# Post-harvest temperature and water status influence post-harvest splitting susceptibility in summer radish (Raphanus sativus L.)

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# Postharvest temperature and water status influence postharvest splitting susceptibility in summer radish (*Raphanus sativus* L.)

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# Abstract

BACKGROUND: Splitting is a problem that seriously affects appearance and marketability in a number of fruit and vegetables. In summer radish (*Raphanus sativus* L.), splitting can occur during growth, harvesting and postharvest. We investigated the factors affecting splitting susceptibility in summer radish cv. Celesta during postharvest handling.

RESULTS: Splitting susceptibility was negatively related to temperature, with higher temperature reducing splitting due to dropping impact. Radish diameter was positively associated with compression failure force, suggesting that larger radishes are more resistant to compressive splitting. An increase in radish hypocotyl water content (WC) was associated with an increase in splitting susceptibility due to impact and decrease in failure force for both compression and puncture forces. Increased hypocotyl WC may increase splitting susceptibility by increasing the water potential of the radish tissue. In agreement, we found that increased hypocotyl WC was associated with higher internal water potential in radish tissue.

CONCLUSIONS: We therefore recommend that the hypocotyl WC of summer radish crops be managed during the harvest and postharvest phases, and that crops are processed at higher, ambient, temperature in order to reduce splitting, before storing at low temperature and high humidity to maintain quality and shelf life.

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Keywords: radish; Raphanus; splitting; temperature; water status; texture analysis; hypocotyl

# INTRODUCTION

Splitting in fruit and vegetables reduces marketability and exposes internal tissue to the external environment and potential pathogens.<sup>1</sup> Splitting occurs when mechanical stress exceeds the ability of the tissue to withstand it,<sup>2</sup> with certain areas of tissue more prone to splitting than others.<sup>3-5</sup> In radish (*Raphanus sativus* L.), severe splitting, leading to deep cracks in the hypocotyl tissue, could result from excessive tensile stress from the integration of all cell turgor or from the exterior pressure and may occur via cell rupture.<sup>6</sup> Radish splitting has been suggested to be due to internal tissues expanding more rapidly than the periderm.<sup>7</sup> Radish growers can experience splitting rates of up to 30%, yet supermarket tolerance to splitting in radishes is typically less than 10%. Excessive splitting not only reduces the marketable yield but also results in batches of radishes requiring time consuming and costly hand processing in order to remove split produce.

Although the entire radish plant is edible, typically radish is grown for the swollen hypocotyl and tap-root. The upper section of the fleshy part of the radish is usually devoid of lateral roots and consists of a swollen hypocotyl and the lower section may be formed of an enlarged taproot and have lateral roots.<sup>8</sup> For ease of reference, the swollen hypocotyl and taproot

will be referred to as the hypocotyl in this article. Vegetable radishes are divided into two distinct types, small summer radishes and large winter radishes. The smaller quick growing summer radishes are popular as salad vegetables in Europe and North America whereas, the larger winter radishes have particular prevalence in Asia.<sup>8-9</sup> Summer radishes are annuals and have a rapid growth cycle, typically taking 3–6 weeks from planting to harvest. Currently, little is known about the environmental and physiological causes of splitting, particularly in summer varieties of radish.

Splitting in radishes and other crops can occur during growth, during harvesting procedures and postharvest. Splitting during growth in a range of crops has been shown to be dependent on both growth stage<sup>1,10,11</sup> and time of day,<sup>1,12</sup> and may reflect changes in growth rate<sup>1</sup> or water status.<sup>12</sup> Water is thought to

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be one of the principal factors affecting growth splitting with relationships found in a number of crops.<sup>12–16</sup> Weather conditions during growth may affect splitting susceptibility by changing turgor pressure within the radish hypocotyl. In winter varieties of radishes, frequent irrigation and fluctuations in soil water potential during growth have been shown to increase splitting.<sup>7,17</sup>

The principle postharvest changes to the radish crop which are thought to affect susceptibility to splitting as a result of mechanical damage are changes in tissue water status and temperature. Post-harvest tissue water status is thought to affect tissue mechanics and splitting susceptibility through turgor pressure. At high turgidity, plant cell walls are believed to be stretched and as a consequence be more easily ruptured.<sup>18</sup> Increased tissue water status has been associated with reduced failure force and higher incidence of harvest splitting in carrot (*Daucus sativus*)<sup>1,3,18,19</sup> and potato (*Solanum tuberosum*).<sup>5,20</sup> However, increased water potential has also been associated with an increase in cut force in carrot and radish.<sup>19</sup> This discrepancy may be due to differences in the type of tissue failure induced by different testing methods.<sup>19</sup>

Low temperature may also increase postharvest splitting by increasing turgor pressure within the hypocotyl. The cytoplasm and cell wall may contract to a greater extent than the vacuole at low temperatures, causing an increase in turgor pressure.<sup>18</sup> Decreased temperature has been associated with reduced tissue toughness and increased splitting in potato,<sup>20</sup> increased stress in carrot<sup>18</sup> and increased firmness in several fruit and vegetable species.<sup>21</sup> In radish, a decrease in temperature from 20 to 10 °C led to an increase in stiffness, reflected by an increased elastic modulus.<sup>22</sup> Other results found the force required to cut carrots was negatively correlated with temperature.<sup>23</sup>

Radishes may be processed in a temperature and humiditycontrolled environment, therefore if temperature or water status has a significant effect on splitting there is the potential to reduce the prevalence of postharvest splitting by changing the temperature and/or humidity of the conditions.

We investigated the relationship between temperature, water status and splitting under controlled conditions in the laboratory. Experiment 1 investigated the effect of temperature and hypocotyl water content (WC) on radish splitting susceptibility as a result of impact from dropping. This approach was chosen due to its relevance to commercial postharvest practices whereby radishes are dropped from heights up to 1.4 m into a metal trailer and then after initial grading are dropped again into hard plastic harvest bins. Experiment 2 further investigated the relationship between hypocotyl WC and splitting through compression and puncture analysis of tissue strength. This method was selected due to the compressive forces experienced by radishes in harvest bins and puncture risk due to harvesting processes. The effect of hypocotyl diameter on tissue strength under compression was also determined for comparison. Experiment 3 investigated the underlying basis for the relationship between hypocotyl WC and tissue strength by determining the effect of WC on internal water potential of the hypocotyl tissue. We hypothesized that both temperature and hypocotyl WC would influence the susceptibility of summer radish to splitting when subjected to impact from dropping, compression and puncture. We also hypothesized that WC would influence internal water potential of hypocotyl tissue.

# EXPERIMENTAL

#### Plant material

Commercially-harvested radishes (*Raphanus sativus* L. cv. Celesta) from Norfolk, UK were couriered on the day of harvest to arrive at

Harper Adams University (HAU), Shropshire, UK the following day. All radishes used were grown under the same agronomic conditions at the same location. The crops were sequentially planted and harvested following commercial practice. Crop growth occurred over July–August and the batches were not exposed to significant variation in temperature or rainfall. The radishes had been topped in the field and harvested into a trailer as per-usual commercial harvesting procedure but had not been washed, graded or trimmed. For transport the radishes were placed into a clear storage bag, which was tied at the top then placed inside a 305 mm  $\times$  230 mm  $\times$  230 mm double wall cardboard removal box which was taped closed. Upon arrival, any split radishes were removed and the remaining radishes washed in distilled water (dH<sub>2</sub>O) and any residual leaf stalks and roots were trimmed to ensure uniformity.

# Impact assessment

#### Temperature

Radishes were stored overnight at 3 °C and 98% relative humidity in a controlled environment chamber. The radishes were placed into individual G3 grip seal bags measuring 75 mm  $\times$  80 mm (Weller Packaging, Lichfield, UK) to prevent contact with the water medium. The bags of radishes were incubated in water baths for 2 h until the required temperature was reached (5, 10, 20, 30 and 40 °C). Twenty radishes were used at each temperature plus an additional three radishes for temperature checking by insertion of a temperature probe into the radish hypocotyl. Impact tests were performed by dropping the radishes down a 1.4 m pipe onto a metal plate and splitting (Fig. 1) recorded as presence of a visual split in the periderm. This height was chosen as it represents the upper limit of drop height into a trailer during commercial harvesting. To keep the radishes at the correct temperature, they were individually removed from the water immediately prior to impact testing.

# Water content (WC)

Radish hypocotyls were placed in groups of three into plastic pots (one experimental unit). Half of the pots were filled with



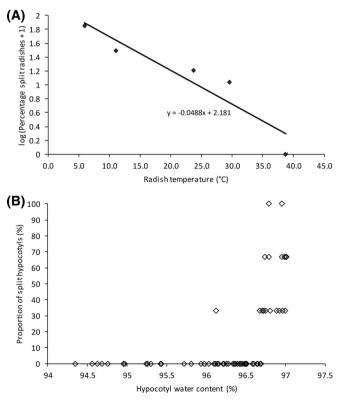
Figure 1. Appearance of split summer radish after impact from dropping.

approximately 100 mL of dH<sub>2</sub>O in order to submerge the radishes and saturate the hypocotyls. The other half were not, in order to obtain air-dried hypocotyls. The pots were placed into a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd, Osaka, Japan) at 90% relative humidity and 4 °C. Radishes were removed from the chamber every 2 to 3 days over the following week and impact tests performed immediately as earlier. After impact assessment the radishes were dried to a constant weight at 105 °C in order to calculate WC.

### **Texture assessment**

### Water content (WC)

In order to create a range of hypocotyl WCs, radishes underwent one of three 1-day treatments upon arrival at HAU: (i) saturation in dH<sub>2</sub>O; (ii) placement in a closed container; and (iii) placement in an open container. All radishes were tested 2 days postharvest. Twenty radishes from each group were used for each type of texture analysis, giving a total of 60 radishes for puncture analysis and 60 for compression analysis [of which 45 were used for texture analysis and 15 were used to determine hypocotyl WC (later)]. Radishes were puncture tested using a TA.HD.plus texture analyser (Stable Micro Systems, Godalming, UK). The texture analyser was fitted with a 2 mm diameter P/2 cylindrical probe in order to penetrate the hypocotyl tissue, the test distance was 16 mm and the test speed was 2 mm S<sup>-1</sup>. Uniaxial compression tests were performed using a 75 mm diameter P/75 compression platen probe fitted to a TA.HD.plus texture analyser. The test speed was 2 mm S<sup>-1</sup> and the test distance was 25 mm. In both



**Figure 2.** The effect of temperature and hypocotyl water content on radish splitting as a result of impact from dropping. (A) Percentage of radishes which split as a result of impact at different temperatures (n = 20). (B) Percentage of split radish hypocotyls in a sample of three which split as a result of dropping down a 1.4 m tube onto an aluminium plate at different hypocotyl water contents (n = 70). cases, probes contacted the hypocotyls at the equator. For puncture analysis, during the experiment a curve was plotted of the force (in kilograms) as a factor of distance (in millimetres). The point at which the periderm of the radish was punctured could be observed on the plotted curve as abrupt decrease in force. For the compression analysis, as with the puncture tests, a curve was again plotted of force (in kilograms) as a factor of distance (in millimetres), as the compression distance increased peaks were observed in the graph profile. Each peak indicated a compression failure in the radish. For the purposes of this experiment the force of the first peak was recorded as the split force.

To measure hypocotyl WC, radishes were weighed [fresh weight (FW)], cut into eight segments and dried in an oven at 65 °C for a minimum of 48 h, until they had reached a constant weight and were then reweighed [dry weight (DW)]. WC was calculated as WC = 100 - (DW/FW).

# Internal water potential

The hypocotyl water potential ( $\Psi_{\rm H'}$  in megapascals) of 20 radish hypocotyls was measured using a digital pressure bomb (SKPM-1400, Skye Instruments Ltd, Llandrindod Wells, UK). The hypocotyl of the radish was placed inside the chamber with the severed petiole protruding, the hypocotyl was fixed to the opening to prevent air loss and the pressure in the chamber increased until internal water was seen to accumulate on the petiole scar.

#### **Statistical analysis**

Data were analysed using linear regression and critical values of Pearson's correlation coefficient. The effect of WC on splitting susceptibility in Experiment 1 was analysed using a logit transformed regression with a binomial distribution. Data were analysed using Genstat 17th Edition (VSN International, Hemel Hempstead, UK).

# **RESULTS AND DISCUSSION**

# Experiment 1 – impact assessment

# Temperature

The five different water baths set to 5, 10, 20, 30 and 40 °C resulted in radishes with mean temperatures of 6.0, 11.0, 23.7, 29.6 and 38.7 °C, respectively. Radish splitting susceptibility as a result of impact was found to be negatively correlated with temperature (r = -0.877, P < 0.001; Fig. 2(A)). The greatest amount of splitting, 70%, was observed at the lowest temperature, 6.0 ° C, and the least amount of splitting, 0%, was observed at the highest temperature, 38.7 °C. The variance in splitting susceptibility accounted for by temperature was 76.9%. These results suggest radishes are more susceptible to splitting due to impact at lower temperatures.

These results are in keeping with a number of previous studies into the effects of temperature on failure force. Decreased temperature is associated with increased firmness in a range of fruits and vegetables<sup>21</sup> and results in significantly decreased compression failure strain and elastic modulus, thought to be associated with increased turgor.<sup>18,20</sup> However, temperature has been found to be negatively correlated with cutting failure force in carrot, with the highest forces being required to cut carrots at 5 °C, and have no effect on cutting failure force in radish.<sup>23</sup>

By affecting the modulus of elasticity, temperature appears to affect the stress but not the strain within the tissue.<sup>18</sup> It is thought the cytoplasm and cell wall may contract to a greater extent than the vacuole at low temperatures causing an increase in turgor pressure and damageability.<sup>18</sup> It is suggested that the effect of

temperature on cutting failure force in carrot, however, is related to tissue properties other than turgor, such as changes in the cell wall.<sup>23</sup> This may be due to the ability of carrots to undergo cold acclimation, changing cell wall properties and tissue stiffness within hours of transfer to cold conditions.<sup>23</sup> These results may also be due to differences in the method of texture analysis employed in the different studies.

These results suggest it may be preferable to harvest, and process radishes at warmer temperatures, such as during the middle of the day, in order to reduce splitting susceptibility and then to chill them after handling to limit respiration and maintain shelf life.

# Water content (WC)

As hypocotyl WC increased, there was a significant (P < 0.001) increase in the number of radishes which split as a result of impact (Fig. 2(B)) and there appeared to be a threshold of hypocotyl WC of 96.5% above which splitting as a result of dropping increased dramatically. Below a hypocotyl WC of 96.5%, the mean percentage of split radishes per pot was 0.8% (n = 42), however, above a mean hypocotyl WC of 96.5%, this increased to 38.1% (n = 28). Further investigation of the data using a logit transformed regression with a binomial distribution provided a model accounting for 66.7% of the variation. The model further suggests that a small increase in WC at around 96.5% results in a rapid increase in splitting, with predicted WCs of 96.25%, 96.49%, 96.59% and 96.9%, for 1%, 5%, 10% and 50% splitting rates, respectively. There would therefore appear to be a level of hypocotyl WC (in this case 96.5%) above which the level of stress on the cell walls is sufficient to cause tissue failure and therefore the likelihood of splitting as a result of an impact event increases suddenly and dramatically. The data suggests that only a very small increase in hypocotyl WC is needed to cause a significant increase in splitting from impact.

We next chose to investigate the effect of WC on hypocotyl texture in more detail.

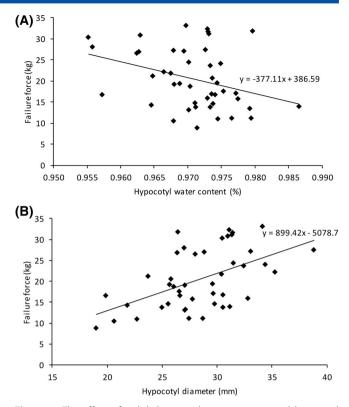
#### Experiment 2 – texture assessment

#### Compression

A range of hypocotyl WCs between a maximum of 98.7% and a minimum of 95.5% was observed at the time of texture analysis. There was a negative correlation between compression failure force and hypocotyl WC (r = -0.33, P < 0.05, 11.3% of the variance being accounted for) indicating that failure force decreased as WC increased (Fig. 3(A)). Consequently, radish hypocotyls are more susceptible to splitting at higher hypocotyl WCs, perhaps due to higher cell wall stress. There was also a positive correlation between compression failure force and hypocotyl diameter (r = 0.527, P < 0.001, 27.8%) of the variance being accounted for) suggesting larger radishes are more resistant to damage from compression (Fig. 3(B)). This may be due to the compressive stress being dissipated to more material when the hypocotyl is larger. When both diameter and hypocotyl WC were included in a multiple linear regression with failure force there was a significant relationship (P < 0.001) and 66.1% of the variation in failure force was accounted for.

#### Puncture

A range of hypocotyl WCs between a maximum of 97.4% and a minimum of 95.6% was observed at the time of texture analysis. There was a negative linear correlation (r = -0.68, P < 0.001) between WC and puncture force with 46.2% of the variance being



**Figure 3.** The effect of radish hypocotyl water content and hypocotyl diameter on failure force due to compression. (A) The force required to compress the hypocotyl of radishes at different hypocotyl water contents (n = 45). (B) The force required to compress the hypocotyl of radishes at different hypocotyl diameters (n = 45).

accounted for (Fig. 4), suggesting radishes are also less resistant to splitting as a result of puncture at higher WCs.

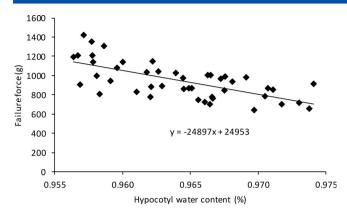
Higher hypocotyl WC may increase radish splitting tendency via an effect on internal water potential. We therefore next investigated the relationship between hypocotyl WC and internal water potential.

#### Experiment 3 - internal water potential

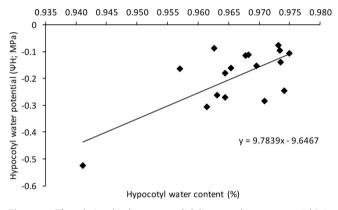
A significant (r = 0.71, P < 0.001) positive linear relationship was observed between hypocotyl WC and hypocotyl water potential, with 51.0% of the variance in hypocotyl water pressure being accounted for by hypocotyl WC (Fig. 5). This would suggest that at higher WC, the cells within the radish hypocotyl are under increased pressure.

Plant mechanical properties are dictated by both their cell structure and internal pressure.<sup>24–27</sup> It would appear that postharvest radish hypocotyl WC may affect splitting susceptibility by affecting the turgor pressure of the cells. Turgor pressure increases with hypocotyl WC as water entry to the cell exerts an outward pressure which is opposed by the cell wall. Under higher turgor pressure, the cell wall is placed under increased tension and so cells may be more susceptible to splitting.<sup>18</sup> The association between cell wall properties and tissue strength is shown, for example, by the down-regulation of cell wall-modifying xyloglucan endotransglucosylase hydrolases (XTHs) in lettuce, which leads to increased leaf strength and shelf-life.<sup>28</sup>

Water status has been associated with tissue strength in a number of crops. In carrot, turgor prior to harvest is positively correlated with splitting<sup>16</sup> and failure force is negatively correlated



**Figure 4.** The effect of radish hypocotyl water content on failure force due to puncture. The force required to puncture the hypocotyl of radishes at different hypocotyl water contents is shown (n = 45).



**Figure 5.** The relationship between radish hypocotyl water potential ( $\Psi_{Hi}$ , MPa) and hypocotyl water content (%) (n = 20).

with tissue turgor and water potential.<sup>3,14</sup> Furthermore, a reduction in turgor pressure caused by partially-lifting carrots was found to reduce splitting susceptibility.<sup>1</sup> In potato, the compressive strength, critical strain and strength of the tissue samples decreases with increasing turgor pressure suggesting potato tubers are more susceptible to splitting when they are more turgid.<sup>5,20</sup> Decreased turgor also significantly reduces tissue stiffness and increases failure strain in lettuce (Lactuca sativa) tissue.<sup>29</sup> In addition, leaves of spinach (Spinacia oleracea L.) processed at low relative humidity (72-85%) in order to reduce turgidity are less prone to damage.<sup>30</sup> It has also been shown that quantitative trait loci (QTLs) for the damage-associated trait of shelf life in lettuce co-locate with those for leaf biophysical properties, including plasticity, elasticity and strength.<sup>31</sup> The similarities between the effects of temperature and turgor on compressive failure strain and tissue toughness in potato has led to the suggestion that the same mechanism must explain both the effects of turgor and temperature.20

However, contradictory data has found water potential to be positively correlated with cutting force using a microtome blade in carrots and radishes.<sup>19</sup> The discrepancy between that study and others, including the results presented here, may be due to differences in the different testing methods. The effects of turgor on the strength of plant tissue are thought to depend on the type of splitting that is occurring. Higher turgor pressure has been shown to reduce tissue strength in the case of plasmoptysis (cell rupture), but increase tissue strength if splitting occurs as a result of cell debonding.<sup>32</sup> This suggests that the mode of tissue failure occurring during compression and puncture, as found in this study, is plasmoptysis, whilst cutting forces<sup>19</sup> may in contrast induce cell debonding. Although higher turgor has been shown to correlate with lower resistance to damage in many cases, it does not explain all variation.<sup>33</sup> The strength of superficial tissues, cell packing, adhesion and cell wall composition could represent additional factors in determining splitting susceptibility.<sup>33</sup> Furthermore, varietal differences may also contribute to determining splitting susceptibility in different crop species. Further investigation of a larger number of radish cultivars would aid in the determination of the extent of variety effect on splitting behaviour.

# CONCLUSIONS

Increased postharvest WC of radish hypocotyls was found to significantly increase the chance of splitting due to impact. We found a significant negative relationship between postharvest WC and failure force due to compression and puncture, suggesting that higher postharvest WC increases splitting susceptibility in summer radishes through pre-loading stress in the cell wall layers, leading to the critical value being reached rapidly upon imposition of external stress. Hypocotyl WC was also significantly correlated with water potential, suggesting their influence on splitting susceptibility may act via an increase in turgor pressure. In addition, radishes with a larger diameter or higher temperature were less prone to splitting. These findings are supported by other studies in a range of root crops. We therefore recommend that the hypocotyl WC of summer radish crops be carefully controlled during the harvest and postharvest phases, for example, through irrigation scheduling, rain shading prior to harvest and selection of harvest time, and that crops are processed at higher ambient temperature where possible in order to reduce splitting, before storing at low temperature and high humidity to maintain guality and shelf life. Further studies will aim to optimize processing conditions to provide reduced splitting while maintaining shelf life.

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# **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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