Padj< 0.05)* identified as key components of enzymatic browning. A total of 6 versions of *PAL* (phenylalanine ammonia lyase) were overexpressed in parsnip tissue with Unigenes of *C4H*, *4C*L, 2 versions of *CCR* and a *CAD* gene also experiencing overexpression. Authors own diagram, created using ChemDraw 19.1.1 (PerkinElmer, 2020).

The analysis identified 2 versions of *PAL1* homologous with parsley located in distinct clusters with different gene lengths (5497 & 742 bp respectively), with overexpression observed in both V1 and V2. Furthermore, 2 versions of *PAL4* were observed to be overexpressed in bruised tissue with one being homologous with carrot and one with parsley and were both overexpressed by V1 and V2. Another version of *PAL* was observed to be homologous with *Camellia sinensis* (tea plant) and was differentially expressed in both V1 and V2, V2 demonstrated a greater log2FoldChange for the 5 homologous *PAL* genes compared to V1, however V1 overexpressed a version of *PAL*

(homologous with *Pinus taeda* (Loblolly pine)) which was not observed in the other two varieties.

In closely related carrot, a total of 3 *PAL* genes were identified by Han *et al.,* (2017), with 2 genes encoding for *C4H* and 4 genes encoding *4CL* acting in close co-ordination regulating the expression of the phenylpropanoid pathway. Following wounding damage to tissue, upregulation of the phenylalanine, tyrosine and tryptophan biosynthesis pathway and phenylpropanoid metabolism pathway were observed as carrot tissue switched from primary metabolism of sugars, to producing antioxidant phenolic compounds as part of the defence response (Han *et al*., 2017). Our analysis identified *PAL4* as homologous with carrot and was upregulated following wounding in 2 varieties of parsnip tissue (V1 & V2). *TYRDC3* was observed to be homologous with parsley and was significantly upregulated in all 3 varieties following mechanical damage. The accumulation of PAL in damaged regions of banana tissue was observed by Couture *et al.,* (1993) whilst Chen *et al.,* (2009) highly correlated mechanical injury in bananas with the accumulation of PAL and subsequent downstream phenolics. Wu *et al.,* (2020) observed significant accumulation of phenolics and PAL (and associated enzymes) in bruise susceptible eggplant tissue indicating that expression of the key *PAL* gene promoted phenolic synthesis in an attempt to increase antioxidant capacity and repair damaged tissue.

The only variety of parsnip to differentially express *C4H* was V1 which upregulated *C4H* with a log2FoldChange of +1.057 (*Padj*=0.005). No version of the *C4H* gene was observed to be upregulated in V2 and V3 following mechanical damage. It has

previously been demonstrated that *C4H* in closely related parsley, is encoded by a single gene (Koopman *et al.,* 1999); this gene was not identified in our analysis. The only *C4H* gene that was differentially expressed following damage was *CYP73A16*, homologous with *Populus kitakamiensis* (poplar).

Expression of *C4H* in closely related parsley *(*Koopman *et al.,* 1999*)* is normally regulated in close coordination with the *PAL* family of genes functioning as the metabolic link between *PAL* and *4CL* during the production of coumaric acid, which is then converted to phenolic compounds such as coumaryl alcohol via CCR and CAD. It is unclear as to the role of *C4H* in V2 and V3, as no differential expression of this key gene in either variety was found. Thus, the mechanism for biosynthesis of coumaric acid in these two varieties is unclear. However, the overexpression of *PAL* genes and *C4H* in V1 suggest a regulation of *C4H*, *4CL* and *PAL* in close co-ordination following mechanical damage, which concurs with the wounding response of fresh produce observed previously (Wu *et al.,* 2020)

Both V1 and V2 exhibited significant upregulation of *4CL1* following mechanical damage. 4CL is implicated in production of precursors for phenolic compounds such as *p*-coumaryl alcohol and coniferyl alcohol via monolignol biosynthesis (Wagner & Ralph, 2012) resulting in the biosynthesis of monolignols via CCR and CAD. Previously, gene suppression experiments in *Pinophyta* (conifers) found that reducing *4CL* activity significantly reduced the amount of lignin produced by up to 63% (Wagner & Ralph, 2012). Wagner *et al.,* (2009) demonstrated a 50% reduction in lignin content and dwarfed phenotypes in *Pinus radiata (*pine) plants that experienced suppression

of *4CL*. This large effect on monolignol production may suggest that manipulations at the entry level of the monolignol pathway (such as PAL, C4H & 4CL) restricts the production of phenolic compounds to a greater extent than manipulations made further downstream. The production of antioxidant phenolics that contribute to tissue browning in V1 and V2 appear to be regulated by PAL, C4H, 4Cl1, CCR and CAD in close co-ordination as a function of the plant defence response to wounding. Tissue browning in V3 seems to be produced by a different mechanism as these genes were not identified as overregulated in response to damage.

Tyrosine/dopa decarboxylase (*TYDC*) was significantly up regulated (*Padj= <0.05)* by all 3 varieties as a response to mechanical damage, with V1 experiencing the greatest log2FoldChange (+6.559) and V3 the lowest (+4.691). The log2FoldChange for V2 was +5.213. TYDC (*TYRDC-3)* represents one of the first steps in the biosynthesis of tetrahydroisoquinoline alkaloids (Facchini & Luca, 1995), such as tyramine (Figure 5.3.9.1). Borg-Olivier & Monties (1993) found that tyramine accounts for 23% of total phenylpropanoid metabolites in wounded potato tubers, where it was undetected in control tissue following RT-QPCR.

In contrast, this study detected *TYDC* reads in control tissue across all 3 varieties (readcount= V1:26.42, V2:89.47 and V3:60.59 respectively) indicating that TYDC may exist in healthy tissue, albeit at a low concentration in comparison to the significant overexpression witnessed following mechanical damage (readcount= V1:2556.79, V2:4196.04 and V3:1556.02).

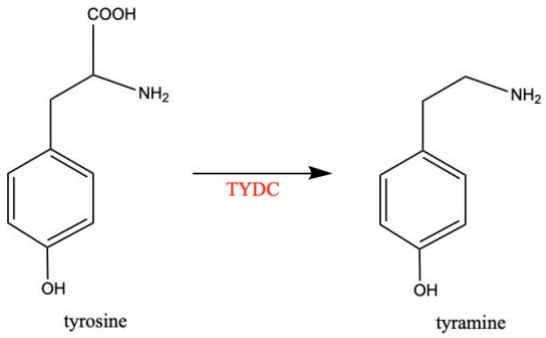


Figure 5.3.9.1) Proposed pathway for biosynthesis of tyramine, from tyrosine in 3 varieties of parsnip tissue via Tyrosine/dopa decarboxylase (TYRDC-3) following mechanical damage to tissue. Authors own diagram, created using ChemDraw 19.1.1 (PerkinElmer, 2020).

Such significant up-regulation indicates that parsnip roots respond to wounding in a similar manner to potato tubers in regards to TYDC, with large amounts accumulating in damaged regions of tissue facilitating discolouration of tissue via the oxidation of phenolic compounds. However, a number of studies have concluded that phenolic concentration (such as tyrosine, chlorogenic acid and caffeic acid) does not always correlate with blackspot bruise susceptibility (Dean *et al.,* 1993; Stevens & Davelaar, 1997). A number of studies report positive correlations between PPO activity and resistance to biotic stresses in tomatoes (Vanitha *et al.,* 2009), *Triticum aestivum* (wheat) (Mohammedi & Kazemi, 2002), and potatoes (Castanera *et al.,* 1996; Urbany *et al.,* 2012).

Previously it has been noted that PPO's from potato tubers have greater specificity against the di-phenolic compound chlorogenic acid, rather than the mono-phenolic

tyrosine. Concentration of tyrosine in bruised tissue has been correlated to browning severity in completely homogenised tissue (Dean *et al.,* 1993), however this correlation is not consistent when employing a bruise replication method such as the falling bolt method (Strehmel *et al.,* 2010). In contrast to the falling bolt method as was employed in this study, total homogenisation of potato tissue found that tyrosine, rather than phenylpropanoids, was the limiting factor in pigment formation and browning (Goyer & Pelle, 2018). This current study also found no significant differences in the inherent oxidative potential of parsnip tissue, but observed significant varietal differences in cell solute leakage with the most bruise susceptible variety (V3), eliciting the greatest leakage.

Identified DEGs involved with PPO production and tyrosine metabolism.				V1		V2		V3	
Gene name & UniGene ID	Gene length	Description	Homologous species	log2FoldChange	Padj	log2FoldChange	Padj	log2FoldChange	Padj
E1.10.3.1 11454.8238	2117	Polyphenol oxidase, chloroplastic	*M. domestica*	+4.618	0.0041	+5.209	0.00546	NA	NA
E1.10.3.1 11454.19188	2306	Polyphenol oxidase, chloroplastic	*M. domestica*	+5.053	3.74×10^{-5}	+5.532	2.49×10^{-5}	NA	NA
E1.10.3.1 11454.25393	2000	Polyphenol oxidase, chloroplastic	*M. domestica*	+3.197	0.0163	NA	NA	NA	NA
TYRDC-3 11454.20608	2257	Tyrosine decarboxylase	*P. crispum*	+6.559	2.26×10^{-31}	+5.213	4.97×10^{-5}	+4.691	0.0294

Table 5.3.9.1) Table displaying differentially expressed genes associated with PPO production and tyrosine metabolism across 3 varieties of parsnip tissue following mechanical damage.

Three chloroplastic PPO encoding genes were identified as overexpressed in bruised tissue in V1, 2 of which were also overexpressed in V2 and were all homologous with apple. V3 did not differentially express any PPO related genes despite suffering the greatest overall susceptibility to bruising. V2 experienced a greater log2FoldChange

for both homologous PPO encoding genes (+5.209, +5.503) in comparison to V1 (+4.618, +5.053 respectively).

Accumulation of PAL is often in tandem in PPO and POD as PAL activates the phelypropanoid pathway producing H_2O_2 and phenolic monolignols which are oxidised by PPO; which then may be oxidatively polymerizsed by POD and H_2O_2 forming lignins which contribute towards the browning of the enzyme substrate.

The analysis highlighted a total of 12 POD encoding genes that were significantly overexpressed in bruised tissue across the 3 varieties (Table 5.3.9.2). V1 and V2 each overexpressed 10 homologous POD genes, and each significantly upregulated a unique POD gene (PNC2 & E1.11.1.7 respectively). V3 only overexpressed 1 POD gene (PNC1) following mechanical damage (log2FoldChange= +5.551), which was homologous with Arabidopsis and was also overexpressed by V1 & V2 (log2FoldChange= +4.993 & +6.883 respectively). The significant upregulation of so many POD genes by V1 & V2 suggests that increased POD activity is a response to mechanical damage in parsnip tissue. The fact that V3 only significantly upregulated a single POD gene suggests varietal differences in the transcriptomic response to bruising in parsnip tissue.

Identified DEG's involved with POD production				V1		V2		V3	
Gene name & UniGene ID	**Gene length**	**Description**	**Homologous species**	**log2FoldChange**	**Padj**	**log2FoldChange**	**Padj**	**log2FoldChange**	**Padj**
poxN1 11454.21266	795	Peroxidase N1	*N. tabacum*	+2.941	0.000125	+3.917	0.000593	NA	NA
poxN1 11454.20343	1459	Peroxidase N1	*N. tabacum*	+3.553	2.46 $x10^{-5}$	+5.218	5.58 $x10^{-5}$	NA	NA
PER44 11454.31869	1245	Peroxidase 44	*A. thaliana*	+5.020	1.95 $x10^{-5}$	+6.207	0.00118	NA	NA
PER12 11454.21074	1290	Peroxidase 12	*A. thaliana*	+3.643	0.000454	+4.208	0.007	NA	NA
PER47 11454.21150	1291	Peroxidase 47	*N. sylvestris*	+6.516	3.45 $x10^{-5}$	+7.360	0.0055	NA	NA
PER73 11454.13794	1549	Peroxidase 73	*A. thaliana*	+4.568	0.0118	+3.962	0.000296	NA	NA
E1.11.1.7 11454.10300	1280	Lignin-forming anionic peroxidase	*N. sylvestris*	NA	NA	+6.642	1.71 $x10^{-6}$	NA	NA
PNC1 11454.21112	3725	Cationic peroxidase 1	*A. hypogaea*	+3.256	2.59 $x10^{-6}$	+6.784	1.37 $x10^{-6}$	NA	NA
PNC1 11454.19467	1565	Cationic peroxidase 1	*A. hypogaea*	+4.993	3.67 $x10^{-8}$	+6.8827	0.0000246	+5.551	0.0272
PNC1 11454.20712	2410	Cationic peroxidase 1	*A. hypogaea*	+1.955	0.0290	+4.168	0.00275	NA	NA
PNC2 11454.21720	751	Cationic peroxidase 2	*A. hypogaea*	+3.256	3.94 $x10^{-8}$	NA	NA	NA	NA
PNC2 11454.20556	1075	Cationic peroxidase 2	*A. hypogaea*	+3.185	7.86 $x10^{-6}$	+3.701	0.000335	NA	NA
At1g30760 11454.20421	UP	Berberine bridge enzyme-like 13	*A. thaliana*	+ 5.814	3.99 $x10^{-20}$	NA	NA	NA	NA
At1g30700 11454.3370	UP	Berberine bridge enzyme-like 8	*A. thaliana*	NA	NA	+7.288	0.00121	NA	NA
At1g30700 11454.30675	UP	Berberine bridge enzyme-like	*A. thaliana*	NA	NA	+6.084	0.000160	+6.452	0.000401

Table 5.3.9.2) Table showing 12 differentially expressed genes associated with peroxidase production (POD) across 3 varieties of parsnip. Included Is the log2FoldChange between bruised and control tissue for each variety and the accompanying *Padj* value.

Previously, root tissue browning in *Pachyrizus erosus* (Mexican turnip) was positively correlated with lignin content, POD and PPO activity as POD activity peaked after 6 days of storage (Aquino-Bolanos & Mercado-Sila, 2004). At a storage temperature of 20 °C a number of phenylpropanoids (for example; coumaric acid) were observed to

be good substrates for POD suggesting that lignification via POD holds an important role in tissue browning (Aquino-Bolanos & Mercado-Sila, 2004).

Due to the overregulation of the phenylalanine, tryptophan and tyrosine biosynthesis pathway by V1 and V2 (*Padj*= 0.0001 & *Padj*= 0.0137 respectively) a number of DEGs were identified as being involved in the shikimate pathway (Table 5.3.9.3), a pathway responsible for the metabolism of phenylalanine and tyrosine. V1 overregulated 6 genes previously identified as part of this pathway, V2 expressed 3 of the 6, whilst V3 did not differentially express any of the 6 genes. Previously, overexpression of this pathway has been linked to bruise formation (Han *et al.,* 2017, Wu *et al.,* 2020), as this pathway biosynthesises metabolites that are precursor molecules for the phenylpropanoid pathway.

Identified DEGs involved with phenylalanine, tyrosine and tryptophan biosynthesis					V1		V2		V3	
Gene name & UniGene ID	Regulation after damage	Gene length	Description	Homologous species	log2FoldChange	Padj	log2FoldChange	Padj	log2FoldChange	Padj
DAHP synthase 1 (SHKB) 11454.19796	UP	4105	3-deoxy-7-phosphoheptulonate synthase	*P. crispum*	+4.222	2.37 x10^{-22}	+4.696	0.0007	NA	NA
DAHP synthase 1 (SHKB) 11454.17888	UP	2039	3-deoxy-7-phosphoheptulonate synthase	*S. tubersom*	+1.690	0.0007	+2.623	0.0198	NA	NA
aroA 11454.18963	UP	2317	EPSP synthase (3-phosphoshikimate 1-carboxyvinyltransferase)	*P. hydriba*	+1.851	4.52 x10^{-9}	NA	NA	NA	NA
CM1 11454.25094	UP	3682	Chorismate mutase 1	*A. thaliana*	+1.313	5.42 x10^{-9}	NA	NA	NA	NA
CM1 11454.11374	UP	2392	Chorismate mutase 1	*A. thaliana*	+1.671	0.00843	NA	NA	NA	NA
CSE 11454.24114	UP	1514	Caffeoyl shikimate esterase	*A. thaliana*	+4.351	7.86 x10^{-6}	+6.415	5.61 x10^{-5}	NA	NA

Table 5.3.9.3) Table showing 6 differentially expressed genes associated with the phenylalanine, tryptophan and tyrosine biosynthesis pathway across 3 varieties of parsnip following mechanical damage.

5.4 Discussion

5.4.1 Physiological response to bruising damage

In order to study the physiological and transcriptional response to mechanical damage in parsnip tissue, roots of all 3 varieties from the second harvest underwent physiological assays, RNA extraction and sequencing.

Significant differences in bruise severity were observed across experimental groups (p=0.0354) following a Kruskal Wallis test, however no significant differences in bruising severity between varieties was observed during H2. V3 displayed the greatest bruising severity witnessed across the study (3.28 ± 0.66) during H2 and was the variety most likely to bruise (85 %). Whilst the severity of V3 bruises were not always significantly greater than the other two varieties, the frequency of bruised V3 roots was consistently higher throughout all harvests, compared to V1 and V2. Thus the overall bruising susceptibility of V3 to a single medium sized impact seems to be greater, and consequently differences during processing may be more pronounced following a greater number of impacts. Results from previous work (chapter 4) concurs with the assumption that V3 is a bruise susceptible variety, where the replicate number was higher than in this section of study, and significant varietal differences in bruising severity were also observed.

Previously, significant differences in browning potential have been observed between parsnip varieties; one of which is present in the current study (V1). V1 was described

by Toivonen, (1992) as a variety less susceptible to browning in comparison to other commercially available varieties in Canada. There has been considerably progression in the genetics of V1 since 1992, but this British study also found V1 to be a bruise resistant variety in comparison to V3.

The differences in browning potential observed between parsnip cultivars was hypothesized by Toivonen, (1992) to be due to variations in solute leakage, rather than enzyme and phenolic content. It was noted that the varietal differences in bruising susceptibility across varieties was due to differences in tissue response to damage, and it was found that calcium chloride dips effectively reduced parsnip browning, thought to be via an increase in membrane stability (Poovaiah *et al.,* 1998).

During the second harvest, fully homogenised *in vitro* V3 tissue exhibited an absorbance at 475nm of 0.191 ± 0.007 which was the lowest witnessed, whilst V1 displayed the greatest absorbance (0.195 ± 0.005). No significant difference between V1 and V3 was observed during H2 (p=0.719) or between V2 and V3 (p=0.935). Whilst variety (p=0.003) and harvest date (p=0.002) were found to be significant factors affecting total oxidative potential in parsnip tissue, no specific significant differences between varieties was observed. However significant differences in bruise severity and percentage likelihood to bruise was observed. Previous work in potatoes has suggested that total oxidative potential is not the determinant for bruising susceptibility (Cantos *et al.,* 2002; Scharf, 2014; Goyer & Pelle, 2018), rather other physiological factors such as solute leakage are more significant for bruise formation. The results from this current study in parsnips may suggest that the observed differences in

bruising susceptibility across varieties is not due to inherent differences in the oxidative potential of tissue. Concurring with previous research, bruising susceptibility *in vivo* tissue does not consistently correlate with results *in vitro*.

The % TSL of plant tissue is a measure of the membrane integrity and/or cell damage, achieved via assessing the amount of solute that exits the cell out over a period of time. Bruising susceptibility has previously been correlated to increased solute leakage in other crop species such as pomegranates (Hussein *et al.,* 2019), bananas (Maia *et al.,* 2011; Bugaud *et al.,* 2014) and tomatoes (Lee *et al.,* 2005). In the current study, during H2 bruised V3 tissue exhibited the greatest % TSL after 4H and 48H of incubation (25.75 & 44.23 %, respectively), the highest values witnessed across all harvest dates. The % TSL of V3:Br after 48H was significantly greater than V3:Con during the second harvest (p=0.006) indicating that mechanical impacts initiate increased solute leakage in V3 tissue, however this observation was not present in V1 or V2 tissue. V3 (control and bruised tissue) displayed greater solute leakage at 4H and 48H across all 3 harvest dates, and consistently displayed both a greater percentage likelihood to bruise and a slightly higher bruise severity than the other 2 varieties. This may indicate that bruise-susceptible parsnip tissue is due to increased solute leakage and not total oxidative potential, concurring with Toivonen, (1992).

It was found that V3 had a greater relative root water content % than that other 2 varieties across all 3 harvests, which concurs with results witnessed previously (chapter 4). During H2, V3 elicited the greatest % relative water content (81.85 ± 0.28) which was found to be significantly greater than that of V1 (p=0.022) but not V2

(p=0.104). The relative water content and resultant turgidity of plant cells are assumed to influence bruising susceptibility (Praeger *et al.,* 2009), with Laerke, (2002) reporting reduced bruising in potato tubers following a decline in turgor pressure and water content over storage. Parsnip roots are not stored for significant lengths of time, typically less than 48 hours, thus the relative water content is unlikely to change too drastically during processing.

Praeger *et al.,* (2009) found no correlation between bruising susceptibility and pressure potential in potato tubers, Corsini *et al.,* (1999) observed lower bruising in tubers harvested from dry fields compared to wetter fields. In avocados. Mazhar, *et al.,* (2018) observed that increasing dry matter content reduced bruising susceptibility as lower firmness was found to increase susceptibility to tissue bruising. In olives, Jiménez *et al.,* (2017) found that fully irrigated olive fruits are more susceptible to bruising as their relative water content was greater than droughted groups.

SEM micrographs have previously been used to analyse microstructural changes in tissue following mechanical impacts (Hussein *et al.,* 2019). Here, due to Covid-19 the number of micrographs produced was severely limited, thus no quantifications were made, just observations. But as no previous research has published SEM micrographs of parsnip tissue, the author of this study thought it important to include this incomplete section of work. The images of damaged tissue show cell rupture, loss of cell compartmentalisation and membrane damage all of which have been illustrated as key indicators of tissue damage, and subsequent enzymatic browning.

5.4.2 Transcriptional response to bruising damage

High throughput sequencing is a powerful technology that has previously been used to identify DEGs involved with the bruising response in crop species (Pertea *et al.,* 2015; Zhu *et al.,* 2017; Xu *et al.,* 2020), but no previous transcriptomic analysis in parsnip existed prior to this study. The construction of a *Denovo* transcriptome and analysis of differential gene expression in parsnip is completely novel, and aimed to investigate the underlying mechanism of tissue browning and bruising.

In this study, DEGs between control and bruised tissue were observed to be involved in oxidation and response to stress, and were enriched into KEGG pathways with phenylpropanoid metabolism being significantly upregulated in V1 (*Padj*= 2.42 $x10^{-16}$) and V2 (*Padj*= 3.99 $x10^{-20}$) tissue. V1 and V2 also significantly upregulated the phenylalanine, tyrosine and tryptophan biosynthesis pathway (*Padj*= 0.0001 & *Padj*= 0.0137 respectively) where precursor metabolites for enzymatic browning are produced. In closely related carrots, Tran *et al.,* (2017) observed that mechanical damage caused carrot tissue to divert energy from sugar metabolism into phenolic compound production. In other species this has been correlated with the plant defence response, as wounded tissue is repaired and antioxidants are produced.

Enzymes that oxidise phenolic compounds (such as PPO and POD) have previously been highlighted to work in co-ordination (Ali *et al.,* 2020), as inhibitive treatments repressed the activity of both enzymes, and subsequently browning. Previously, PPO and POD activity in potato tubers has been artificially induced via the employment of

a methyl jasmonate treatment (Zhou *et al.,* 2019) whilst Liu *et al.,* (2019) significantly retarded expression of genes involved in phenolic compound metabolism (for example, *PAL*), thus inhibiting PPO and POD activity. Gonzelez *et al.,* (2020) demonstrated significant reductions in PPO activity and subsequent tissue browning in potato tubers via the silencing of one *PPO* gene (*StPPO)*. In eggplant tissue, Wu *et al.,* (2020) described 4 *PPO* encoding genes and 2 *POD* encoding genes in bruise susceptible varieties and observed significant upregulation of phenolic compounds via the phenylpropanoid and shikimate pathways, as observed in V1 and V2 bruised parsnip tissue. In bruise susceptible eggplant varieties, overexpression of *PAL* and associated genes in the phenylpropanoid pathway resulted in increased accumulation of downstream phenolic compounds (Wu *et al.,* 2020).

In the current study, it was found that the two bruise resistant varieties (V1 & V2) overexpressed pathways associated with the production of phenolic substrates and PPO, PAL and POD enzymes. It was also observed that the bruise susceptible variety (V3) did not significantly differentially express any KEGG pathways or the majority of enzymatic browning genes (for example *PAL* or *PPO*) found in bruised tissue from the other two varieties. However, the total oxidative potential of tissue did not significantly differ across varieties during H2, thus the mechanism as to which V3 tissue discolours is not as clear as the other varieties. It should be noted however that V3 significantly upregulated *TYRDC-3* (log2FoldChange= +4.691) but to a less degree than the other varieties. Tyrosine has been observed as a significant substrate for PPO activity (Goyer & Pelle, 2018), thus overregulation of this gene by V3 indicates a transcriptional response to damage. Furthermore, cell properties and membrane

leakage indicate that V3 may be more physiologically susceptible to bruising, which may explain the observed differences of *in vivo* and *in vitro* results from this study in parsnips.

POD enzymes are key in a number of metabolic processes in plant cells such as oxidation of cinnamyl alcohols during lignification and forming bridges within cell walls (Aquino-Bolanos & Mercado-Sila, 2004). Aquino-Bolanos *et al.,* (2000) found that PPO activity did not account for total observed bruising of jicama tissue thus POD, in combination with PPO, account for the majority of enzymatic browning in plant tissue. The analysis highlighted a single *POD* gene in V3 tissue (*PNC1)* that was significantly differentiated following mechanical damage, this gene was also significantly upregulated by the other 2 varieties. Whilst V3 did not overexpress any of the other parsnip *POD* identified and described here, the overregulation of *PNC1* and *TYDC* indicates a response to mechanical damage, and may highlight the browning mechanism in V3 tissue. More work is required to quantify phenolic compound quantity and enzymatic activity in damaged tissue across parsnip varieties, significant upregulation of *TYDC* indicates that tyramine may be a significant substrate for enzymatic browning. Identification of other substrates that contribute towards bruising, such as chlorogenate acid, would provide a diagnostic marker to breeders to implement during selection.

The identification of damage inducible *PPO, PAL* and *POD* genes in parsnip tissue provides breeders and researchers with the opportunity to implement gene silencing studies to ascertain how the different *PPO* genes contribute to phenolic degradation.

There has been success in producing bruise resistant potato and apple cultivars via the silencing of key *PPO* genes (Waltz, 2015), with hopes of expanding this research into other crops. Following the identification of 3 *PPO* genes homologous with apple; parsnip may now be included as a crop with commercial hopes of implementing such techniques going forward in the future.

Chapter 6: General discussion

This study has used a novel combination of approaches to investigate the causes of bruising in parsnips. Broadly the main findings were that bruising occurs due to mechanical impacts with harvesting and processing machinery and induces the expression of damage inducible genes, there are also a number of pre and post-harvest factors that affect bruising susceptibility.

The aim of this project was to support parsnip producers and breeders by investigating the causes of post-harvest damage and to assess factors that exacerbate bruising susceptibility. Working with producers on site to identify destructive processes that cause damage and assess mitigation strategies was attempted, as reductions in impact forces has been achieved in other crops. Furthermore, field trials and laboratory assays to investigate how agronomic and biological factors affect bruising in parsnips were undertaken. The development of diagnostic tools that identify physiological traits that correlate to bruising susceptibility would aid breeders to select for bruise resistant lines during screening programmes. Finally, transcriptomic analysis of parsnip tissue was performed to identify differentially expressed genes associated with enzymatic tissue discolouration, in other plant species that suffer from excessive bruising damage.

6.1. Post-harvest factors affecting bruising susceptibility in parsnips

Following the development and employment of the falling bolt methodology to replicate industrial impacts, this study found that increasing the magnitude of impacts caused more severe bruising, and caused a greater frequency of bruised roots. Irrespective of storage temperature, impact magnitude and storage duration both significantly affected bruising severity. The most severe bruises across all magnitudes were observed following 72 hours of storage, however bruises were present after only 12 hours.

It would appear that there is a bruising threshold in parsnips that when exceeded, low storage temperatures can reduce the bruising severity witnessed, but not the % likelihood to bruise. However for impacts under that threshold, temperature can limit the % of roots that bruise, but not the severity of bruises that do form. Future work is recommended to study bruising responses in parsnip tissue over a greater range of impact magnitudes to fully represent industrial damage to roots. This study employed V2 roots, thus the bruising responses and thresholds witnessed are likely to vary across varieties. Significant differences in bruise severity and % likelihood to bruise were witnessed across varieties in chapter 4, and chapter 5, following a single falling bolt impact.

Since the inception of this study, producers have made logistical changes to their operation to facilitate faster processing of roots in an attempt to improve post-harvest quality, and have publicly identified it as an area for improvement. Frederick Hiam, Ltd

(@FrederickHiam) noted that they had managed to get roots topped, harvested, washed and packed into cold storage within an hour of leaving the field (Twitter, 06/08/2020). The results of this study suggest that such behaviour may limit bruising susceptibility and should be encouraged, however reducing the impact forces exerted onto roots during processing is also a key factor in mitigating post-harvest damage, and future studies should focus on reducing impact magnitudes.

Based on this data, industrial impacts that exceeded 15 g may cause bruising as severe as caused by extreme impacts in chapter 2; where an average of 95 % of roots bruised after a single impact. Whilst impacts that do not exceed 4.49 J still elicit bruising, they bruise less severely and at a lower frequency than those that exceed 4.49 J.

6.2. Harvesting and processing of parsnip roots in the UK

This study found that bruising accumulates throughout harvesting and post-harvest processing, whilst manual inspection effectively removed the majority of scuffed roots, sub peridermal bruising is often not visible, thus goes undetected. Parsnip roots exhibit a significant bias to bruise on their crown end, rather than the tail end, which is presumed to be a result of differences in surface area and weight dispersion down the root.

The novel employment of a pseudo-parsnip, capable of quantifying industrial impact forces has facilitated an industry wide study in post-harvest processing. The device

was able to identify significant differences in total impact force (g) exerted by processes across packhouse B, with polishing and grading being the two processes exerting the greatest mean total impact force (g). By analysing the distribution of the impacts exerted via splitting the impact magnitudes into 4 groups, 0 – 5 g, 5 – 10 g, 10 – 20 g and 20+ g, it was possible to analyse how many impacts of each size were inflicted during each process. Polisher 1, grader 1, polisher 2 and grader 2 all exerted a significant number of impacts that exceeded 10 g, which have previously been demonstrated to result in severe bruising in a significant number of roots (chapter 2).

A total of 5 of the 8 processes in packhouse B inflicted a peak impact magnitude that exceeded 15 g (categorized as the extreme impact magnitude (6.13 J)), the largest of which were inflicted by polishers. Harvesting, intake and loose packaging were the only processes whose peak impact magnitude did not exceed 15 g, the lowest peak magnitude observed across packhouse B was inflicted by bagged packaging. The number of larger impacts exerted by polishing and grading indicate that these processes may cause significant post-harvest damage to parsnips, and subsequently increase wastage.

After visiting a number of packhouses throughout mainland Britain and Northern Ireland, processes were compared against their industrial competitors to identify any significant differences in destructive potential. This section of the study analysed 7 harvesting methods, soil removal, 2 intake methods, 10 polishers, 7 graders and 2 packing methods to try and identify the least destructive working practises for each process across the industry. This data was fed back to participating producers via

individualised, anonymised reports, or at various national conferences such as the British Carrot Growers Association (BCGA). Analysis of the impact force (g), VC ($m.s^{-2}$), time (seconds), peak impact force (g) and the distribution of impacts based on size, highlighted a number of significant differences existing between the same processes from different producers. Future studies are recommended to maximise the number of industrial participants across a respective crop species; to gain a full representation of industrial impacts, and amplify the robustness of the research.

Polishing was identified as the process which exerted the greatest total impact force (g) and mean total VC ($m.s^{-2}$) onto parsnip roots, all polishers exerted a mean peak impact magnitude that exceeded 15 g. Across the 10 polishers significant differences in mean total impact force (g), mean total VC ($m.s^{-2}$), mean time (secs) and mean peak impact magnitude (g) were observed, indicating significant industrial variance in destructive potential. Reasons for this may include variance in polisher model employed, age of polishers, running settings and desired level of root polish. Vegetable root polishers are employed to improve the post-harvest quality of produce by cleaning, polishing and buffing roots; in parsnips this maximises the cream coloured appearance that is desired. The results of this study suggest however that polishing may also hinder the post-harvest quality of roots, via exerting significant impact forces, which result in bruises and exacerbated scuffs. The use of pseudo-produce whilst designing processing equipment has been used in potato's, when designing the next generation of parsnip polisher, manufacturers are recommended to take advantage of this proven technology, to reduce the bruising load exerted onto roots. Previous studies suggest that padding contact surfaces effectively reduces bruise occurrence, the uptake of

which in parsnip processing during harvesting, intake, grading and packing may reduce post-harvest damage.

Given that polishing was identified as the most destructive process, it was decided to revisit and reanalyse polisher G, and an average performing polisher (F) to assess mitigating strategies to try and reduce the destructive potential of processing. It was found that running polisher G on modified settings with the exit door down reduced the total impact force (g) experienced by 55 %, compared to the standard operating settings. Running Polisher G on this modified setting significantly reduced the frequency of impacts sized between 5 - 10 and 10 - 20 g exerted onto roots, in comparison to the standard operating settings. Applying the methodology employed here across all industrial processes in a greater number of packhouses is recommended for future study.

The employment of the electronic parsnip device has facilitated a novel, industry wide study analysing the mechanical post-harvest processing of parsnip roots. The quantification of impact forces exerted by processes has allowed the identification of destructive processes, compared them against their industrial competitors, and introduces an effective method of testing handling settings. This was done in an effort to reduce the forces parsnips experience during harvesting and processing.

6.3. Agronomic factors affecting bruising susceptibility in parsnips

To investigate whether harvest date, irrigation scheme and variety significantly affect bruising susceptibility, a novel study employing a poly-tunnel and mesocosm set up was employed, to facilitate measurement and control over the soil water content of the field trial. No significant difference in SWD was observed to a depth of 40 cm, or 70 cm at the experiment initiation, during H1, H2 or H3. It therefore appears as though the irrigation schemes (control, 1 week drought and 2 week drought) were not sufficient to elicit a significant effect on SWD. Furthermore, no significant differences in SWD were found between varieties, indicating that cultivars did not exhibit differential water usage. Despite this, V3 was found to possess a greater relative root water content % than V1 and V2 across all 3 harvest dates, irrigation scheme was not observed to significantly affect relative root water content % in V1 or V2 roots. The evidence of effect of relative water content, or conversely dry matter content, on bruising in fresh produce is mixed, with some crop species experiencing greater susceptibility, whilst others greater resistance to bruising following changes in relative water content. Further work is needed to investigate whether irrigation can impact bruising susceptibility in parsnip roots and to elucidate why relative water content varies across harvest dates.

V3 was observed to be overall a more bruise susceptible variety compared to V1 and V2 across the 3 harvests. Whilst the bruising severity of V3 roots was found to be greater than V1 and V2 during all harvests, the difference was not always significant. However, V3 roots also bruised at a greater frequency than the other varieties in the

majority of cases following a single impact, thus this difference in susceptibility is likely to be significantly amplified if variety testing was to occur in a packhouse, where a significant number of large impacts occur (chapter 3). The results of this study concur with the industry reported varietal differences in susceptibility, where V1 and V2 are viewed as more bruise resistance, whilst V3 is more bruise susceptible (personal communications with industrial participants during packhouse visits).

6.4. Physical and transcriptional changes in parsnip tissue

This section of study was designed to investigate physiological characteristics, such as solute leakage %, to ascertain what are the determinant factors in bruising susceptibility in parsnips. Bruised and control tissue from roots during the second harvest were removed for RNA sequencing and analysis, which involved the novel *denovo* construction of a parsnip transcriptome.

Following bruise replication and analysis, the results of this chapter classified V3 as a bruise susceptible variety, whilst V1 and V2 were described as more bruise resistant varieties. V3 roots bruised at the greatest frequency, and the bruises that formed were more severe than those found in V1 and V2 tissue, however these differences in severity were not consistently significant. It should be noted that bruising susceptibility was ascertained following a single falling bolt impact of 4.49 J, therefore differential responses are likely to be amplified in industry as roots suffer from a large number of impacts that exceed 4.49 J (chapter 3).

Whilst the oxidative potential of homogenised tissue did not significantly differ across varieties, the % tissue solute leakage did, concurring with previous research where it has been observed that *in vivo* bruising doesn't correlate with *in vitro* browning. The inherent discolouration potential of parsnip tissue does not seem to vary across varieties, but the bruising response does. This may indicate that cell physics limit total bruising, thus are responsible for varietal differences in bruising susceptibility. V3 exhibited the greatest % TSL after 4, and 48 hours of incubation across all 3 harvests, and was the only variety where bruised tissue exhibited significantly greater % TSL than control tissue after 48 hours of incubation. Mechanical impacts did not initiate significantly greater % TSL in bruised tissue compared to control tissue in V1 and V2, whereas in the bruise susceptible variety this was the case.

In addition, V3 exhibited a greater relative root water content % than the other two varieties across all 3 harvest dates, concurring with results witnessed in chapter 4. Differences in bruising severity and % likelihood found between varieties is likely to be due to differences in cell solute leakage, cell wall characteristics, turgidity and relative water content, rather than differences in the inherent oxidative potential of tissue. Bruising susceptibility in parsnips is therefore hypothesized to be determined not by phenolic and enzymatic activity, but rather solute leakage, concurring with previous research (Toivonen, 1992). However, employing methodologies (such as gene silencing) to limit the discolouration of parsnip tissue may limit the inherent discoloration potential, thus contributing to the production of more bruise resistant lines.

The production of SEM micrographs illustrates microstructural changes to parsnip parenchyma cells in response to mechanical impacts as has been witnessed in other crops. Mechanical impacts initiated cell rupture, loss of compartmentalisation and membrane leakage in parenchyma tissue, releasing the contents of the vacuole into the intracellular space. The bruise susceptible parsnip variety (V3), was the only variety where mechanical impacts initiated significant changes in tissue integrity, defined as % TSL, compared to control tissue.

Differential gene analysis highlighted a number of differentially expressed genes associated with the bruising response in other crop species, involved in phenylpropanoid biosynthesis and the production of lignins. There was differential expression of these genes witnessed between varieties, as V1 and V2 expressed a similar number of DEG's associated with tissue browning, whilst V3 only differentially expressed 2 genes, identified as encoding important enzymes in other crop species.

A total of 6 *PAL* genes were found across V1, 5 were upregulated by V2 whilst V3 upregulated none. A number of downstream genes associated with *PAL* such as *C4H, 4Cl1, CCR* and *CAD* were also observed to be upregulated in V1 and V2 bruised tissue. This study found that all 3 varieties upregulated *TYDC* as a response to mechanical impacts, suggesting that tyrosine is an important substrate for enzymatic browning in parsnip tissue. A total of 3 *PPO* genes were identified as upregulated in V1 bruised tissue, 2 were homologous with V2 whilst V3 did not differentially express any *PPO* genes.

The significant upregulation of the phenylalanine, tyrosine and tryptophan biosynthesis, and the phenylpropanoid metabolism pathways by parsnip tissue as a response to damage, concurs with research conducted in closely related crop species. Thus, a molecular mechanism for parsnip tissue browning is suggested via PPO, POD, TYDC and PAL following cell rupture and membrane leakage. Investigating and quantifying enzymatic activity in bruised parsnip is a natural next stage of study, furthermore studying phenolic content may highlight reasons for the differential molecular responses witnessed between varieties here.

The identification of candidate genes involved in parsnip bruising offers novel information to breeders so they can screen lines for bruise resistant individuals, and improve parent lines. The advent of next generation technologies, such as CRISPR, have been employed in other crop species to produce bruise resistant varieties, the identification of candidate genes in parsnip provides valuable information for future studies.

The United Kingdom's self-sufficiency of fresh vegetables has fallen by 16% over the last 2 decades. In 2019, the UK only produced 55% of her total fresh vegetable consumption; relying on imports to fill the gap of 45% between demand and supply. Coupled with a rapidly growing population to feed, it is clear that agricultural capacity in the UK must increase in the future as producers, breeders and agronomists strive to maximise yields and efficiencies. In parsnips, the currently observed pack out losses of over 50% are a barrier for increasing production and quality, with bruising currently contributing approximately 20% towards losses.

6.5. Conclusions

Mechanical impacts during harvesting and post-harvest processing cause bruising damage in parsnip roots. Quantification of impact forces via the electronic parsnip facilitated the identification of destructive processes, comparison of processes across industry, and testing of modifications to find the least destructive working practises to improve post-harvest quality. The agronomic or pre-harvest factors that significantly affected bruising were harvest date and variety, whilst impact force, storage duration and storage temperature were post-harvest factors that significantly affected bruising. Physiological differences in relative water content % and % TSL were observed between bruise susceptible and bruise resistant varieties. All 3 varieties exhibited a transcriptional response to mechanical damage, however the bruise susceptible response was muted in comparison to the bruise resistant varieties. A number of genes associated with PPO, PAL, TYDC and POD production were identified in bruised parsnip tissue, highlighting the molecular mechanism of parsnip bruising following mechanical impacts.

6.6 Recommendations for growers.

- The falling bolt methodology employed in this study is a low cost, robust and portable method of replicating industry relevant bruises onto parsnip roots. Using this method in situ is a suitable method of screening and assessing bruising susceptibility for a specific growing operation.
- As impact magnitude increases, the bruising susceptibility of parsnip roots also increases, therefore reducing the number and size of impacts exerted onto roots should be a priority.
- The electronic parsnip is a suitable method of assessing impact forces and identifying destructive processes across a grower's operation. In addition, the electronic parsnip was capable of testing modifications to equipment, designed to reduce impact forces (g).
- As time since impact increases, bruising severity also increases, therefore producers should aim to harvest and process as quickly as possible and get roots into temperature controlled storage.
- Low storage temperatures reduced the severity of bruises, but not the % likelihood of a root to bruise. Therefore, reducing impact forces, processing roots quickly and storing roots at low temperatures must be done in combination to maximise post-harvest quality and reduce losses.
- Significant impact forces were observed throughout all processing operations, therefore a systematic effort across all processing stages is recommended as bruises accumulate throughout processing.
- Polishers and graders were the most destructive processes so initial focus should be placed on reducing the forces experienced by roots during these

processes. Modifications to running settings (e.g barrel speed) are low cost, effective methods of reducing post-harvest damage to roots, and can be quantified with impact force loggers.

- Reducing the amount of time that roots spend in each process (e.g polishing) reduces the number of impacts exerted onto roots, therefore reduces the likelihood of bruise formation.
- Reducing the drop height roots experience across all processing stages would reduce the impact force exerted, and thus reduce bruise severity.
- Running polishers with the exit door open increases the flow rate of roots, reducing the amount of time roots spend being polished. This reduces the number of impacts being exerted onto roots and therefore the likelihood of severe bruising damage.
- Harvesting roots after a period of heavy rainfall should be avoided as less control over harvesting equipment is available, which may increase the impact forces exerted onto roots.
- There are varietal differences in bruising susceptibility in parsnips, therefore when selecting cultivars this should be taken into consideration. Employing the falling bolt methodology in situ would be suitable during screening trials to select for bruise resistant cultivars.
- Harvest date significantly affects bruising in parsnips, with later harvested roots (November onwards) displaying a greater susceptibility to bruising. This should be considered when planning harvesting schedules, and extra care should be taken when harvesting and processing later crops.

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