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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Rainfall, temperature and relative humidity during growth and/or at harvest all affect the rates of splitting in commercially grown radishes. Splitting rates at harvest were correlated with substrate moisture content at the radish growth stage 41.
- Post-harvest splitting susceptibility can be decreased by reducing hypocotyl water content and increasing temperature during handling.

Background

Splitting is a problem for radish growers as rates can be up to 30%, exceeding supermarket tolerances which are typically about 10%. It usually occurs pre-harvest or shortly post-harvest (1-2 days) during storage. To remove the split radish prior to packing, batches have to be sorted by hand, a process which is costly and time consuming. Despite these problems, little is known about the environmental and physiological conditions which result in split radishes. Identification of the factors affecting splitting or splitting susceptibility may allow the development of field production, harvesting and handling practices which minimise the issue.

Summer radishes (*Raphanus sativus*), which are predominantly grown in the UK as opposed to winter radishes grown more commonly in Asia, have a rapid growth cycle. In the field they can take less than 4 weeks from drilling to harvest. Previous field scale research into splitting in larger, slower growing winter radish types has shown that both irrigation frequency (Wan and Kang 2005) and quantity (Kang and Wan 2005) affect splitting at harvest, suggesting that water availability and irrigation management are an important influence on splitting in summer radishes. The timing of water availability or periods of stress as a result of drying down may also affect splitting rates, which was shown in carrots by Salter and Goode 1967 and Sorensen and Harker 2000.

Irrigation and water availability during growth may affect hypocotyl water contents at harvest. Marcelis reports that increased salinity (which consequently decreases water availability) during growth results in lower percentages in the water contents of the radish hypocotyl at harvest (Marcelis *et al.* 1997). Likewise, the post-harvest practice of e.g washing radishes can lead to water absorption, resulting in changes to hypocotyl water contents. Increased hypocotyl water contents could lead to more post-harvest splits as the turgor pressure increases. Literature has it that the failure force (the force required to split a carrot with a metal wedge driven into it at a constant speed) is negatively correlated with tissue turgor and water potential (McGarry 1993, 1995). There have been no reported investigations into the effects of hypocotyl water content on splitting susceptibility in summer radishes.

The temperature of the radish hypocotyls during harvest and post-harvest processes may also have an effect on splitting susceptibility. In a review of the effects of temperature on a range of fruits and vegetables, which did not include radishes, Bourne (1982) showed that for the majority of the crops tested, an increase in temperature was associated with decreasing failure force suggesting that produce is less susceptible to damage at high temperatures. Bajema *et al.* (1998) also found a decrease in compressive failure strain and tissue toughness with increasing temperature in potatoes. In this investigation the effects of turgor were investigated and a similar pattern was observed. The similarities between the effects of temperature and turgor led the investigators to conclude that the same mechanism must explain both the effects of temperature and turgor (Bajema *et al.* 1998).

The aim of this research was to identify the pre- and post-harvest factors affecting splitting in radish hypocotyls. The specific objectives were to investigate:

- the splitting trends in commercial radishes;
- the splitting susceptibility of different cultivars;
- the effects of water availability during growth on splitting;
- the effects of hypocotyl water content on post-harvest splitting susceptibility;
- the effects of hypocotyl temperature on post-harvest splitting susceptibility and to;
- establish a key for the development stages of radish.

Summary

Objective 1: Investigating splitting trends in commercial produce

Commercial quality assessment data for the cultivar ‘Celesta’ from 2012, 2013 and 2014 was analysed for correlations between splitting and weather. Analysis showed a significant effect of rainfall during growth, temperature during growth and at harvest and relative humidity during growth and at harvest. Temperature both during growth and at harvest tended to be negatively correlated with splitting suggesting lower temperatures were associated with increased splitting. Rainfall during growth and relative humidity during growth and at harvest were both positively correlated with splitting indicating higher levels of rainfall or higher relative humidity were associated with increased splitting.

In 2014 additional measurements were made. The number of split radishes measured by the quality assessment team at G's was correlated with the number of split radishes which arrived at Harper Adams University (HAU). The number of radishes which were split on arrival at HAU was highly correlated with the number of radishes which split as a result of impact texture analysis. Impact texture analysis was conducted by dropping the radishes from a height of 1.4 m onto a metal plate, which is the maximum height radishes are dropped from under commercial harvest conditions when they are dropped into the metal trailer. Correlation between the number of radishes which were split on arrival at HAU and the number of radishes which split after dropping indicated this as an accurate, rapid and repeatable way of testing splitting susceptibility. Splitting which occurred as a result of impact texture analysis was significantly correlated with relative water content suggesting relative water content may affect splitting susceptibility. Splitting observed by G's was significantly correlated with weather during growth and at harvest. Again a negative correlation was observed between splitting and temperature suggesting low temperatures result in more splitting and a positive correlation was observed between precipitation and relative humidity with splitting suggesting high levels of rainfall and relative humidity increase splitting.

Results from this investigation suggest the weather conditions associated with splitting are cold, wet weather during growth with a high relative humidity. These conditions are expected to result in radishes with a high relative water content and turgor pressure. In support of this theory, analysis at HAU showed radishes which were more susceptible to splitting as a result of being dropped had higher relative water contents.

Objective 2: To establish a key for the development stages of radish

A key was established for the growth stages of radishes and growth stage 41 was identified (see Table 1).

Table 1: Example pictures of whole radish and free-hand cross-sections of radishes at key growth stages. Principle growth stages 1 and 4 occur simultaneously.

Days post drilling	Growth Stage	Whole plant	Cross section of hypocotyl
2	01: Radicle emerged from seed		
5	10: Cotyledons completely unfolded; true leaf initial visible (diameter 1.2 mm)		
13	11: 1^{st} true leaf or pair of true leaves unfolded (diameter 1.9 mm)		
15	11/41(start): 1^{st} true leaf or pair of true leaves unfolded / The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (diameter 2.4 mm)		
17	12/41(end): 2^{nd} true leaf or pair of true leaves unfolded / The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (diameter 3.5 mm)		

Growth stage 41 marks the start of rapid hypocotyl expansion. During this growth stage the exodermis and outer cortex rupture and slough away exposing the periderm. Growth stage 41 is important as it is splitting of the periderm which is a problem therefore, all splitting must occur after this point.

Timings for growth of the cultivar *Raphanus sativus* 'Rudi' were determined and growth stage 41, which can be identified non-destructively, was found to occur between days 15 and 17 under glasshouse conditions (Figure 1).

Figure 1 Growth stage 41 can be identified non-destructively

Objective 3: To investigate the splitting susceptibility of different radish cultivars

Cultivars were shown to have different splitting susceptibilities. Of the cultivars tested 'Rudi' displayed the most splitting at harvest and 'Celesta' showed the least. A non-significant trend was observed linking the thickness of the periderm with the splitting susceptibility. The cultivars with a thinner periderm tended to split less and within the same cultivar the radishes with a thicker periderm also split more.

Objective 4: To investigate the effects of water availability during growth on splitting

High volumetric water content during growth was shown to result in high levels of splitting in the cultivars ‘Rudi’, ‘Celesta’ and ‘Saxa 2’.

Splitting was found to correlate with volumetric water content (VWC) on day 17 which was shown in objective 2 to be the time when growth stage 41 occurs. High levels of water at this point resulted in high levels of splitting.

Marketable yield was greatest for radishes which received a drying down period from day 8 to day 17 and then irrigation to harvest.

Objective 5: To investigate the effects of hypocotyl water content on post-harvest splitting susceptibility

High hypocotyl water contents were shown to result in radishes being more susceptible to damage from dropping, puncture and compression. As hypocotyl water content increased the force required to cause a fracture in the periderm as a result of crushing or puncture decreased and a sharp increase was observed in the number of radishes which split as a result of a 1.4 m drop at hypocotyl water contents at or in excess of 96.5 %. Hypocotyl water contents in excess of 95.6 % have been found at harvest in trials carried out for Objective 4 and in commercial radishes (data not shown). The compression force required to fracture a radish was shown to be affected by the size of the radish, larger radishes were more resistant to compression damage, therefore smaller radishes are more likely to split when packed together.

Objective 6: To investigate the effects of hypocotyl temperature on post-harvest splitting susceptibility

As radish temperature decreased splitting susceptibility measured by dropping increased. The greatest amount of splitting, 65%, was observed at the lowest temperature, 5.6°C, and the least amount of splitting, 0%, was observed at the highest temperature, 39.0°C. These results suggest radishes are more susceptible to splitting at lower temperatures.

Financial Benefits

UK radish producers encounter frequent problems with post-harvest splitting in radish which has a significant commercial impact in terms of product wastage. Identification and removal of splits is both time consuming and wasteful and is not always successful which results in depot rejection and further cost. The direct impact on the consumer is not known, but it is thought that the split radishes and drying/deterioration of the split surfaces may also deter sales.

Action Points

1. Avoid high soil moisture contents during growth. High water contents during growth resulted in more splitting in all cultivars tested.
2. In particular do not irrigate plants for the period from day 7 to growth stage 41. Water requirement at this stage is less than later in development and excessive water prior to growth stage 41 increases the number of splits at harvest.
3. Ensure plants have adequate water in later growth. Increased irrigation in the final 10 days increases yield but does not increase splitting.
4. Avoid having very high hypocotyl water contents post-harvest as radishes are more prone to splitting as a result of puncture, compression or dropping at high hypocotyl water contents.
5. A preference should be made for larger radishes as these are more resistant to splitting when crushed.
6. Avoid having low temperatures during post-harvest handling as radishes are more prone to splitting as a result of dropping at low temperatures.
7. Cultivar selection was shown to have a significant effect on splitting susceptibility. Radishes with a thicker periderm tended to split more.

SCIENCE SECTION

Introduction

Radish (*Raphanus sativus*) is an economically important member of the mustard family, Brassicaceae. Hypocotyl splitting in radish is typically characterized by a radial longitudinal fracture which usually occurs pre-harvest, growth splits, or shortly (1-2 days) post-harvest, harvest splits, during storage. Splitting is a problem for growers as the amount of splitting can be as high as 30 % which exceeds supermarket tolerance to splitting which is usually 10 %. Splitting reduces the marketable yield as split radishes have to be removed by hand prior to packing which is time consuming and costly. Despite these problems, little is known about the environmental and physiological causes of splitting particularly in the smaller summer radishes which are predominantly grown in the UK. Identification of the factors governing splitting susceptibility may allow the development of field production, harvesting and handling practices which can minimise this damage.

Growth and harvest splitting is likely to depend both on the environmental conditions which the radish hypocotyl is exposed to and the physiological predisposition to splitting. The radish hypocotyl will split more not only if it is less strong but also if it is exposed to more stressful conditions. These are not separate factors and environment will affect the way the radish grows and therefore its physiology. Physiological weakness to splitting may also be affected by cultivar. Therefore, genetics and the choice of cultivar may affect the amount of splitting which occurs. In fruit such as cherry (Demirsoy and Demirsoy 2004) and tomato (Dorais *et al.* 2004) and in vegetables such as kohlrabi (Lippert 1999) and carrot (Hole *et al.* 1999, Hartz *et al.* 2005) cultivar has been shown to affect splitting susceptibility, it is likely this may also be true for radish although there is a lack of research in this area. In fruit cuticle thickness has been investigated as a cause of genetic differences in splitting susceptibility. Demirsoy (2004) found a negative correlation between cuticle thickness and splitting in 8 cultivars of sweet cherry. In tomato (Dorais *et al.* 2004) epicarp thickness has been shown to be an important factor related to splitting resistance. There is no reported research into the effects of periderm thickness and splitting susceptibility in radishes.

As radishes are a field grown crop in the UK the weather conditions they are exposed to during growth and at harvest will affect how the radishes grow and the stresses they are exposed to and this may in turn affect the amount of splitting which is observed. Rainfall, relative humidity and temperature during growth are likely to be important factors in determining growth splitting as they may affect the available water content of the soil, transpiration rates of the radish plants, rate of growth and turgor pressure within the hypocotyls. Rainfall shortly prior to harvest, temperature at harvest and relative humidity at harvest may affect the amount of harvest splitting which is observed. Radishes are harvested, washed, trimmed, placed into Dolavs and quality assessed at ambient temperature therefore the hypocotyl temperature during this will be affected by the temperature on the day of harvest. The relative humidity on the day of harvest is likely to affect the rate of moisture loss from the hypocotyls as they are not kept under controlled atmosphere conditions. Rainfall in the week prior to harvest is likely to affect the soil moisture content at harvest and potentially affect the relative water content of the radish hypocotyls. Radishes are grown on Fenland peat which has high organic matter content and is moisture retentive.

Soil moisture content is increased by rainfall and irrigation and decreased by evaporation and transpiration. Rainfall, temperature and relative humidity will all therefore affect the available water content during growth and at harvest. Radishes are harvested, washed and trimmed at ambient temperature therefore the temperature on the day of harvest will affect the temperature of the hypocotyls during these processes. Hypocotyl moisture content during harvest and post-harvest processing may be affected by the rainfall prior to harvest. Marcelis (1999) found increased salinity and consequently decreased water availability during growth resulted in a lower percentage water content, in the radish hypocotyl at harvest. These results suggest water availability during growth may affect hypocotyl water content and increased dry matter at harvest, though salinity effects on osmotic potentials can be complex. The ambient relative humidity and temperature may also affect the rate of moisture loss and therefore the post-harvest hypocotyl water content.

Previous field scale research into splitting in the larger Asian radish types has shown irrigation frequency (Wan and Kang 2005) and quantity (Wan and Kang 2005) to have significant effects on splitting at harvest but there has been no reported investigation into the effects of irrigation on splitting in European radishes. Irrigation and water availability during growth may also have effects on hypocotyl water content at harvest.

There is evidence that timing of water availability during growth may affect splitting. Salter (1967) found dry conditions during mid-season carrot growth followed by rain prior to harvest resulted in an increased proportion of split carrots and significantly decreased marketable yield. Sorensen (1997) also found the timing of water stress had an effect on splitting in carrot, with carrots grown under fully irrigated conditions, or with an early drought stress, splitting more than carrots grown with a period of drought stress mid-growth when rapid radial expansion is occurring. Similar results have been found in tomato with cracking rates being at their highest when fruit growth is at a maximum (Dorais *et al.* 2004). Timing of water availability during growth may also affect splitting in summer radishes but this needs investigation. For this work to be carried out in a way which can be replicated, specific growth stages for radishes will need to be defined.

It should be noted another aspect of marketable yield is size. Uniformity in radish diameter is desirable as supermarkets typically require radishes which are between the sizes of 18 mm and 32 mm, anything outside of this range is too small or too large for commercial sale. It is known that water availability affects radish growth and drought stress can have a detrimental effect (Joyce *et al.* 1983). Therefore to maximise marketable yield, any treatments which reduce splitting at harvest must not as a consequence also be damaging to uniform growth of the hypocotyl.

Post-harvest washing may also increase hypocotyl water content as preliminary experiments have shown radishes are able to take up water through the periderm. Hypocotyl water content may affect splitting post-harvest by affecting turgor pressure. McGarry (1993, 1995) found failure force in carrot tissue was negatively correlated with tissue turgor and water potential. There have been no reported investigations into the effects of hypocotyl water content on splitting susceptibility in summer radishes.

The temperature of the radish hypocotyls during harvest and post-harvest processes may also have an affect on splitting susceptibility. In a review of the effects of temperature on a range of fruits and vegetables, which did not include radishes, Bourne (1982) showed for the majority of crops tested, increased temperature was associated with decreasing firmness. This was measured as failure force with a texture analyser. This relationship was represented by an approximately linear relationship. Bajema et al (1998) also found a decrease in compressive failure strain and tissue toughness with increasing temperature in potatoes. In this investigation the effects of turgor were also investigated and a similar pattern was observed. The similarities between the effects of temperature and turgor lead the investigators to conclude that the same mechanism must explain both the effects of temperature and turgor.

Aims:

To identify pre and post-harvest factors which affect splitting in radish hypocotyls

Objectives:

Objective 1: Investigating splitting trends in commercial produce

Objective 2: To establish a key for the development stages of radishes

Objective 3: To investigate the splitting susceptibility of different radish cultivars

Objective 4: To investigate the effects of water availability during growth on splitting

Objective 5: To investigate the effects of hypocotyl water content on post-harvest splitting susceptibility

Objective 6: To investigate the effects of hypocotyl temperature on post-harvest splitting susceptibility

Materials and methods

Objective 1: Investigating splitting trends in commercial produce

By investigating commercial splitting trends and correlating these with weather data it should be possible to determine if weather appears to affect splitting. The factors which appear to be correlated with splitting can then be investigated under controlled conditions in future experiments to determine if they have an effect on splitting.

The aims of Objective 1 are:

1. To determine the magnitude of variation in splitting between years (Objective 1a)
2. To determine if rainfall, temperature and relative humidity during growth and temperature and relative humidity at harvest are correlated with splitting (Objective 1a and 1b)
3. To determine if the rainfall, temperature or relative humidity during growth or temperature and relative humidity at harvest has a greater correlation with the amount of splitting i.e. do growing conditions or harvest conditions appear to have a greater effect on splitting (Objective 1a)
4. To determine if dropping can be used to accurately represent commercial splitting (Objective 1b)
5. To determine if relative water content is correlated with splitting (Objective 1b)
6. To determine if relative water content is correlated with rainfall, temperature or relative humidity during growth or at harvest (Objective 1b)

Objective 1a: To investigate trends in commercial quality assessment data for splitting in 2012, 2013 and 2014 to see how these correlate with weather conditions during growth and at harvest

It is thought weather conditions during growth may affect splitting susceptibility by affecting the turgor pressure within the radish hypocotyl. High turgor pressure has been shown to be related to increased splitting susceptibility in other crops (McGarry 1993, 1995). The theory is that less force is required to rupture cells which are already under some degree of stress. Large amounts of rainfall during growth may increase the hypocotyl water content, increase turgor pressure within the hypocotyl and increase splitting susceptibility. High relative humidity may decrease rates of evapotranspiration, increase turgor pressure within the hypocotyl and increase splitting. Low temperature may increase turgor pressure with the hypocotyl and increase splitting. The conditions which the radishes are exposed to during harvest and post-harvest handling may also affect splitting susceptibility. Cold temperature during harvest and handling may increase splitting susceptibility by increasing turgor and high relative humidity may decrease evaporation, maintaining turgor and increasing splitting susceptibility.

In commercial production, after radishes have been harvested, washed and trimmed, they are placed into large containers called Dolavs. Quality assessment of the produce is conducted at this point. To do this, 100 radishes are taken from the Dolav and assessed by trained employees for quality attributes including the number of split radishes. This information is recorded along with a unique batch number. From the batch number, the drilling date and harvest date can be determined. The number of Dolavs which have the same batch number can vary.

Data from G's Growers for the years 2012, 2013 and 2014 was analysed for correlations between the amount of splitting, which was recorded by the quality assessment team, and weather data at harvest and during the growth of the radishes. The quality assessment data which was used has been summarised in

Table 2. Weather data for RAF Marham, which is approximately 14 km from the site where the radishes were grown, was provided by BADC and the Met Office.

Table 2 Summary of commercial data used for analysis

Year	No. Dolavs	No. batches	First drill	First harvest	Final drill	Final harvest
All	34228	646	01/02/2012	10/04/2012	11/09/2014	23/10/2014
2012	9168	152	01/02/2012	10/04/2012	18/09/2012	31/10/2012
2013	12189	213	05/02/2013	26/04/2013	06/09/2013	19/10/2013
2014	12871	281	05/02/2014	08/04/2014	11/09/2014	12/10/2014

Before statistical analysis was performed the accumulated precipitation from drilling to harvest, the mean temperature from drilling to one day before harvest, the mean relative humidity from drilling to one day before harvest, the mean temperature on the day of harvest and the mean relative humidity on the day of harvest were calculated (

Table 3). To avoid duplication of data and to ensure the variables were as independent as possible, the same weather data was not used twice for the same radishes, mean temperature and mean relative humidity during growth did not include the data for temperature and relative humidity on the day of harvest. Mean temperature and relative humidity on the day of harvest were calculated because it was thought these might affect the splitting susceptibility of the radish hypocotyl during harvesting and handling. Harvesting and handling on the day of harvest is conducted under non-environmentally controlled ambient conditions, therefore the temperature and relative humidity may affect the temperature and water content of the radishes. Radish water content may be affected by differing rates of evaporation which may be affected by both temperature and relative humidity.

As the size of the batches and therefore the number of Dolavs harvested on any one day can vary, the mean data for radishes with the same batch number, i.e. radishes which were drilled and harvested on the same day was used for statistical analysis rather than individual Dolavs.

Table 3 Summary of variables analysed for correlations between commercial splitting and weather

	Variable
S	Mean number of split radishes (%)
R	Total accumulated rainfall during growth (mm)
T_g	Mean temperature during growth excluding harvest day (°C)
RH_g	Mean relative humidity during growth excluding harvest day (%)
T_h	Mean temperature on the day of harvest (°C)
RH_h	Mean relative humidity on the day of harvest (%)

Using GenStat software (Payne, 2015), multiple linear regression with step-wise deletion was used to analyse the correlations between the weather variables and splitting and to determine a model to describe the relationship between the weather variables for 2012, 2013, 2014 and all three years together. The contributions of each variable towards the model were estimated as the total proportion sum of squares accounted for by the accumulated sum of squares.

Objective 1b: Seasonal variation in splitting susceptibility and relative water content for 2014

Additional measurements were made in 2014. On 23 occasions throughout the growing season, a box of radishes from G's growers, Feltwell, was couriered on the day of harvest to arrive at Harper Adams University (HAU), Shropshire the following morning. The first delivery was made on 16th April 2014 and the final delivery was made on 14th October 2014. 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 (Waitrose, Berkshire, UK) which was tied at the top then placed inside a 305 mm x 230 mm x 230 mm double wall cardboard removal box which was taped closed.

Upon arrival at HAU, 100 radishes were removed from the box. These were briefly washed in tap water to get rid of soil residue and trimmed using a knife to remove any remaining leaf petioles and fibrous roots. The radishes were assessed for splits at this point for comparison with commercial quality assessment data. At G's Growers radishes are assessed after they have been washed and trimmed. The maximum diameter of the radishes was measured using digital callipers (Draper Expert 46610, Draper Tools Ltd., Hampshire, UK) they were weighed using a PCB 2500-2 balance (Kern and Sohn GmbH, Balingen, Germany). Any radishes which were split were cut into eight pieces and placed in a pot of approximately 100mL of deionised water and placed into cold storage at 2.5°C for 48 hours to saturate. The remaining radishes were individually dropped from a height of 1.4m onto a metal plate to test splitting susceptibility. A metal plate and a height of 1.4m were used as this was the maximum distance observed which radishes were dropped into the metal trailer during commercial harvest. The number of radishes which split as a result of dropping was counted. A record was made of the weight, diameter and if the radishes were split on arrival, split due to dropping or not split. Next, additional non-split radishes which were of commercial size, between 18 and 32mm in diameter, were taken from the box. These were washed, trimmed and dropped as before, until a total of 100 commercial sized non-split radishes had been dropped. Commercial sizes were used to make the results as relevant as possible to growers. Up to a maximum of 25 radishes which split as a result of dropping, were cut into 8 segments and placed into individual pots of approximately 100mL of deionised water and placed into cold storage at 2.5°C to saturate. A further 25 radishes which did not split were also each cut into 8 pieces, placed into a container of approximately 100mL of water and stored in a controlled environment at 2.5°C to saturate.

The temperature in the controlled environment was logged using a TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK) every half hour during the period from 16/04/2014 when the first radishes were delivered and 17/10/2014 when the final lot of radishes was removed from the cold store. The mean temperature during this period was 2.5°C with a standard error of 0.004.

After 48 hours all the radishes which had been placed in storage to saturate were removed from the water, patted dry using paper towel and weighed using the same scales which they were initially weighed with, this was the turgid weight (TW). The radishes were then placed in a drying oven at 65°C for a minimum of 48 hours, until they had reached a constant weight. The radishes were re-weighed giving the dry weight (DW). The relative water content (RWC) was then calculated (see equations).

Throughout the season, the mean number of split radishes which G's recorded was compared to the number of split radishes upon arrival at HAU. The number of radishes which were split on arrival at HAU was compared to the number of radishes which split as a result of dropping and the number of radishes which split as a result of dropping was compared to the RWC. The lot number for the radishes was supplied by G's and from this the drill date and harvest date could be determined. Using weather data from RAF Marham which was supplied by BADC and the Met Office, the rainfall during growth, the relative humidity during growth and at harvest and the temperature at growth and at harvest were correlated with RWC, the number of radishes which arrived split and the number of radishes which split as a result of dropping.

Objective 2: To establish a key for the development stages of radish

A total of 29 radishes were planted over 3 days: 17th April 2013, 29th April 2013 and 1st May 2013. Radish (*Raphanus sativus* 'Rudi') plants were grown under glasshouse conditions in 7.5L rhizotrons measuring 50 x 300 x 500mm (Figure 2) and in 1.75L plant trays (G18B half sized seed trays, Garland Products Ltd., Kingswinford, UK). The trays and rhizotrons were filled with John Innes No. 2 compost (Keith Singletons Horticultural products, Cumbria, UK). The radishes in rhizotrons were watered regularly and the radishes in the plant trays were placed on capillary matting with irrigation tubing. The bench irrigation was delivered over three periods a day each with the duration of 5 minutes totalling 17mm day^{-1}.

Figure 2 Radish plants growing in a rhizotron allowing measurement of roots

The glasshouse was set to 20/5ºC day/night temperature and achieved a mean temperature of 18.2 °C. The mean relative humidity was 57.9%. Relative humidity and temperature were logged using a TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK).

Hypocotyl diameter and leaf number were recorded regularly for all plants and the root length of the plants grown in rhizotrons was measured. Destructive samples were taken to enable free-hand cross-sections of the hypocotyl to be made.

Objective 3: To investigate the splitting susceptibility of different radish cultivars

Replication: There were eight experimental pots and three destructive pots for each cultivar.

Planting date: The seeds in this experiment were planted on 26th October 2012.

Experiment duration: Plants were harvested on day 29.

Glasshouse conditions: In the glasshouse the mean temperature was 17.0°C with a range of 28.5°C to 5.3°C. The mean relative humidity was 52.8% ranging between 90.9% and 22.1%.

Cultivar selection: This experiment investigated the splitting susceptibility of three different radish (*Raphanus sativus*) cultivars, 'Rudi', 'Celesta' and 'Topsi'. 'Celesta' is a variety grown commercially and it is thought by growers to split less than other varieties, 'Rudi' is a cultivar which is grown commercially although is being phased out as it is thought to split more than 'Celesta' (Pers. Comm. Scott Watson, G's Growers). 'Topsi' is described as having a thin periderm (Mr Fothergill's Seeds Ltd., Suffolk, UK) which may affect its splitting rate.

Growing conditions: Radishes were grown in 4.2L pots (TEKU VCA 21, Pöppelmann GmbH & Co. KG, Lohne, Germany) arranged in a randomised block design on the glasshouse bench. The pots were filled with a 1:1 mix of horticultural sand and John Innes No. 2 growing medium (both from Keith Singletons Horticultural products, Cumbria, UK). The sand and compost were mixed together in a cement mixer. Then the pots were filled to the rim of the pot. Once the pot was full, the compost and sand mixture was consolidated and smoothed level with the rim of the pot using a wooded pot tamper.

In each pot 12 seeds were planted in six evenly spaced pairs 25mm from the rim of the plant pot at a depth of approximately 7mm, this is the planting depth which is used commercially. On day 7 the cotyledons were showing on the majority of seedlings. At this point seedlings were thinned to leave the six most uniform evenly spaced seedlings remaining; the experimental unit was one pot containing six radish plants (Figure 3).

Figure 3 The experimental unit was one pot of 6 evenly spaced seedling, positioned 25mm from the edge of the pot

All pots were watered by hand to the weight at 103% pot capacity. The weight at pot capacity was calculated by saturating pots, covering the surface and allowing water to drain freely from the bottom of the pot. Pots were then weighed until a constant weight was reached; this was the weight at pot capacity. Pots were watered just over pot capacity to ensure they had the greatest water content for as long as possible. As a result of the pots being saturated a small amount of water flowed from the base of the pots. This water was caught in the saucer and had always been absorbed and/or had evaporated when the pots were checked after 6 hours and before they were next weighed. Pots were irrigated using a water bottle with a fine nozzle to ensure even distribution of water over the surface without damaging the seedlings (Figure 4). As irrigation was based on weight, compensation was made for the increasing weight of the radish in the pots by performing destructive harvests prior to irrigation three times a week. For this experiment radishes were grown under wet conditions as these are the conditions considered most likely to cause splitting. Split radishes were required for this experiment so that comparisons could be made between the splitting susceptibility of different cultivars.

Figure 4 Pots were surface irrigated using a water bottle with a fine nozzle to ensure even distribution of water over the surface without damaging the seedlings

Volumetric water content (VWC) (%) was measured using a Theta Probe ML2x (Delta T Devices, Cambridge, UK) connected to a HH2 moisture meter (Delta T Devices, Cambridge, UK). The measuring probes of the Theta Probe were 60mm long, therefore the top 60mm of the pot was classed as the surface. The pot VWC was calculated from the gravimetric water content of the pot by multiplying the gravimetric water content by the bulk density of the compost in the pot. The bulk density is the dry mass of the compost divided by the volume; this was calculated in a preliminary experiment and found to be 0.49g cm^{-3}.

Harvest: At harvest the radish hypocotyls were washed in tap water at ambient temperature (average temperature in the glasshouse at harvest was 21.6°C) and examined for splits. The diameter and length of the hypocotyl to the first root hair measured. The radishes were weighed then defoliated, the white roots removed and the remaining radish was reweighed.

Storage: The radish were put into a labeled cryovac bag to simulate commercial packaging and moved to a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd., Japan).

The environmental test chamber achieved an average temperature of 3.1°C ± 2.9°. The average relative humidity was 98.3% ± 1%.

Sectioning: On day 10 of storage the radish were weighed again and the number of splits counted. Six non-split radishes from each cultivar type were sealed in separate plastic G3 grip seal bags measuring 75 x 80mm (Weller Packaging, Lichfield, UK). At harvest splits were 'fresh' or 'healed', fresh splits have white tissue exposed whereas healed splits have a red scar showing where a split has healed. Six 'Rudi' radishes with fresh splits and four 'Topsi' with fresh splits were also sealed in separate grip seal bags. At harvest there were only four fresh split 'Topsi' and no 'Celesta' with fresh splits. All the radishes which had been placed into bags were then sent for professional sectioning and staining (Finn Pathologists, Norfolk, UK). The non-split radishes were sectioned through the widest point of the hypocotyl and the split radishes were sectioned through the splits. Sections were along the radial latitudinal axis. On arrival a segment through the widest or split part of the radish was taken and placed in a cassette. They were then placed in 70% industrial methylated spirit (IMS) for 24 h. The segments were cleared in a gradient of IMS, followed by xylene and embedded in wax (Table 4).

Table 4 Step-by-step procedures for clearing and embedding radish hypocotyl sections

Step	Reagent	Time (mins)	Temperature (ºC)
1	70% IMS	90	RT
2	95% IMS	30	RT
3	95% IMS	45	RT
4	100% IMS	30	RT
5	100% IMS	60	RT
6	100% IMS	120	RT
7	Xylene	30	RT
8	Xylene	60	RT
9	Xylene	90	RT
10	Wax	30	61
11	Wax	60	61
12	Wax	90	61
13	Histowax	-	61

*RT = room temperature

Once embedded in Histowax 10µM sections were made which were stained with 1% w/v toluidine blue made up in 50% v/v isopropanol and de-ionised water (dH_2O) (Table 5).

Table 5 Step-by-step procedure for staining radish hypocotyl sections with 1% Toluidine Blue

Step	Reagent	Time (mins)
1	70% IMS	
2	1% Toluidine Blue	30
3	Blot dry	
4	Isopropanol	1
5	99% IMS	1
6	Xylene	
7	Mount / coverslip	

The sections were returned and then analysed using an Infinity 2 22C camera (Lumenera, Ottowa, Canada) with CX31 compound microscope (Olympus, Tokyo, Japan). Pictures were analysed using infinity capture software, release: 6.0, 2011 (Lumenera, Ottawa, Canada), the image was calibrated using a 1 mm graticule slide. The water content of the remaining radishes was calculated on a wet weight basis post-storage by drying all radishes at 105°C to a constant weight.

Objective 4: To investigate the effects of water availability during growth on splitting

Five experiments designed to investigate the effects of water availability during growth were conducted in a glasshouse at HAU. These were:

4a: To investigate the effects of water availability on splitting in *Raphanus sativus* 'Rudi',

4b: To determine the effects of water availability on splitting in three different cultivars,

4c: To investigate the effects of the timing of a period of drying-down on splitting,

4d: To investigate the effects of a period of drying-down on plant responses,

4e: To determine the factors in a period of drying-down which result a reduction in splitting.

For each experiment 1.75L G18B half sized seed trays (Garland Products Ltd., Kingswinford, UK) were used to grow the radish plants in. The seed trays measured 230 mm in length, 170mm in diameter and 60mm in depth. All trays were filled level with the rim of the pot, to a weight of 1.5kg, with John Innes No. 2 compost (Keith Singletons Horticultural products, Cumbria, UK). The compost in each pot was consolidated and levelled using a wooden pot tamper.

For these experiments glasshouse conditions were: 20/5 ºC day/night temperature. From October to March supplementary lighting was provided for 16 hours a day from 400W SON/T lamps (Thermoforce Ltd., Cumbria, UK). Relative humidity and temperature were logged in the glasshouse using TGP 4500 TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK).

Experimental trays were arranged in a randomised block design.

With the exception of objectives 4a and 4b for all experiments 20 'Rudi' radish seeds were planted in pairs at a depth of 7mm (commercial practice) into 1.75L trays. Plants were irrigated for the initial 7 days for even establishment. After 7 days the seedlings were thinned to 10 in two rows of 5 with a spacing of 40mm between plants (commercial practice) and the edge of the pot and 90mm between the two rows of plants (Figure 5).

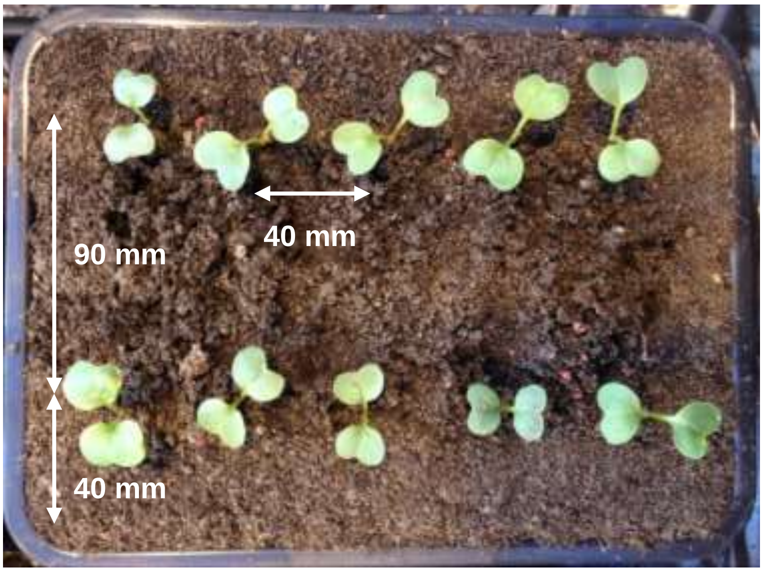

Figure 5 Plant pot containing 10 radish seedlings after radish plants had been thinned.

Post-sowing all seedling trays received irrigation via capillary matting for the first 7 days. The capillary irrigation was programmed to turn on for five minutes three times a day for the duration of each experiment, 17 mm/day. Drying-down treatments (from day 8 onwards) were imposed by placing trays on upturned pot saucers (Figure 6).

Figure 6 Plant trays were raised on upturned saucers to prevent irrigation from the capillary matting

Volumetric water content was measured using an ML2x Theta Probe (Delta T Devices, Cambridge, UK) connected to a HH2 moisture meter (Delta T Devices, Cambridge, UK). The prongs of the Theta Probe measured 60 mm long, the same as the depth of the pot (Figure 7).

Figure 7 Measuring the volumetric water content of the compost in a plant pot using a Theta Probe

At harvest the radish hypocotyls were washed in tap water at ambient temperature, weighed and examined for splits. The radishes were then defoliated and trimmed removing the white roots and the remaining trimmed radish was reweighed.

The water content of the radishes was calculated on a wet weight basis by drying all radishes at 105°C to a constant weight.

Methods which are specific to individual experiments from objective 4 are described below.

Objective 4a: To investigate the effect of substrate water content during growth on splitting

Unlike the rest of the experiments under objective 4, for objective 4a and 4b, radish seeds were initially planted and grown for seven days in seed trays measuring 350mm in length by 210mm in diameter and 55mm in depth. These trays were watered by bench capillary matting for 2 minutes three times a day giving a total of 17mm day $^{-1}$. After seven days the majority of seedlings had germinated and the most evenly sized plants were transplanted, using the spacing described previously, into trays which had been prepared as previously described but with the exception that half the trays had been placed on the bench for watering and half of the trays had been placed in saucers to allow them to dry down. The trays which the plants were transplanted into were at the correct volumetric water content (VWC) for the treatments to begin. Transplanting was used to ensure even germination of seedlings and to allow treatments to begin immediately without the trays requiring a period of drying down.

During harvest, postharvest splitting susceptibility was tested using impact texture analysis as this was shown to be an accurate indicator of commercial splitting susceptibility in Objective 1. Immediately after harvest, the radishes were all individually dropped from a height of 1.4 m onto a metal plate to test splitting susceptibility. The number of radishes which split as a result of dropping was recorded for each tray.

Replication: 20 plus 5 extra dry pots for destructive harvests.

Planting date: The seeds were planted on 5th February 2014. The seedlings were transplanted and treatments started on 11th February 2014.

Experiment duration: Plants were harvested from each treatment when more than 50% of the plants were a minimum of 25mm in diameter. This is the median commercial size. Plants from the wet treatment were harvested on day 26 and the plants from the dry treatment were harvested on day 31.

Glasshouse conditions: In the glasshouse the mean temperature was 18.15°C with a range of 3.9°C to 35.0°C. The mean relative humidity was 63.9% ranging between 31.3% and 98.8%.

Treatments: Two treatments were used, wet and dry. For the first treatment the compost in trays was maintained at high water content close to pot capacity using capillary irrigation, this was the wet treatment. The dry treatment was maintained at low water content by hand watering to a low water content which was above permanent wilting point.

Objective 4b: To determine the effects of water availability on splitting in three different cultivars

Replication: 10 plus 3 extra dry pots for each cultivar for destructive harvests.

Planting date: The seeds were planted on 15th July 2014. The seedlings were transplanted and treatments started on 21st July 2014.

Treatments: Three cultivars 'Rudi', 'Celesta' and 'Saxa 2' were all grown under wet and dry conditions similar to those used for Objective 4a to determine if the effects of water availability during growth were similar for different cultivars.

Experiment duration: Treatments were harvested when more than 50% of plants were 25 mm in diameter or greater. This was to ensure there were no effects on splitting due to size. The diameter 25mm was chosen because this is the median commercial hypocotyl diameter. There were differences in rate of growth between cultivars and treatments therefore they were harvested on different days. 'Celesta' and 'Saxa 2' which were grown under wet conditions were harvested first on day 27, followed by 'Rudi' grown under wet conditions on day 29. The three dry treatments were harvested last, 'Saxa 2' was harvested on day 34 then 'Rudi' and 'Celesta' were both harvested on day 36 (Table 6).

Table 6 Days to harvest, the number of days taken from drilling for 50 % of the plants of each cultivar grown under wet or dry conditions to reach 25 mm in diameter

Cultivar	Treatment	Days to harvest
Saxa 2	Wet	27
	Dry	34
Celesta	Wet	27
	Dry	34
Rudi	Wet	29
	Dry	34

Glasshouse conditions: In the glasshouse the mean temperature was 23.7°C with a range of 41.9 °C to 10.7 °C. The mean relative humidity was 68.9 % ranging between 100 % and 28.5 %.

Objective 4c: To investigate the effects of the timing of a period of drying-down on splitting

The design for this experiment was a refinement of a previous experiment (CP 083 Annual Report 2011/2012) in which a non-significant trend was observed suggesting high levels of splitting were associated with high volumetric water content (VWC) from days 8 to 17 and that yield was determined by high VWC in the final 10 days prior to harvest. In this experiment several improvements to the method which had been used in the previous experiment were made. The number of replications was increased from 6 to 32 and the number of radishes in a pot was increased from six to ten plants. The substrate was changed from a 1:1 mix of sand and John Innes No. 2 to just John Innes No. 2 as the addition of sand made it more difficult to achieve similar conditions in all pots. The sand appeared to be mobile within the compost when it was watered heavily resulting in an uneven mix in some pots. The depth of the pots was decreased from 183 to 60mm which is the same length as the Theta Probe pins (Delta T Devices, Cambridge, UK) which were used to measure the volumetric water content during the experiment. Decreasing the pot depth allowed more accurate measurements of the conditions the radish plant was experiencing. It became apparent, and was demonstrated in the results for Objective 1, that within the taller pots there was a gradient of water contents down the pot and as it was unclear where the radishes were taking water from. Therefore, with the taller pots it was impossible to determine exactly what the water availability to the radish plants was.

Replication: n = 34.

Planting date: Seeds were planted on 19th February 2013. Treatments commenced on day seven.

Glasshouse conditions: In the glasshouse the mean temperature was 16.8 °C with a range of 4.7 °C to 30.5 °C. The mean relative humidity was 60.6 % ranging between 29.5 % and 100 %.

Experiment duration: Plants were harvested in block order on days 28 and 29.

Treatments: The experimental period, which commenced after the initial seven day establishment period, was divided into two treatment periods each lasting 10 days. There were three treatment groups. The first group received irrigation for the duration of the experiment, the second group received no irrigation for the first 10 days and irrigation for the final 10 days, the third group received no irrigation for the initial 10 days and irrigation for the final 10 days of the experiment (Table 7).

Table 7 Irrigation regimes for the three treatment groups

Treatment	Day 7 to 17	Day 18 to harvest
W/W	Irrigation	Irrigation
D/W	No Irrigation	Irrigation
W/D	Irrigation	No Irrigation

Additional measurements: VWC of all pots was measured three times a week using a Theta Probe ML2x (Delta T Devices, Cambridge, UK) connected to a HH2 moisture meter (Delta T Devices, Cambridge, UK). The maximum hypocotyl diameter was measured using Draper Expert 46610 digital vernier callipers (Draper Tools Ltd., Hampshire, UK) for each radish.

Objective 4d: To investigate the effects of a period of drying-down on plant responses

Replication: For the main harvest n = 34. In addition 20 plants were grown under the same conditions and were used to measure stomatal resistance, leaf area and leaf temperature on day 18, when treatments were changed and day 28 when plants were harvested.

Planting date: Seed were sown on 14th May 2013, treatments began on day 7.

Glasshouse conditions: In the glasshouse the mean temperature was 19.0^{o}C with a range of 7.0°C to 41.0°C. The mean relative humidity was 68.7% ranging between 23.2% and 100%.

Experiment duration: Plants were harvested in block order on day 28 and 29.

Treatments: Treatments began after the initial 7 days of irrigation for establishment. There were two treatment periods each lasting 10 days. Treatment group W/W received irrigation for the duration of the experiment; treatment group D/W received no irrigation for the first 10 days and irrigation for the final 10 days (Table 8). When irrigated plants were placed on capillary matting, the irrigation for the capillary matting was programmed to last for five minutes three times a day giving a total of 17mm day $^{-1}$.

Table 8 Irrigation regimes for the three treatment groups

Treatment	Day 8 to 17	Day 18 to harvest
W/W	Irrigation	Irrigation
D/W	No Irrigation	Irrigation

Additional measurements taken during growth: The leaf area (cm^2) was measured using a Li-3000A leaf area meter (Li-Cor Lincoln, NE, USA), temperature (°C) of the youngest fully opened leaf was measured using a 66 infrared thermometer (Fluke, WA, USA) and stomatal resistance (m^2 s mol^{-1}) of the youngest fully opened leaf was measured using an AP4 porometer (Delta-T Devices, Cambridge, UK).

Additional measurements taken at harvest: The maximum hypocotyl length and diameter were measured using Draper Expert 46610 digital vernier callipers (Draper Tools Ltd., Hampshire, UK).

Objective 4e: To determine the factors in a period of drying down which result a reduction in splitting

The experimental design for this experiment was similar to that of previous experiments (objective 4c and 4d) in which significant differences were observed in radishes which were irrigated for the duration of the experiment and radishes which were given a drought period of 10 days early in growth. The previous experiments in combination with work on the growth stages of radishes suggest high levels of splitting are associated with high VWC at growth stage 41, when rapid expansion of the hypocotyl begins following the rupture of the exodermis and outer cortex which exposes the periderm, and that yield is determined by high volumetric water content (VWC) in the second half of the radish growth period. The objective of this experiment was to determine whether it is having a period of drying down at the point of growth stage 41 or the duration of the period of drying down which has resulted in the reduction in splitting in previous experiments.

Replication: n = 24.

Planting date: Seeds were planted on 22nd May 2013, treatments commenced on day 7.

Experiment duration: 27 to 29 days.

Glasshouse conditions: In the glasshouse the mean temperature was 19.5°C with a range of 7.0°C to 41.0°C. The mean relative humidity was 71.4% ranging between 23.2% and 100%.

Treatments: Following the initial 7 days of irrigation for seedling establishment a total of five treatment regimens were imposed. T1 received irrigation for the duration of the experiment; T2 received no irrigation for the first 10 days and irrigation for the final 10 days, T3 received no irrigation for the first five day and irrigation for the final 15 days, T4 received no irrigation for the first 15 days and irrigation for the final five days andT5 received irrigation for the first five days, no irrigation for the following 10 days and then irrigation for the final five days (Table 9). It was expected that T2 and T4 would be driest at the point when the hypocotyl begins to swell, treatments T1 and T3 would have the greatest VWC at the point when the hypocotyl swells and treatment T5 would have a VWC between the other groups. If splitting was related to the VWC at the point of hypocotyl expansion then it was expected there would be a difference in the treatments which have different VWCs at this point. Conversely if differences in splitting were related to the duration of the drought period it was expected there would be differences between the treatment groups with different drought durations. Treatment group T4 had the longest drought period of 15 days, treatment groups T2 and T5 had the second longest drought period of 10 days, treatment group T3 had the shortest drought period of 5 days and treatment group T1 had no drought period.

Table 9 Irrigation regimes for the five treatment groups

<table>
<tr><th>Treatment</th><th>Day 1-7</th><th>Day 8-12</th><th>Day 13-17</th><th>Day 18-22</th><th>Day 23 -27</th></tr>
<tr><td>T 1</td><td colspan="5">Irrigation</td></tr>
<tr><td>T 2</td><td>Irrigation</td><td colspan="2">No Irrigation</td><td colspan="2">Irrigation</td></tr>
<tr><td>T 3</td><td>Irrigation</td><td>No Irrigation</td><td colspan="3">Irrigation</td></tr>
<tr><td>T 4</td><td>Irrigation</td><td colspan="3">No Irrigation</td><td>Irrigation</td></tr>
<tr><td>T 5</td><td colspan="2">Irrigation</td><td colspan="2">No Irrigation</td><td>Irrigation</td></tr>
</table>

Additional measurements taken during growth: The VWC of the compost was measured at the start of the experiment on day 1 and again on day 8, day 12, day 17, day 22 and day 27 before treatments were changed and before plants were harvested.

Objective 5: To investigate the effects of hypocotyl water content on post-harvest splitting susceptibility

Splitting susceptibility at different water contents was measured using three different tests. The tests used were impact, puncture and compression. These tests were used as they were considered most likely to replicate commercial harvesting and packing processes. During harvest radishes are dropped from heights up to 1.4 m into a metal trailer and then after the initial grading they are dropped again into Dolavs. In the trailers used to transport the radishes from the field and during washing the radishes may experience puncture from any stones or other foreign bodies collected from the field. Once in the Dolavs, the radishes experience compression from the weight of the other radishes in the Dolav. The pressure inside the hypocotyl was also measured at different water contents as it is hypothesised that any differences in splitting susceptibility may be as a result of differences in pressure within the hypocotyl.

Radishes from a commercial grower in Norfolk, England were couriered on the day of harvest (17th June 2014) to arrive at HAU, Shropshire, England the following morning (18th June 2014). Upon arrival radishes were briefly washed in dH_2O to remove soil residue and trimmed to remove any remaining leaf stalks and roots.

The experimental unit for the puncture, compression and pressure tests was an individual radish, for impact analysis the experimental unit was three radishes. The method for the preparation of radishes used for impact analysis was slightly different and has been described separately. To ensure there was a range of hypocotyl water contents, the radishes which were to be used for the puncture, compression and pressure tests, were divided into three groups. The first groups were individually placed into pots of approximately 100 mL of deionised water to increase the hypocotyl water content, the second group were placed into individual pots with closed lids to maintain a high humidity within the pot and maintain the hypocotyl water content, the third and final group were placed into individual pots without a lid to allow them to air dry slightly. All the pots were placed into a controlled environment for 1 day before analysis was carried out. The mean temperature in the controlled environment for the duration of storage was 2.6°C with a maximum of 2.7°C and a minimum of 2.3°C the mean relative humidity was 100% with a maximum of 10% and a minimum of 99.1 %.

Prior to testing the diameter of the radishes was measured and they were weighed. After testing, the radishes were dried and weighed to enable a calculation of water content (WC) to be made.

Impact:

Once the radishes had been washed and trimmed, they were placed into plastic pots in groups of three, the experimental unit was one pot of three radishes. The pots of radishes were then placed into a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd., Japan) where they were either allowed to air dry or the pots were filled with approximately 100 ml of dH_2O to saturate the hypocotyls. The chamber achieved an average relative humidity of 83.5% ranging from 62.0% to 100.0%, and an average temperature of 4.5°C ranging from 2.5ºC to 7.4°C. Radishes were removed from the chamber every two to three days over the following week, weighed and subjected to impact texture analysis. The variations in temperature and relative humidity are thought to have been due to the opening and closing of the chamber to remove samples.

After impact texture analysis the radishes were dried to a constant weight at 105ºC to calculate the water content at the point of analysis.

Impact tests were performed using the method described by Hartz et al (2005) with a slight modification. In this experiment radishes were dropped down a pipe onto a metal plate from a height of 1.4m. The height was increased from the 1m used by Hartz et al (2005) to 1.4m to ensure some splitting was observed. This height is at the upper limit of what would be observed commercially when the first radishes are harvested into the trailer.

Puncture:

Puncture tests were performed on 54 radishes using a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England). The texture analyser was fitted with a P/2 cylindrical probe, the test speed was $2mmS^{-1}$ and the test distance was 16mm. During the experiment a curve was plotted of the force (kg) as a factor of distance. The point at which the periderm of the radish was punctured could be observed on the plotted curve as abrupt decrease in force. This is a slight modification of the method reported in the CP 083 2012/2013 annual report, the test speed was doubled after preliminary experiments showed the change in speed did not affect the results but enabled more tests to be conducted in the same amount of time.

Compression:

Uniaxial compression tests were performed on 44 radishes using a P/75 probe fitted to a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England). The test speed was 2mmS^{-1} and the test distance was 25mm. During the test a curve was plotted of force (kg) as a factor of distance (mm). As the compression distance increased peaks were observed in the graph profile. Each peak indicates a compression failure in the radish. For the purposes of this experiment the force of the first peak was recorded as the force required to split the radishes. This is a slight modification of the method reported in the CP 083 2012/2013 annual report, the test speed was increased from 0.05mmS^{-1} to 2mmS^{-1} to allow a greater number of samples to be processed. In addition liquid had been observed to be lost from the radishes at the slower speed making it impossible to determine exactly what the water content at the point of failure was. In addition the test distance was increased from 16 to 25mm. This was because a number of radishes did not break with the shorter distance and it was felt an increased test distance would reduce the likelihood of this occurring.

Pressure:

The water potential (bar) of 20 radish hypocotyls was measured using a digital pressure bomb (SKPM-1400, Skye Instruments Ltd, Powys, UK). The pressure bomb was used as it would be with a leaf but the hypocotyl of the radish was placed inside the chamber instead of the leaf with a severed petiole protruding as usual (Figure 8).

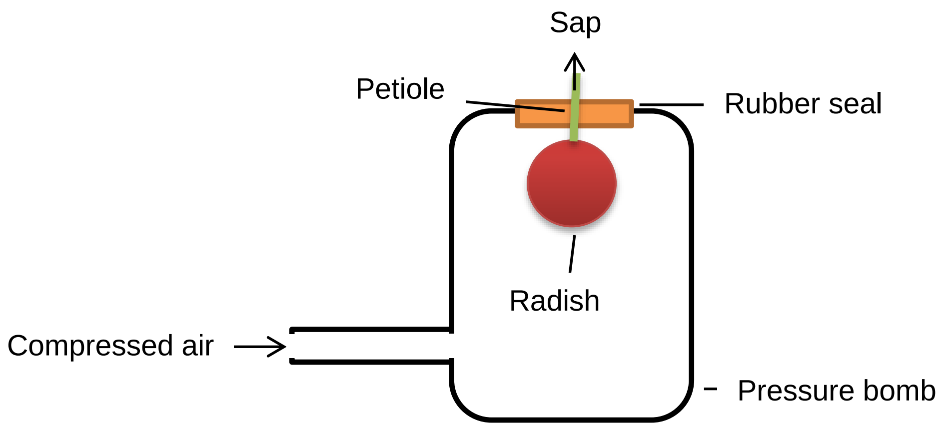

Figure 8 Diagram showing how the radish hypocotyl was positioned in the pressure bomb

Objective 6: To investigate the effects of hypocotyl temperature on post-harvest splitting susceptibility

Radishes from a commercial grower in Norfolk, England were couriered on the day of harvest, 22nd July 2014, to arrive at HAU, Shropshire, England the following day, 23rd July 2014. Upon arrival radishes 115 radishes were washed in dH_2O to remove soil residue and trimmed to remove any remaining leaf stalks and roots. They were then placed in a controlled environment chamber overnight ready for testing the following day. The temperature and relative humidity in the controlled environment were measured with a TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK) every half hour. The mean temperature in the cold store during this period was 2.6°C ranging from a maximum of 3.4°C to a minimum of 1.8°C. The mean relative humidity was 98.4% with a maximum of 100% and a minimum of 93.0%.

On the day of testing, the radishes were placed into individual G3 grip seal bags measuring 75 x 80mm (Weller Packaging, Lichfield, UK). The bags of radishes were placed into baths of water at the required temperature.

Figure 9). The temperature of the water in the 5 baths was 5, 10, 20, 30 and 40°C. The experimental unit was one radish hypocotyl and 20 replicates were used for each temperature.

Figure 9 Water bath set to 40°C containing radishes in grip seal bags prior to texture analysis

Impact testing for splitting susceptibility at the five temperatures was performed once the radishes had acclimatised to approximately the temperature of the water bath, this took roughly two hours. This was measured by inserting a digital thermometer into three additional destructive radishes which had also been placed into grip seal bags in each of the five water baths. Impact tests were performed as previously described, by dropping the radishes down a 1.4m pipe onto a metal plate. In an effort to keep the radishes at the correct temperature, they were individually removed from the water immediately prior to testing leaving the remaining radishes in the water bath. If the radish split or not was recorded. The number of split radishes in each water bath was correlated with the mean temperature of the destructive harvest radishes using simple linear regression.

Equations

For all experiments, relative water content (RWC) was calculated using the equation:

$$\mathrm{RWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})}$$

Water content (WC) was calculated on a wet weight basis using the equation:

$$\mathrm{WC} = \frac{(\mathrm{FW} - \mathrm{DW})}{\mathrm{FW}}$$

Where, FW = fresh weight, DW = dry weight and TW = turgid or saturated weight.

Statistical analysis

All data was analysed using GenStat for Windows 15th Edition (Payne 2012).

For objective 1 the response variable, mean percentage splitting, and explanatory variables, accumulated rainfall, mean temperature during growth, mean relative humidity during growth, mean temperature on the day of harvest and mean relative humidity on the day of harvest were measured on a continuous scale. Multiple regression was appropriate for the analysis. A correlation matrix was performed in Excel to identify which parameters were correlated using Pearson product-moment correlation coefficients to determine the critical values. Due to the nature of weather data, it was found the explanatory variables were often correlated. In GenStat a full model containing main effects and interaction terms was fitted and the significance of terms tested by stepwise deletion. Due to the correlation between factors, to ensure the data was independent the same data was not used twice, therefore mean relative humidity during growth and mean temperature during growth did not include the data from the day of harvest. Analysis of variance (ANOVA) was used to test for differences in the amount of splitting observed each year. Significant differences in annual splitting at the 5 % confidence limit were identified by Tukey pair wise comparison. For the additional analysis which was conducted throughout the season in 2014 simple linear regression was used to compare, the mean number of split radishes which G's recorded for each lot to the number of split radishes upon arrival at HAU. The number of radishes which were split on arrival at HAU was compared to the number of radishes which split as a result of dropping and the number of radishes which split as a result of dropping was compared to the relative water content of the radishes. Using analysis of variance (ANOVA) the relative water content of the radishes which were split on arrival at HAU, which split due to dropping, which had suffered mechanical damage and were intact were compared.

For objectives 2, 5 and 6 regression was used to analyse the data. When the response variable and explanatory variables both contained continuous data, simple or polynomial regression was used to estimate their relationship. When the response variable consisted of presence absence data, for example split or not split radishes, linear regression with a probit link function was used.

For objectives 3 and 4 the response variants, including number of split radishes, were tested for normality using a Shapiro-Wilk test for normal distribution. Parametric data was analysed using ANOVA. Where data was not normally distributed, with or without transformation, Friedman's test was used. When a P value of less than 0.05 was observed multiple comparisons were made using a Tukey test for parametric data and a Mann-U Whitney test was used for non-parametric data.

Results

Objective 1: Investigating splitting trends in commercial produce

Objective 1a: To investigate trends in commercial quality assessment data for splitting in 2012, 2013 and 2014 to see how these correlate with weather conditions during growth and at harvest

There was significantly less splitting in 2014, 2.14%, compared to 2012, 2.76% and 2013, 2.73%, but the difference was not large (Table 10).

Table 10 Mean number of splits observed each year

Year	Mean Splits[1]
2012	2.76 b
2013	2.73 b
2014	2.14 a
P	<0.001
SEM	0.154

[1] a, b Denotes significant differences at the 5% confidence limit.

The accumulated precipitation during the commercial growing season, determined as the time from the first drilling of the season to the final harvest, was least for 2014 and greatest for 2013. The mean temperature was the greatest for 2014 and least for 2012 and 2013. The mean relative humidity was greatest for 2012 and least for 2013 (Table 11).

Table 11 Accumulated precipitation, mean temperature and mean relative humidity during the commercial growing season for each year

Year	Accumulated precipitation (mm)	Mean temperature (°C)	Mean relative humidity (%)
2012	301.9	11.1	81.0
2013	313.3	11.1	77.7
2014	227.9	12.8	78.5

Despite the limitations of using commercial data, significant correlations between the amount of splitting observed and the weather variables tested were found for all years (Table 12).

Table 12 Correlation matrices between commercial splitting rates observed at G's Growers and weather data measured at RAF Marham for 2012, 2013, 2014 and all three years together (numbers in bold are significantly different at the 5% level).

Year		**S**[1]	**R**	**T_g**	**RH_g**	**T_h**	**RH_h**
All years	**S**	1					
n = 646	**P**	**0.251**	1				
PPMC	**T_g**	**-0.138**	-0.178	1			
(5%) ~	**RH_g**	**0.301**	0.222	-0.301	1		
0.088	**T_h**	**-0.272**	-0.283	0.620	-0.265	1	
	RH_h	**0.247**	0.193	-0.052	0.297	-0.214	1
2012	**S**	1					
n = 152	**P**	**0.226**	1				
PPMC	**T_g**	-0.008	0.082	1			
(5%) ~	**RH_g**	0.002	-0.166	-0.862	1		
0.139	**T_h**	**-0.216**	-0.236	0.305	0.086	1	
	RH_h	**0.240**	-0.113	-0.134	0.152	-0.010	1
2013	**S**	1					
n = 213	**P**	**0.389**	1				
PPMC	**T_g**	0.104	-0.479	1			
(5%) ~	**RH_g**	**0.577**	0.442	-0.020	1		
0.139	**T_h**	**-0.168**	-0.644	0.728	-0.279	1	
	RH_h	**0.288**	0.275	0.050	0.374	-0.263	1
2014	**S**	1					
n = 281	**P**	**0.113**	1				
PPMC	**T_g**	**-0.431**	0.189	1			

(5%)	~	**RH_g**	**0.216**	0.004	-0.046	1		
0.113		**T_h**	**-0.419**	0.016	0.654	-0.145	1	
		RH_h	**0.225**	0.146	-0.054	0.217	-0.184	1

[1] **S** = Mean number of split radishes (%), **P** = Mean accumulated precipitation during growth (mm), **T_g** = Mean temperature during growth excluding harvest day (°C), **RH_g** = Mean relative humidity during growth excluding harvest day (%), **T_h** = Mean temperature on the day of harvest (°C), **RH_h** = Mean relative humidity on the day of harvest (%). [2] **ρ** = Pearson product-moment correlation coefficient

Fitted models

The parameters which were correlated with splitting varied between years and the amount of variation the weather data was able to explain varied between a maximum of 39.6% in 2013 and a minimum of 12.3% in 2012. The amount of variance in splitting explained by the weather variables over all three years combined was 16.6 %. In all years combined and in 2012 all of the variables were included in the final model whereas in 2013 and 2014, variable 7, the mean relative humidity on the day of harvest, was not included (Table 13).

Table 13 Model determined by multiple linear regression and stepwise deletion for each and all years

Year	Model fitted	P	Variance accounted for	SE
All	T_g+RH_h+P+T_h+RH_g	<0.001	16.4	1.75
2012	T_h+T_g+RH_g+RH_h+P	<0.001	12.9	1.64
2013	RH_g+P+T_g+T_h	<0.001	39.6	1.67
2014	T_g+P+RH_g+RH_h+T_h	<0.001	27.9	1.49

All of the weather variables which were analysed had some correlation with splitting suggesting weather during growth and at harvest has an effect on splitting (

Table 14).

There did not appear to be a pattern of weather during growth or weather at harvest affecting the amount of splitting to a greater extent than the other. Temperature during growth did account for a greater proportion of the total sum of squares when compared to temperature at harvest for 2012, 2013 and 2014 but temperature at harvest accounted for the greatest proportion of the total sum of squares for all years combined. In 2012 relative humidity on the day of harvest accounted for the greatest proportion of the total sum of squares, in 2013 relative humidity during growth accounted for the greatest proportion of the total sum of squares, in 2014 temperature during growth accounted for the greatest proportion of the total sum of squares and over all the years relative humidity during growth accounted for the greatest proportion of the total sum of squares. Relative humidity at harvest was the only variable which was not included in all of the final models after stepwise deletion had been performed, it was not included in either the model for 2013 or 2014 (

Table 14).

Temperature both during growth and at harvest tended to have a negative parameter estimate when correlated with splitting suggesting radishes are more likely to split with decreasing temperatures. Relative humidity and rainfall both had exclusively positive parameter estimates when correlated with splitting suggesting radishes are more likely to split with increasing rainfall and relative humidity (

Table 14).

Table 14 The significance of variation for splitting, parameter estimate, mean, standard error of the mean and proportion of total sum of squares accounted for by the accumulated sum of squares for each weather variable in each and all years

Variable	Year	P	Parameter estimate	Mean	S.E.M	Proportion of TSS (%)
Accumulated precipitation (mm)	**All**	<0.001	0.013	37.88	0.003	3.55
	2012	0.052	0.021	43.46	0.011	2.22
	2013	<0.001	0.167	38.87	0.007	2.25
	2014	0.002	0.012	34.14	0.004	3.93
Mean temperature during growth excluding harvest day (°C)	**All**	0.093	0.045	13.11	0.027	1.91
	2012	0.013	0.917	10.61	0.363	0.36
	2013	<0.001	0.029	13.68	0.042	5.51
	2014	<0.001	-0.213	14.04	0.042	18.59
Mean relative humidity during growth excluding harvest day (%)	**All**	<0.001	0.095	78.69	0.018	3.44
	2012	0.017	0.739	83.64	0.306	4.83
	2013	<0.001	-0.053	76.35	0.033	33.24
	2014	0.004	0.095	77.80	0.033	3.75
Mean temperature on the day of harvest (°C)	**All**	<0.001	-0.103	14.05	0.025	2.37
	2012	0.002	-0.162	12.62	0.050	4.65
	2013	0.297	0.224	14.58	0.051	0.31
	2014	0.035	-0.081	14.42	0.038	1.16
Mean relative humidity on the day of harvest (%)	**All**	0.002	0.027	78.76	0.009	5.74
	2012	0.023	0.043	80.62	0.019	3.06
	2013	-[1]	-	77.26	-	-
	2014	0.022	0.027	78.89	0.012	1.81

[1]- = variable not included in model

Objective 1b: Seasonal variation in splitting susceptibility and relative water content for 2014

The mean number of split radishes for each batch recorded by the quality assessment team at G’s was correlated with relative humidity and temperature on the day of harvest suggesting harvest conditions influenced the splitting susceptibility of the radishes. There was also a correlation between the environmental conditions during growth, namely temperature and accumulated precipitation, and the amount of splitting observed at G’s suggesting weather conditions during growth may also effect splitting (

Table 15).

Temperature both during growth and at harvest was negatively correlated with splitting suggesting lower temperatures may result in an increased splitting susceptibility. Accumulated precipitation and relative humidity at harvest were positively correlated with splitting recorded at G’s suggesting higher levels of rainfall and higher relative humidity result in more splitting (

Table 15).

The amount of splitting which was observed on arrival at Harper Adams was correlated with the splitting recorded at G's and was negatively correlated with temperature during growth and at harvest.

The number of radishes which split as a result of dropping was correlated with relative water content but the number of radishes recorded as split by G's and the number of radishes which arrived split at HAU were not (

Table 15).

Relative water content (RWC) was not correlated with any of the weather conditions during growth or at harvest (

Table 15).

Table 15 Correlation matrix showing the relationships between splitting, relative water content and weather during growth for radishes grown in 2014 (n=23)

	GS	FS	DS	RWC	P	T_G	RH_G	T_H	RH_H
GS[1]	1								
FS	**0.569****	1							
DS	0.360	**0.629****	1						
RWC	-0.092	0.227	**0.698*****	1					
P	0.398	0.005	0.287	0.160	1				
T_G	-0.453	-0.362	0.054	0.315	0.284	1			
RH_G	0.075	0.263	0.116	0.190	-0.328	-0.021	1		
T_H	**-0.619****	-0.398	-0.058	0.211	-0.031	**0.703*****	-0.096	1	
RH_H	**0.605****	0.144	0.133	-0.212	0.333	-0.269	0.267	-0.314	1

[1]GS = mean number of split radishes recorded by the quality assessment team at G's, FS = number of radishes which were split on arrival at HAU, DS = number of radishes which split after dropping, RWC = relative water content of the radishes, P = total accumulated

precipitation during radish growth, T_G = mean temperature during growth, RH_G = mean relative humidity during growth, T_H = mean temperature on the day of harvest, RH_H = mean relative humidity on the day of harvest. *** = denotes significance at the 1 % level (PPMC = 0.652), **= denotes significance at the 5 % level (PPMC = 0.537)

Fitted models

A multiple linear regression of the weather data which was correlated with the amount of splitting recorded at G's resulted in a model which accounted for 57% of the variation in splitting. The model included accumulated precipitation during growth, mean temperature and relative humidity on the day of harvest and mean temperature during growth (

Table **16**).

Table 16 Model determined by multiple linear regression and stepwise deletion for the relationship between splitting observed at Gs and weather

Split type	Model fitted	P	Variance accounted for	SE
GS[1]	$T_g+RH_h+T_h+P$	<0.001	57.0	1.20

[1] GS = Number of splits recorded at G's, T_g = Mean temperature during growth(°C), RH_h = Mean relative humidity on the day of harvest (%), T_h = Mean temperature on the day of harvest (°C), P = Accumulated precipitation (mm)

In the model the temperature during growth accounted for the greatest proportion of the total sum of squares (38.32%) followed by mean relative humidity on the day of harvest (18.75%) then mean accumulated precipitation (7.75%) and mean temperature on the day of harvest (0.003%) (Table 17).

Table 17 The significance of variation for splitting recorded at G's, parameter estimate, mean, standard error of the mean and proportion of total sum of squares accounted for by the accumulated sum of squares for each weather variable used in the model determined by multiple linear regression

Parameter	P	Parameter estimate	Mean	S.E.M	Proportion of TSS[1] (%)
Temperature during growth	0.364	-0.124	14.13	0.134	38.32
RH harvest day	0.062	0.0731	80.18	0.0367	18.75
Temperature harvest	0.102	-0.245	14.01	0.142	0.003

day					
Accumulated precipitation	0.062	0.0441	29.23	0.0221	7.75

[1] TSS is the total sum of squares

Using simple linear correlation showed the number of radishes which were split on arrival at Harper Adams (FS) was correlated (P<0.001) with the number of radishes which were recorded as split by the quality assessment team at G's (GS), although fewer splits tended to be recorded at G's than at Harper Adams. FS was significantly correlated (P<0.001) with the number of radishes which split after they were dropped from a height of 1.4 m (DS), the number of radishes which split after dropping was greater than the number of radishes which were split on arrival at Harper Adams. GS and DS were not highly correlated but there was a trend (P=0.091) (Table 18).

Table 18 Correlations between different split types in 2014

Variables correlated	P	Variance accounted for (%)	Standard error	Model
GS[1] + FS	<0.001	29.2	1.54	GS = 0.24 FS + 1.77
DS + FS	<0.001	36.7	6.72	DS = 1.20 FS + 6.63
GS + DS	0.091	8.8	1.75	GS = 0.08 DS + 1.73

[1] refers to the type of split, FS = the number of radishes which were split on arrival at HAU, DS = the number of radishes which split after they were dropped, GS = the number of radishes recorded by the quality assessment team at G's

The number of radishes which split after dropping was highly correlated (P<0.001) with hypocotyl RWC (Table 19).

Table 19 Correlation between the number of radishes which split when dropped and relative water content

Variables	P	Variance accounted for (%)	Standard error	Model
DS[1] RWC	<0.001	46.3	6.19	DS = 207.89 RWC – 174.4

[1]DS = Number of radishes which split after dropping from a height of 1.4 m, RWC = relative water content

The RWC of the radishes which did not split and split as a result of dropping was significantly different at the 5% level. The RWC of the radishes which were split on arrival at Harper Adams was mid-way between the RWC of the radishes which did not split and the radishes which split as a result of dropping but was not significantly different from either. The water content on the radishes which did not split was significantly lower than the water content of the radishes which were split on arrival at HAU and those which split after dropping. There was no significant difference in the water content of the radishes which were split on arrival at HAU and those which split as a result of dropping (

Table **20**).

Table 20 Relative water content and water content of radishes which were not split, which were split on arrival at Harper Adams and which split after they were dropped

Split type	n	RWC	WC
Not Split	575	0.888 a[2]	96.57 a
Split on arrival	77	0.895 ab	96.74 b
Split after dropping	245	0.912 b	96.91 b
P		<0.001	<0.001

[1]a, b denotes differences at the 5% significance level

Objective 2: To establish a key for the development stages of radish

The proposed scale for the growth stages of radishes is based on the BBCH Monograph for root and stem vegetables (Meier 2001) and uses three of the eight principle growth stages identified for root and stem vegetables. Commercially grown radishes are harvested prior to maturity and this scale only includes the growth stages which are relevant to commercial growers. It should be noted that principal growth stages 1 and 4 occur simultaneously and progress concurrently. In the UK radishes are required by supermarkets to be between 18 and 32mm in diameter giving a median size of 25mm. This enables the median diameter for the hypocotyl during growth stage 4 to be calculated and has been included in the scale (

Table **21**).

Table 21 Proposed growth stages for radishes during the commercial growing period including median hypocotyl diameters for principle growth stage 4

Principal growth stage 0: Germination	
00	Dry seed
01	Radicle emerged from seed
09	Emergence: cotyledons break through soil surface
Principal growth stage 1: Leaf development	
10	Cotyledons completely unfolded; true leaf initial visible
11	1st true leaf or pair of true leaves unfolded
12	2nd true leaf or pair of true leaves unfolded
1.	Stages continue until…
19	9 or more true leaves or pairs of true leaves unfolded
Principal growth stage 4: Development of harvestable vegetative plant parts	
41	The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (~ 2.5mm)
42	20% of the final hypocotyl diameter reached (5mm)
43	30% of the final hypocotyl diameter reached (7.5mm)
44	40% of the final hypocotyl diameter reached (10mm)
45	50% of the final hypocotyl diameter reached (12.5mm)
46	60% of the final hypocotyl diameter reached (15mm)
47	70% of the final hypocotyl diameter reached (17.5mm)
48	80% of the final hypocotyl diameter reached (20mm)
49	Expansion complete; typical form and size of hypocotyl reached (25mm)

The difference between the general root and stem growth stages included in the BBCH root and stem vegetable scale and the growth stages in this piece of work are the expansion of the description of growth stage 41. Here growth stage 41 is described as: The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (> 2.5mm) but was described as: Roots beginning to expand (diameter > 5mm) in the BBCH root and stem vegetable scale. This description has been altered for a number or reasons. Firstly, the portion of the radish which is sold commercially is predominantly a swollen hypocotyl not a root. Secondly, as radishes are harvested when they are between 18 and 32mm in diameter, they are not exclusively > 5mm in diameter when they are less than 20 % of the harvest size. Finally the original description did not mention the change in physiology which occurs at this point, namely how the periderm becomes the outer layer of the radish. This growth stage is of significance to radish splitting because splitting is observed as ruptures of the periderm. The periderm is only fully formed and exposed after growth stage 41 (

Table **22**) therefore, all splitting must happen after this point.

Table 22 Example pictures of whole radish and free-hand cross-sections of radishes at key growth stages. Principle growth stages 1 and 4 occur simultaneously.

Days post drilling	Growth Stage	Whole plant	Cross section of hypocotyl
2	01: Radicle emerged from seed		
5	10: Cotyledons completely unfolded; true leaf initial visible (diameter 1.2mm)		
13	11: 1st true leaf or pair of true leaves unfolded (diameter 1.9mm)		
15	11/41(start): 1st true leaf or pair of true leaves unfolded / The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (diameter 2.4 mm)		
17	12/41(end): 2nd true leaf or pair of true leaves unfolded / The exodermis and outer cortex rupture and slough away exposing the periderm. The hypocotyl begins to expand (diameter 3.5mm)		

Growth stage 41 can be identified non-destructively during growth as the radish hypocotyl is visible above the surface of the growing medium (Figure 10).

Figure 10 Growth stage 41 can be identified non-destructively

Objective 3: To investigate the splitting susceptibility of different radish cultivars

Substrate water content: Each cultivar group was exposed to similar ranges in volumetric water content (VWC) (Table 23); there were no significant differences between the average water content at the surface for the different cultivar types (P=0.836). The pot was calculated from the gravimetric water content (GWC).

Table 23 The average volumetric water content (VWC) at the surface and for the whole pot in each treatment group. Surface readings were taken before irrigation. Pot readings were taken before and after irrigation

Cultivar	Maximum VWC (%)		Minimum VWC (%)		Average VWC (%)	
	Surface	Pot	Surface	Pot	Surface	Pot
Rudi	25.9	26.5	21.1	22.3	23.7	25.1
Celesta	26.0	26.5	21.1	22.2	23.9	25.1
Topsi	25.9	26.5	21.2	22.5	23.8	25.1

There was a greater range in VWC at the surface of the pot; 4.8%, compared to the whole pot which had a range of 4.2% on average. The compost at the surface had a lesser VWC than the pot as a whole for the duration of the experiment.

Splitting: There was a significant difference (P=0.001) in the number of split radish between cultivars at harvest. At harvest 'Rudi' had significantly more split radish on average per pot than 'Topsi' or 'Celesta' which did not have significantly different numbers of splits from each other (Table 24).

Table 24 The mean number of split hypocotyls for three different cultivars of radish. Each pot contained 6 radish plants (n=8)

Cultivar	All Splits
Topsi	0.1a[1]
Celesta	0.1a
Rudi	3.3b
P	<0.001

[1]Denotes difference at the 5% level

Periderm thickness: After 10 days of storage there was no significant difference in the average thickness of the periderm between cultivars (Figure 11).

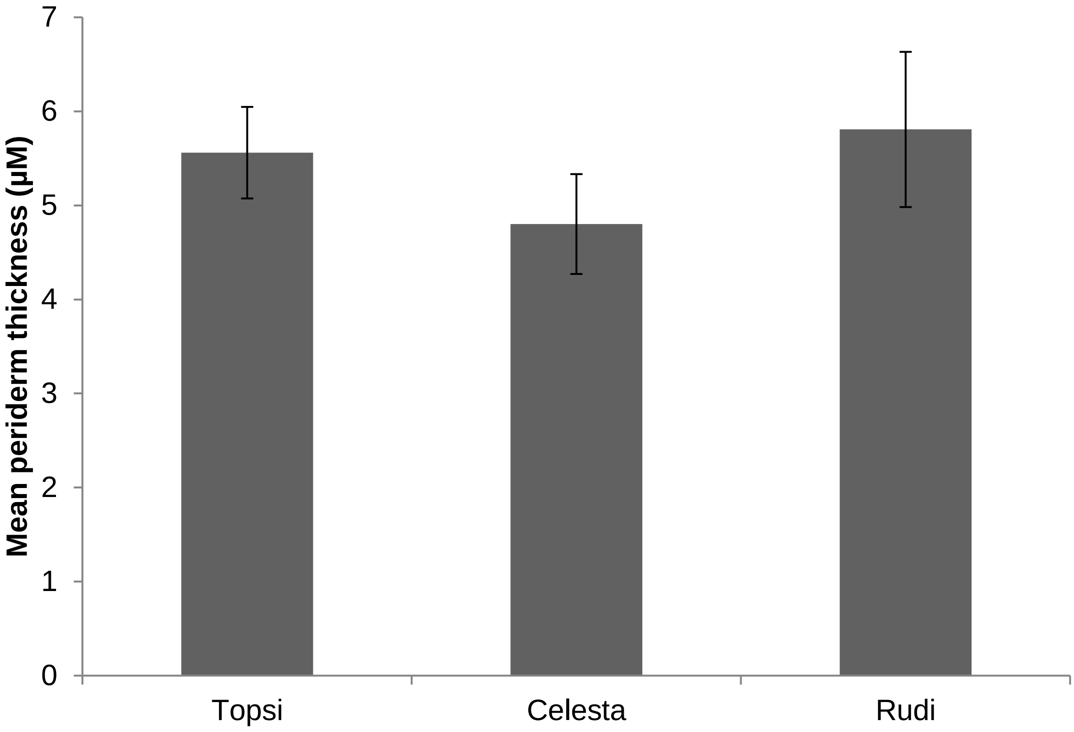

Figure 11 The mean periderm thickness of radish of different cultivars. For 'Topsi': n=10, for 'Rudi': n=12, for 'Celesta': n=6. Bars represent ± the standard error of the mean for each group, P=0.674

It was observed the thickness of the periderm tended to be greater for the split radishes than the non-split radishes of the 'Topsi' and 'Rudi' cultivars (Figure 12) although significant differences between the periderm of split and non-split radish was only observed in the cultivar 'Rudi'.

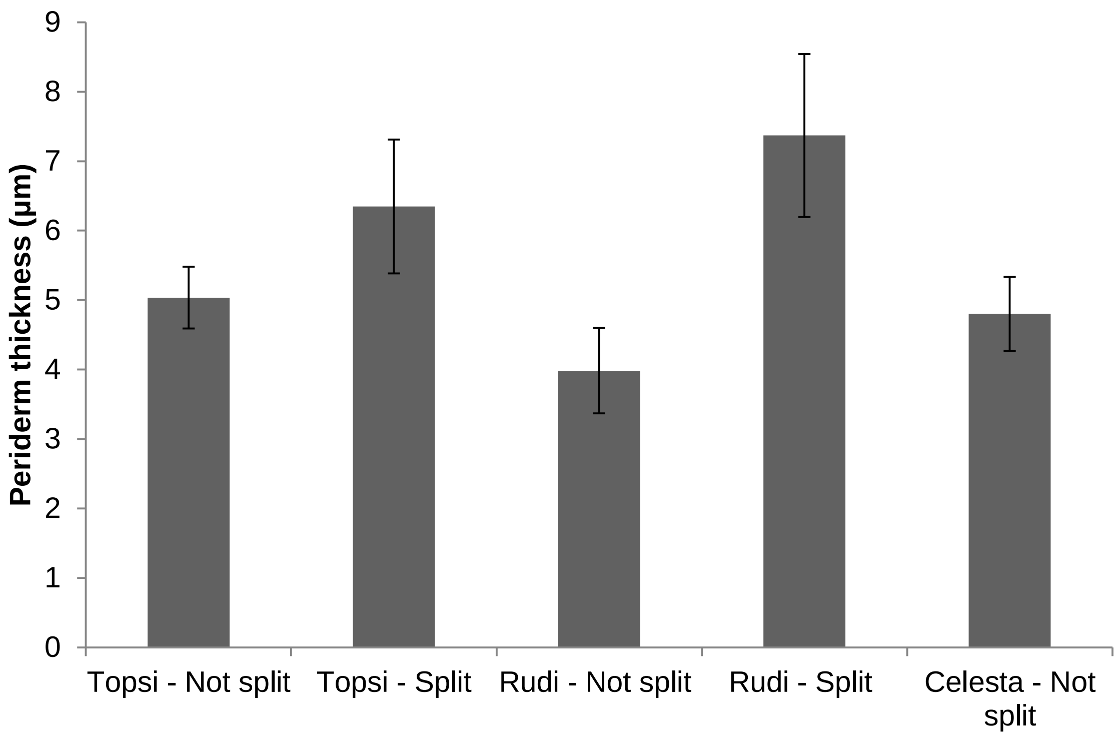

Figure 12 The average epidermal thickness for fresh split and non-split radish of difference cultivars. For non-split 'Topsi': n=6, for non-split 'Rudi': n=6, for non-split 'Celesta': n=6. For split 'Topsi': n= 4 for split 'Rudi' n=6. There were not enough split 'Celesta' radishes for analysis. Bars represent ±the standard error of the mean. P=0.045.

Objective 4: To investigate the effects of water availability during growth on splitting

The results from each experiment under Objective 4 have been summarised separately below.

Objective 4a: To investigate the effect of substrate water content during growth on splitting

Substrate water content:

The different irrigation methods successfully created a difference in volumetric water content (VWC) between the two treatment groups. The wet treatment had an average VWC of 61.2% with a maximum of 65.5% and a minimum of 57.2%. The dry treatment, which was watered by hand, had a greater range with an average VWC of 15.8%, a maximum of 24.8% and a minimum of 8% (Table 25).

Table 25 Mean substrate volumetric water content (VWC) of the trays from the two treatments during the experiment

Treatment	Mean VWC (%)	Max VWC (%)	Min VWC (%)
Wet	61.2	65.5	57.2
Dry	15.8	24.8	8.0

Splitting:

Substrate water content during growth had a significant effect both on the number of radishes which split during growth (P<0.001) but also on splitting susceptibility of radishes postharvest (P<0.001). Both growth and harvest splits were significantly higher for radishes grown under wet conditions (

Table **26**).

Table 26 Mean number of split radishes per tray at harvest (growth split) and as a result of dropping from 1.4m (drop split)

Irrigation	Growth split (%)	Drop split (%)
Wet	63.7	8.9
Dry	1.6	0.5
P	<0.001	<0.001
LSD (5%)	8.44	4.33

Harvest size:

There was no significant different in the hypocotyl diameter of radishes from the two treatments (

Table **27**), as radishes were harvestest when the treatment reached a commercial hypocotyl harvest size rather than on a specific day this result was expected. The radishes grown under dry conditions were harvested five days later than the radishes which were grown under wet condition. Having radishes of the same size for both treatment gives the advantage that any differences observed were not due to differences in the hypocotyl diamter of radishes. Having radishes of a commercial harvest size has the advantage that the results are relevant to growers.

The radishes grown under wet conditions were significantly (P<0.001) longer than the radishes grown under dry conditions ans were less round as a result. The diamter to length ratio for radishes grown under wet conditions was 0.77 compared to 0.90 for the radishes grown under dry condition. There was no significant difference (P=0.359) in fresh weight between the two treatment groups but the radishes grown under wet conditions had a greater water content at harvest (P<0.001) (

Table **27**).

Table 27 The mean hypocotyl diameter, length, fresh weight and water content for radishes grown under different irrigation treatments

Irrigation	Hypocotyl diameter (W) (mm)	Hypocotyl length (L) (mm)	Roundness (W/L)	Hypocotyl fresh weight (g)	Hypocotyl water content (%)
Wet	23.99	31.26	0.77	90.0	95.09
Dry	25.42	28.40	0.90	84.7	94.17
P	0.258	<0.001		0.359	<0.001
LSD (5%)	2.572	1.380		11.97	0.2727

Substrate water content during growth had a significant effect on radish leaf growth. Despite being harvested five days earlier, the radishes which were grown under wet conditions had a greater leaf area (P<0.001), number of leaves (P=0.009), leaf fresh weight (P<0.001) and leaf water content (P<0.001) at harvest when compared to radishes grown under dry conditions (Table 28).

Table 28 The mean leaf area, number, fresh weight and water content for radishes grown under different irrigation treatments

Irrigation	Leaf area	No. leaves	Leaf fresh weight	Leaf water content
Wet	147.3	5.74	78.55	91.92
Dry	81.0	4.89	41.53	91.19
P	<0.001	0.009	<0.001	<0.001
LSD (5%)	23.07	0.61	3.44	0.27

Objective 4b: To determine the effects of water availability on splitting in three different cultivars

Substrate water content:

The different irrigation methods successfully created a difference in volumetric water content (VWC) between the wet and dry treatment groups but there was not a large difference in VWC between cultivars. The dry treatments, which were watered by hand, had a greater range in water contents than the wet treatments which were watered by capillary irrigation. The 'Rudi' wet treatment had an average VWC of 65.0% with a maximum of 70.5% and a minimum of 58.6%. The 'Rudi' dry treatment had an average VWC of 17.2%, a maximum of 23.3% and a minimum of 7.8%. The 'Saxa 2' wet treatment had an average VWC of 64.9 % with a maximum of 69.5% and a minimum of 55.2%. The 'Saxa 2' dry treatment had an average VWC of 16.0%, a maximum of 23.0% and a minimum of 8.5 %. The 'Celesta' wet treatment had an average VWC of 64.6% with a maximum of 69.9% and a minimum of 58.5%. The 'Celesta' dry treatment had and average VWC of 16.3%, a maximum of 22.9% and a minimum of 7.9% (Table 29).

Table 29 Mean volumetric water content (VWC) of the trays from the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta') during the experiment

Cultivar	Treatment	Mean VWC (%)	Max VWC (%)	Min VWC (%)
Rudi	Wet	65.0	70.5	58.6
Saxa 2	Wet	64.9	69.5	55.2
Celesta	Wet	64.6	69.9	58.5
Rudi	Dry	17.2	23.3	7.8
Saxa 2	Dry	16.0	23.0	8.5
Celesta	Dry	16.3	22.9	7.9

Splitting:

Substrate water content during growth had a significant effect on the number of radishes which split during growth ($P<0.001$). As in Objective 4a, splits were significantly higher for radishes grown under wet conditions. Cultivar had no effect on splitting ($P=0.746$) (Table 30).

Table 30 Mean number of split radishes per tray at harvest for the two irrigation treatments (wet and dry) and the three cultivars (‘Rudi’, ‘Saxa 2’ and ‘Celesta’)

Treatment	Rudi	Saxa 2	Celesta	Mean
Wet	37.94	35.56	33.33	35.61
Dry	1.00	6.56	10.78	6.11
Mean	19.47	21.06	22.60	20.86
	P		**L.S.D.**	
Treatment	<0.001		5.59	
Cultivar	0.746		6.85	
Interaction	0.118		9.68	

Hypocotyl:

There was no significant difference in diameter between cultivars, treatments or interaction between the two (data not shown). As radishes were harvestest when the treatment reached a commercial hypocotyl harvest size rather than on a specific day this result was expected. Like Objective 4a the radishes from the cultivar ‘Rudi’ which were grown under wet conditions were harvested five days before those grown under dry condition. For the cultivars ‘Saxa 2’ and ‘Celesta’, the period between the harvest of the radishes grown under wet conditions and those grown under dry conditions was seven days. As before the radishes grown under wet conditions grew the most rapidly and were harvested first. Having radishes of the same size for all treatments gives the advantage that any differences observed were not due to differences in the hypocotyl diamter of radishes. Having radishes of a commercial harvest size has the advantage that the results are relevant to growers.

Hypocotyl fresh weight was significantly (P<0.001) affected by irrigation treatment with the radishes which received more water having a greater weight. This result was consistent for all cultivars. There was no effect of cultivar on hypocotyl fresh weight (P=0.189) (

Table **31**).

Table 31 Hypocotyl fresh weight (g) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean
Wet	100.9	119.7	86.6	12.4
Dry	66.8	72.1	75.3	71.4
Mean	83.9	95.9	80.9	86.9
	P		**L.S.D.**	
Treatment	<0.001		14.3	
Cultivar	0.189		17.9	
Interaction	0.111		24.31	

Hypocotyl water content was significantly (P<0.001) affected by irrigation treatment with the radishes which received more water having a greater water content at harvest. This result was consistent for all cultivars. There was no effect of cultivar on hypocotyl water content (P=0.594) (Table 32).

Table 32 Hypocotyl water content (%) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean
Wet	89.86	90.57	89.80	90.08
Dry	86.84	87.40	87.68	87.31
Mean	88.35	88.98	88.74	88.69
	P		**L.S.D.**	
Treatment	<0.001		1.02	
Cultivar	0.594		0.660	
Interaction	0.660		1.76	

Leaves:

Number of leaves was not affected by irrigation treatment or cultivar (data not shown).

Leaf area was significantly (P<0.001) affected by irrigation treatment with the radishes which received more water having a greater leaf area at harvest. This result was consistent for all cultivars. There was no effect of cultivar on leaf area at harvest (P=0.982) (Table 33).

Table 33 Leaf area (cm^2) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean
Wet	199.2	211.1	204.4	204.9
Dry	147.0	136.6	148.0	143.9
Mean	173.1	173.9	176.2	174.4

	P	L.S.D.
Treatment	<0.001	28.06
Cultivar	0.982	34.37
Interaction	0.788	48.60

Leaf fresh weight was significantly (P<0.001) affected by irrigation treatment with the radishes which received more water having a greater leaf fresh weight at harvest. This result was consistent for all cultivars. There was no effect of cultivar on leaf fresh weight at harvest (P=0.396) (

Table **34**).

Table 34 Leaf fresh weight (g) for the two irrigation treatments (wet and dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean
Wet	88.7	93.2	97.0	93.0
Dry	65.5	66.3	64.0	65.2
Mean	77.1	79.7	80.5	79.1
	P		**L.S.D.**	
Treatment	<0.001		4.30	
Cultivar	0.396		5.26	
Interaction	0.181		7.44	

Objective 4c Results

Substrate water content: There was no significant difference in volumetric water content (VWC) of the substrate between treatments (P=0.092) at the start of the first treatment period or between the W/W and W/D treatments (P=0.399) at the end of the first treatment period. The VWC of D/W was significantly (P<0.001) dryer by day 9 when the first substrate moisture readings were taken after treatments had begun on day 8. The VWC for D/W continued to decrease throughout the first treatment period as the growing medium dried-down. The VWC of W/D fell for the duration of the second treatment period and was ultimately below the minimum VWC for D/W in the first treatment period, D/W dried down to a minimum VWC of 15.1% compared to a minimum of 6.6 % for W/D. By the end of the experiment D/W had not rehydrated to the same level as W/W, having an average VWC of 63.2% compared to an average VWC of 68.1% for W/W (Figure 13). The mean VWC for W/W was 64.1%, for D/W was 49.0% and for W/D was 47.3%.

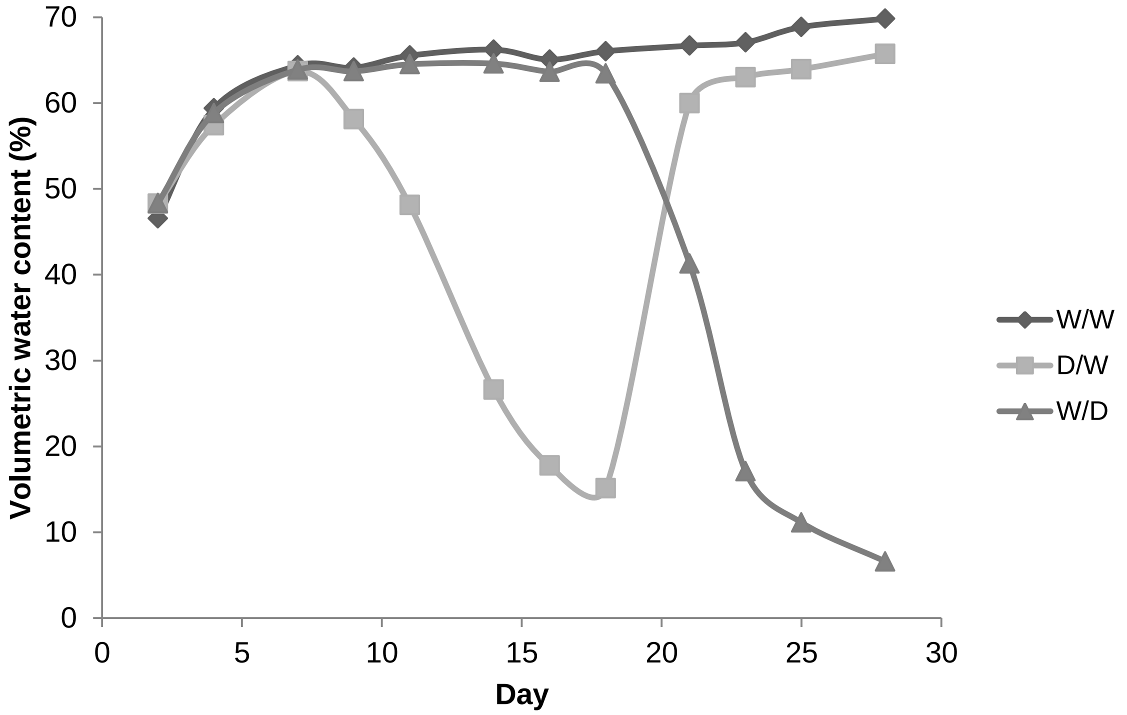

Figure 13 The volumetric water content of pots undergoing different irrigation treatments. Readings were taken with a field domain reflectometer probe

Splits: The average number of split radish per tray was significantly (P<0.001) lower for treatment D/W (9.7%) than both W/W and W/D (42 and 36% respectively) (Table 35).

Size: Both W/W (mean diameter of 27.3mm) and D/W (mean diameter of 25.9mm) radishes were significantly (P<0.001) larger in diameter than the W/D (mean diameter of 11.2mm) radishes. There was no significant difference in diameter between W/W and D/W radishes. However, D/W radishes were more uniform in shape and diameter than the other two treatments (Figure 14), 91% of radishes from D/W were of commercial diameter compared to 67% of W/W and 0% of W/D (Figure 15).

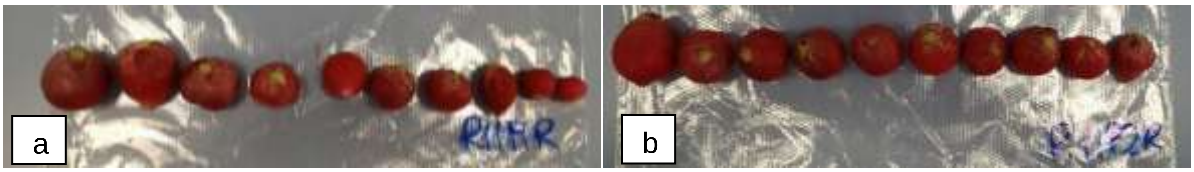

Figure 14 Ten radishes from one experimental tray from W/W (a) and D/W (b). The radishes from W/W (a) appeared to be less uniform than the radish from D/W (b)

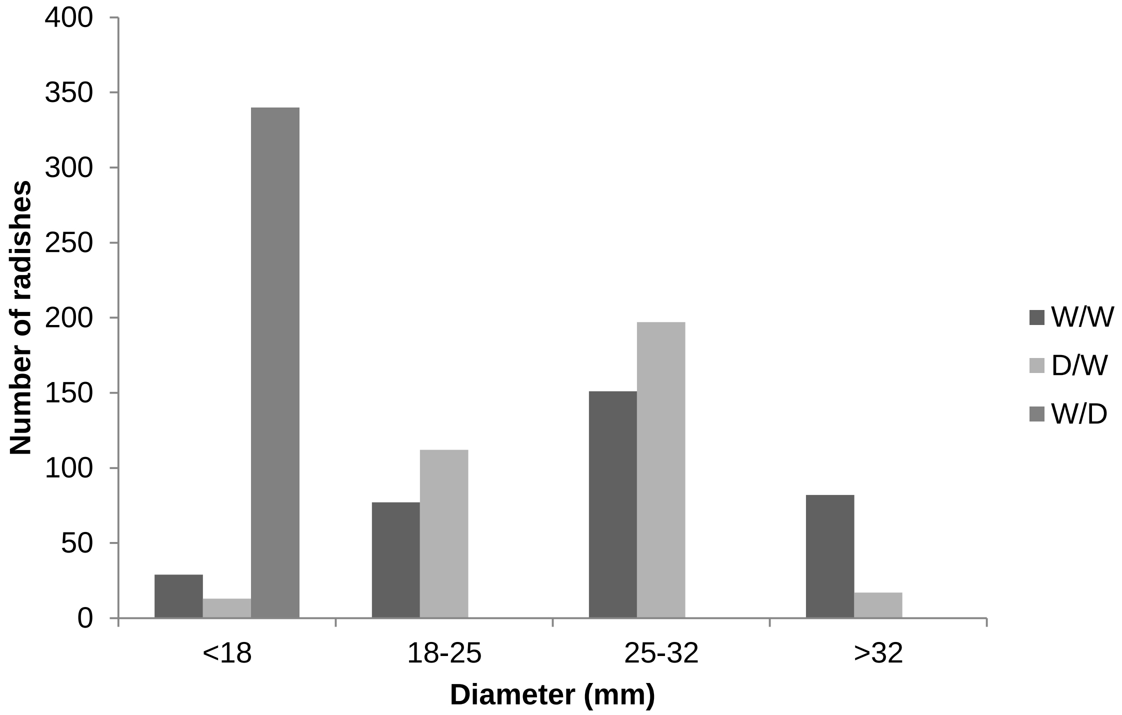

Figure 15 Distribution of sizes of radishes from different treatment groups. Radishes below 18 mm in diameter and above 32mm in diameter are outside of the typical commercial range. Commercial radishes are usually graded into two groups 18 to 25mm and 25 to 32mm.

Weight: The trimmed hypocotyl weight at harvest was significantly different (P<0.001) for all treatments. Hypocotyl weight was heaviest for treatment W/W with a mean weight of 116.0g per tray, D/W radishes were second heaviest with a mean weight of 95.7g and W/D radishes were the lightest with a mean weight of 13.4g (Table 35).

Marketable yield: The calculated marketable yield for W/D was greatest at 78.6g per tray, the marketable yield for W/W was 45.1g per tray and there was no marketable yield for treatment W/D as the radishes were all <18 mm diameter.

Hypocotyl water content: The water content of the hypocotyls at harvest was significantly (P<0.001) lower in the W/D treatment (78.7%) compared to both the W/W (94.7%) and D/W (95.5%) treatments (Table 35).

Table 35 Significant effects of irrigation treatment on splitting and yield at harvest (n=34, d.f.=66)

Treatment	Number of split radish per tray	Total weight (g)	Trimmed hypocotyl weight (g)	Diameter (mm)	Hypocotyl water content (%)
Wet/Wet	4.2b[1]	185.6c	116.0c	27.3b	94.7b
Dry/Wet	1.0a	139.8b	95.7b	25.9b	95.5b
Wet/Dry	3.6b	25.6a	13.5a	11.2a	78.7a
P	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Denotes difference at the 5% level

Objective 4d Results

Substrate water content: There was no significant difference in volumetric water content (VWC) when seeds were planted. After treatments began the dry treatment, D/W was already significantly (P<0.001) dryer than W/W by the first soil moisture reading (day 11) and continued to dry down until irrigation was restarted at day 18 when the VWC of D/W increased. The VWC of D/W was no longer significantly different from W/W by the second reading (day 25) after treatments changed (P=0.561). The average VWC for W/W was 63.1% with a maximum of 65.5 % and a minimum of 60.0%. The average VWC for D/W was

52.9% with a maximum of 66.2 % and a minimum of 20.2% (Figure 16).

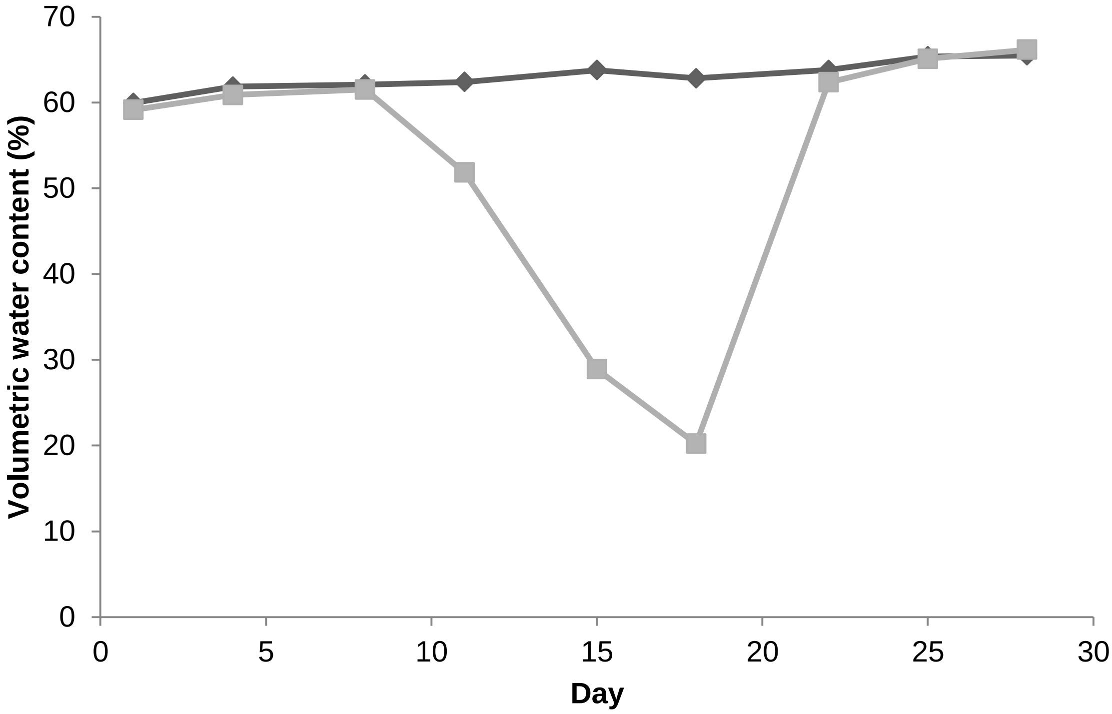

Figure 16 The volumetric water content of pots undergoing different irrigation treatments. Readings were taken with a ML2X Theta Probe. W/W pots (dark grey line) were irrigated for the duration of the experiment. D/W pots (light grey line) received no irrigation for 10 days between day 8 and 18 (n=3)

Stomatal resistance, leaf area and leaf temperature: By day 18 there was no significant differences between treatments in the stomatal resistance (P=0.697) or the leaf area of the radishes (P=0.923). A significant difference was observed in leaf temperature (P=0.034) with W/W plants having significantly cooler leaves at 21.6°C compared to 22.5°C for D/W plants (Table 36).

Table 36 Measurements taken on day 18 prior to irrigation of D/W. W/W was irrigated but D/W had not received any irrigation for 10 days (n=20)

Treatment	Stomatal resistance (m^2 s mol^{-1})	Leaf temperature (ºC)	Leaf area (cm^2)
W/W	1.1a[1]	21.6a	12.8a
D/W	1.1a	22.5b	12.6a
P	0.697	0.034	0.923

[1] Denotes difference at the 5 % level

On day 28 there was no significant difference in the stomatal resistance (P=0.208), the temperature of the leaves (P=0.091) or the leaf area of the radishes (P=0.369) between the two treatments (Table 37).

Table 37 Measurements taken on day 28. W/W had received irrigation for the duration of the growing period but D/W received no irrigation from day 8 to 17 (n=20)

Treatment	Stomatal resistance (m^2 s mol^{-1})	Leaf temperature (ºC)	Leaf area (cm^2)
W/W	1.0a[1]	15.9a	128.5a
D/W	1.0a	15.6a	130.7a
P	0.208	0.091	0.369

[1]Denotes difference at the 5 % level

Harvest: At harvest there were significant differences in the number of split radish per pot, the total weight of the radish, the trimmed radish weight and the diameter of the hypocotyls. No significant difference was observed in hypocotyl length between treatments. The average number of split radish per pot was significantly lower (P=0.001) for D/W (6.5 split radish) than W/W (7.7 split radish). Radishes were significantly larger in D/W than W/W for total weight (P=0.001), trimmed weight (P=0.011), and hypocotyl diameter (P=0.024). The length was not significantly different (P=0.491) between the two groups. The fresh weight of the hypocotyl was significantly greater (P=0.011) for D/W, but dry biomass of the hypocotyls was found not to be significantly different (P=0.539). Radishes from D/W were found to have significantly greater hypocotyl water content than W/W (

Table **38**).

Table 38 Effects of irrigation treatment on splitting and yield at harvest (n=34, d.f.=66)

Treatment	Number of split radish per pot	Total weight (g)	Hypocotyl water content (%)	Trimmed hypocotyl weight (g)	Hypocotyl diameter (mm)	Hypocotyl length (mm)
W/W	7.7a[1]	93.7a	94.8a	41.3a	16.9a	27.6a
D/W	6.5b	113.5b	95.7b	53.8b	18.6b	28.1a
P	<0.001	<0.001	<0.001	0.011	0.024	0.491

[1] Denotes difference at 5% level

Marketable yield: The calculated marketable yield for D/W was greatest at 10.7g per tray compared to 4.1g per tray for W/W.

A greater proportion of radishes from D/W were of a commercial size at harvest (Figure 17).

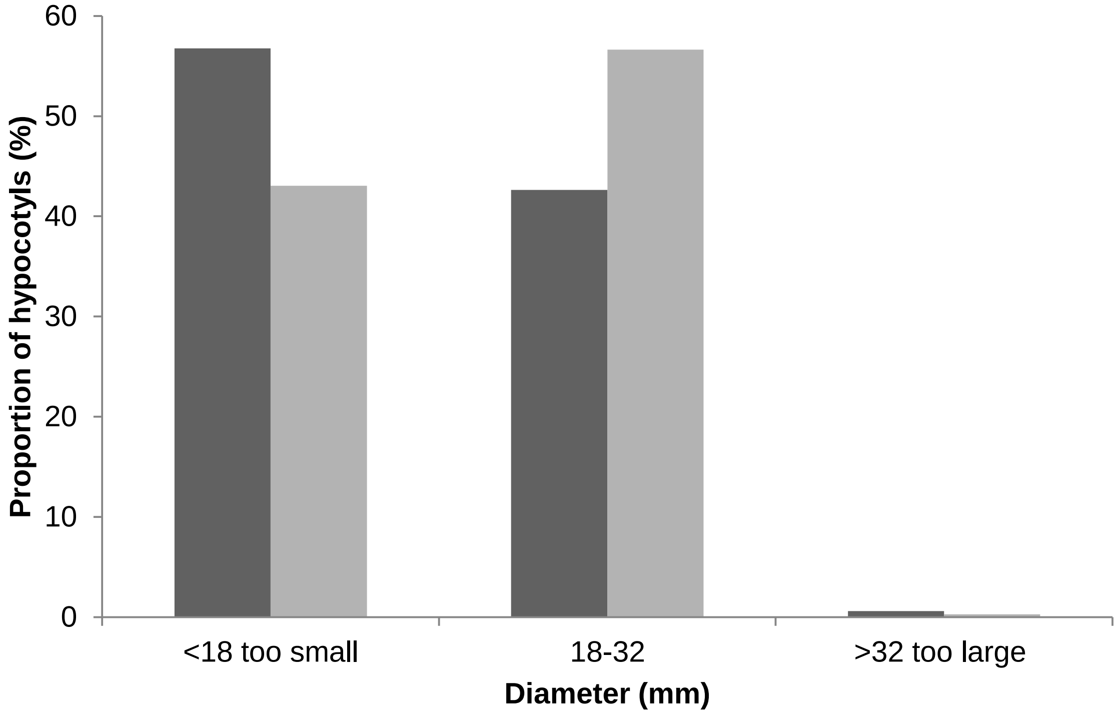

Figure 17 The size distribution of radishes grown with different irrigation regimes. W/W (dark grey bars) were irrigated for the duration of the experiment but D/W (light grey bars) received no irrigation for 10 days from day 8 to day 17 (n=340)

Objective 4e: To determine the factors in a period of drying down which result a reduction in splitting

Substrate water content: There was no significant difference (P=0.204) in volumetric water content (VWC) at the start of the experiment when seeds were planted. At harvest there was a significant difference (P<0.001) in VWC between the pair T1 (66.8%) and T3 (65.6%), with the pair T2 (61.1%) and T3 (65.6%) with T4 (55.8%) (Figure 18).

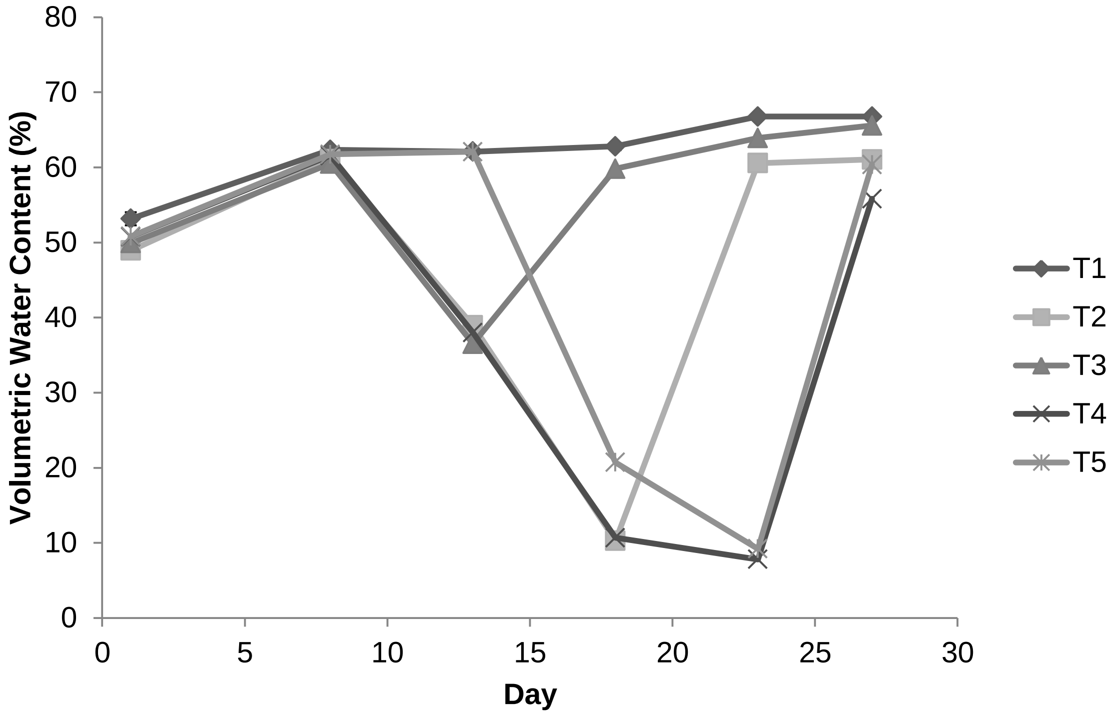

Figure 18 The volumetric water content of pots undergoing different irrigation treatments. T1 pots were irrigated for the duration of the experiment. T2 pots received no irrigation for 10 days between day 8 and day 17, T3 pots received no irrigation for 5 days between day 8 and day 12, T4 pots received no irrigation for 15 days between day 8 and day 22 and T5 pots received no irrigation for 10 days between day 13 and day 22. n=24.

Harvest: Significant differences were observed between treatments for the number of split radishes per tray, the total weight of the radishes, the trimmed hypocotyl weight, the length and diameter of the hypocotyls and the water content of the hypocotyls (Table 39). The average number of split radish per pot was significantly lower ($P<0.001$) for plants in T2 and T4 (1.38 and 1.67 split radish on average respectively) than T5 (3.79 split radish on average) and T5 radishes had significantly fewer ($P<0.001$) splits than radishes from T1 and T3 (6.5 and 7.5 split radishes on average respectively). The size was significantly different between treatment groups in terms of total weight ($P<0.001$), trimmed weight ($P<0.001$) and hypocotyl diameter ($P<0.001$). Radishes grown in T4 were the smallest for all of these parameters and radishes from T1 and T3 were the largest for all these parameters. All treatments produced similarly round radish except the radishes in T4 ($P<0.001$) which received the longest period of drying down and produced radishes which were proportionally longer in length than diameter than the other treatment groups (Table 39).

Table 39 Significant effects of irrigation treatment on splitting and yield at harvest. Different letters, within columns, indicate that values are significantly different (P<0.05) (n=24)

Treatment	Total weight (g)	Trimmed hypocotyl weight (g)	Hypocotyl diameter (D) (mm)	Hypocotyl length (L) (mm)	Roundness (L/D)	Number of split radishes per tray	Hypocotyl water content (%)
T1	197.2d[1]	98.8c	23.3c	30.3c	1.2a	6.50c	96.0a
T2	138.1c	66.1b	21.5c	26.4b	1.2a	1.38a	96.6b
T3	196.9d	104.8c	25.7d	31.4c	1.2a	7.54c	96.2ab
T4	51.7a	10.4a	9.7a	19.2a	2.1b	1.67a	96.6b
T5	104.9b	59.9b	18.7b	24.2b	1.3a	3.79b	96.6b
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004

[1]Denotes difference at the 5 % level

The number of split radishes was found to be positively correlated with the VWC (%) on day 18 but there was only a slight negative correlation between splits and duration of dry period (

Table **40**). The duration of the dry period and the trimmed hypocotyl weight at harvest were found to be negatively correlated.

Table 40 Number of split radishes compared to duration of dry period and volumetric water content (VWC) on day 18 when the hypocotyl has begun to expand.

Treatment	VWC (%) on day 18	Duration of dry period (days)	Number of split radishes per tray
T1	62.8c[1]	0	6.50c
T2	10.a	10	1.38a
T3	59.8c	5	7.54c
T4	10.7a	15	1.67a
T5	20.7b	10	3.79b
P	<0.001		<0.001

[1]Denotes difference at 5 % level

Marketable yield: The calculated marketable yield for T2 was greatest at 43.5 g per tray, the marketable yield for T1 was next largest at 29.1g per tray, then for T5 was 22.0g per tray, for T3 was 21.7g per tray and the marketable yield for T4 was least at 0.3g per tray.

Objective 5: To investigate the effects of hypocotyl water content on post-harvest splitting susceptibility

Impact:

By air drying and saturating in dH_2O a range in hypocotyl water contents between a minimum 93% and a maximum of 97% at saturation was achieved. All radishes were considered to be commercially viable by examiners.

There appeared to be a threshold at a hypocotyl water content of 96.5% above which splitting as a result of dropping occurred. The average percentage of split radish per pot below a hypocotyl water content of 96.5% was 0.8% (n=42), above 96.5% this number increased to 38.1% (n=28) (Figure 19).

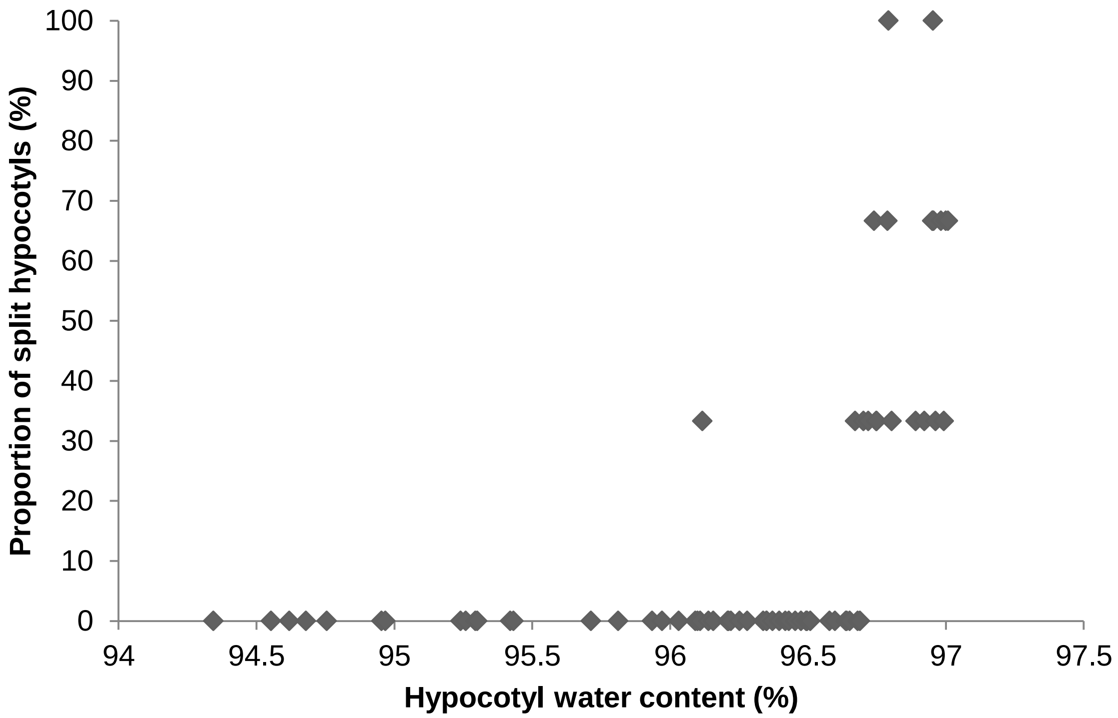

Figure 19 The percentage of split radish hypocotyls in a sample of three which split as a result of dropping down a 1.4m tube onto an aluminium plate at different hypocotyl water contents. n = 70.

Puncture:

A narrow range in hypocotyl water contents between a maximum of 97.4% and a minimum of 95.6% was observed at the time of texture analysis. All radishes used for texture analysis were considered commercially viable by examiners. There was a negative linear correlation between water content (WC) and puncture force (P<0.001) with 44.4% of the variance being accounted for (Figure 20). These results would suggest radishes are less resistant to splitting as a result of puncture at higher water contents.

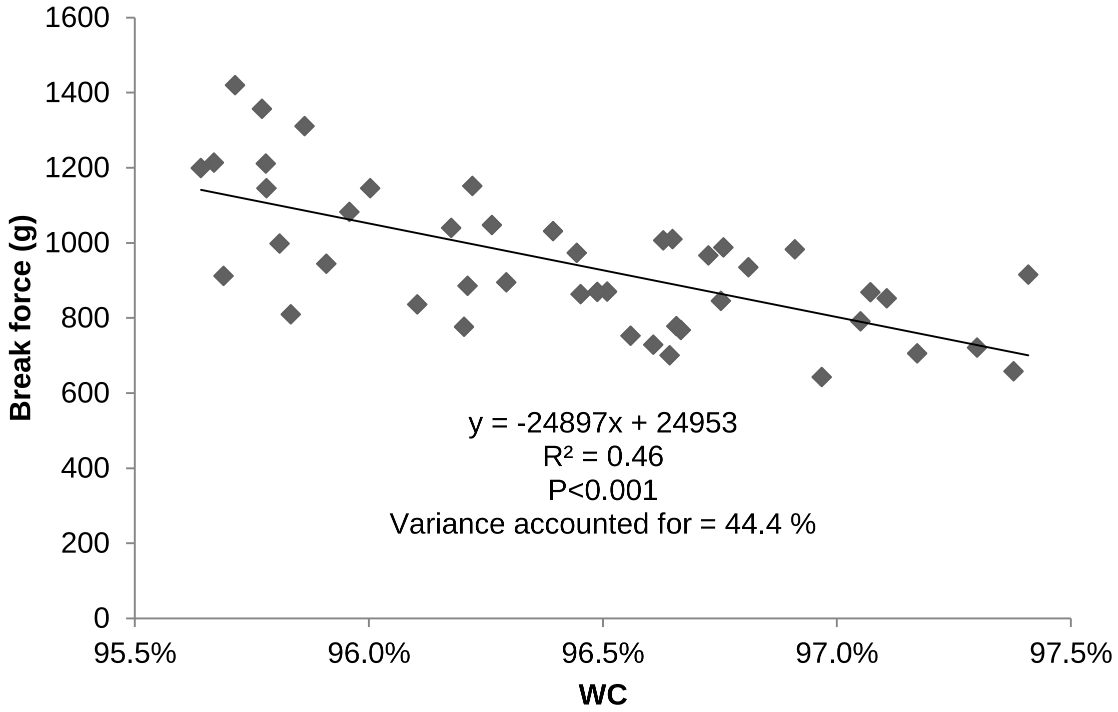

Figure 20 The force required to puncture the hypocotyl of radishes at different relative water contents (RWC) (n=55)

Compression:

A narrow range in hypocotyl water contents between a maximum of 98.7% and a minimum of 95.5% was observed at the time of texture analysis. All radishes used for texture analysis were considered commercially viable by examiners.

When a correlation matrix between the force required to split the radish and the water content (WC) and diameter of the radish was conducted it was found both the size of the radish and its water status were correlated with break force. There was a negative correlation between break force and WC suggesting splitting susceptibility increased as water content increased. There was a positive correlation between diameter and break force suggesting larger radishes are more resistant to damage from crushing (Table 41).

Table 41 Correlation matrix for break force due to crushing, water content (WC) and hypocotyl diameter

	Diameter (mm)	WC	Break force (Kg)
Diameter (mm)	1		
WC	0.180	1	
Break force (Kg)	0.527	-0.337	1

When both diameter and WC were included in a multiple linear regression with break force there was a significant relationship (P<0.001) and 66.1% of the variation in break force was accounted for.

Table 42 Model determined by multiple linear regression and stepwise deletion for the relationship between splitting observed at G's and weather

	Model fitted	P	Variance accounted for
Break force (Kg)	Diameter + WC	<0.001	66.1

Pressure:

A narrow range in hypocotyl water contents between a maximum of 97.4% and a minimum of 97.5% was observed at the time of analysis. All radishes used were considered commercially viable by examiners.

A negative linear relationship was observed between water content and pressure (P=0.034) with 18.4% of the variance being accounted for. This would suggest at higher water contents the cells within the radish hypocotyl are under increased pressure.

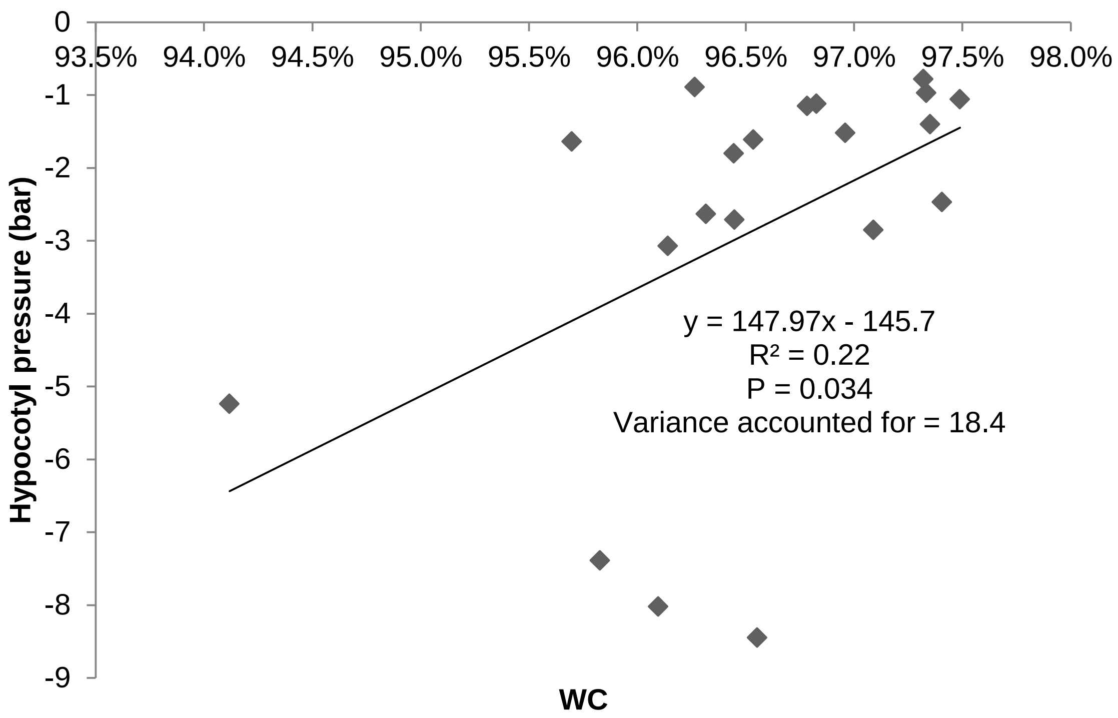

Figure 21 Linear correlation between hypocotyl pressure (bar) and water content (WC)

Objective 6: To investigate the effects of hypocotyl temperature on post-harvest splitting susceptibility

The five different water baths set to 5, 10, 20, 30 and 40°C resulted in radishes with mean temperatures of 5.6, 10.8, 22.0, 29.7 and 39.0°C respectively.

Radish splitting susceptibility as a result of impact was found to be negatively correlated with temperature (P=0.008). The greatest amount of splitting, 65%, was observed at the lowest temperature, 5.6°C, and the least amount of splitting, 0%, was observed at the highest temperature, 39.0°C. These results suggest radishes are more susceptible to splitting at lower temperatures (Figure 22).

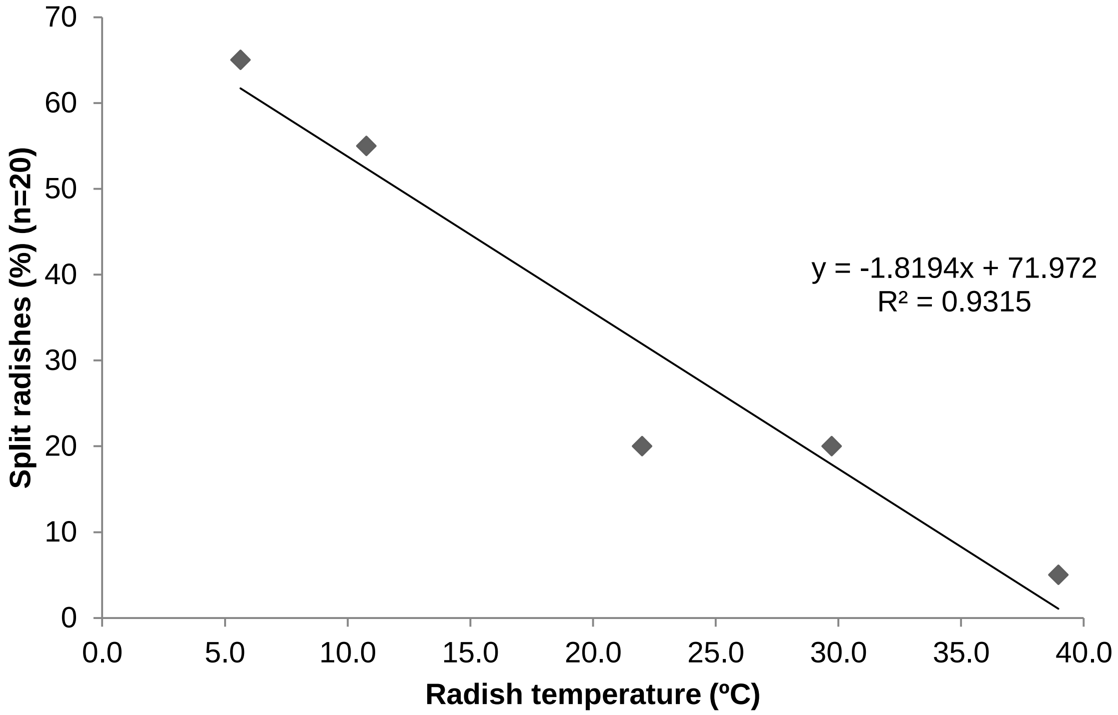

Figure 22 Percentage of radishes which split as a result of dropping at different temperatures

Discussion

Objective 1: Investigating splitting trends in commercial produce

Objective 1a: To investigate trends in commercial quality assessment data for splitting in 2012, 2013 and 2014 to see how these correlate with weather conditions during growth and at harvest

In support of the hypothesis that weather conditions during growth and at harvest affect splitting, correlations were observed between splitting in commercially grown radishes and the weather recorded 14 km away. As this was an observational study, there are limitations to this investigation as this was not a scientific experiment and factors which may have had an effect on the results were not controlled. In addition due to the nature of weather data all of the parameters were highly correlated therefore it is impossible to determine exactly which factors were affecting splitting without conducting controlled experiments where each factor can be tested individually. Nevertheless, the results are encouraging and support further investigation into the effects of environment during growth and post-harvest during handling.

The results suggest weather did have an effect on splitting. Rainfall during growth and at harvest tended to be positively correlated with splitting suggesting an increase in rainfall or relative humidity will increase splitting and hence soil plant water interactions have an important role in splitting. It is hypothesised increased rainfall during growth would lead to increased hypocotyl water content and turgor pressure resulting in increased splitting susceptibility as has been shown in other crops (McGarry 1993, 1995). Similarly low temperatures are also thought to increase splitting susceptibility by increasing turgor pressure. Low temperatures have been shown to decrease failure force in other crops (Bourne 1982). Controlled experiments should be carried out to confirm the validity of the correlations observed.

A limitation of using rainfall to indicate soil moisture is that it fails to include water added to the soil in the form of irrigation. Radishes are usually irrigated when they are drilled in an attempt to prevent scab. Following this the crop tends not to be routinely irrigated, with water only being applied when absolutely necessary. However, for a more accurate indication of soil moisture this information should be included. Rather than attempting to predict soil moisture from the amount of water added, it would be preferable to measure soil moisture directly. The amount of water in the soil is affected by many variables. The amount of water added to the soil will be determined by rainfall and irrigation, but many other factors, such as the maturity and density of plants, will affect how rapidly the soil dries.

The exact time of harvest for each lot of radishes was unknown therefore the mean temperature and relative humidity on the day of harvest were used to indicate what the conditions were. It would have been more accurate to measure the temperature of the radishes directly during harvest and handling. The relative humidity while the radishes were being harvested and handled could have been logged to give more accurate results.

Objective 1b: Seasonal variation in splitting susceptibility and relative water content for 2014

In 2014 further analysis of commercially produced radishes was conducted to determine, if the amount of splitting observed by G's can be predicted by the weather conditions during growth and harvest of the radishes, if the weather conditions correlate with the relative water content (RWC) of the radishes, if the RWC of the radishes correlates with the amount of splitting recorded by G's and finally if impact texture analysis can be used to test splitting susceptibility. Correlation matrices and regression were used to determine the parameters which were correlated and to investigate trends in splitting.

There were more splits on arrival at Harper Adams compared to the number of radishes which were recorded as split at G's. This suggests radishes continued to split after harvest while they were being couriered. There is anecdotal evidence from growers that radishes continue to split during the first couple of days of storage and these results would appear to support this. It would be expected that water lost from the hypocotyl after harvest would decrease splitting susceptibility by decreasing the pressure within the hypocotyl. If keeping the radishes under conditions of high relative humidity maintains the hypocotyl water content above a critical value they would still be susceptible to splitting as a result of impact from being moved around and compression from being stored in large Dolavs.

More radishes split as a result of dropping than were recorded as either split at G's or which were split on arrival at Harper Adams. This result is as would be expected as it is unlikely commercial radishes would all experience drops of this magnitude. The number of radishes which split as a result of dropping and the number of radishes which were split on arrival at Harper Adams was highly correlated ($P<0.001$) and there was trend ($P=0.091$) between the number of radishes which split as a result of dropping and the number of radishes which were recorded as split by G's. These results suggest impact texture analysis may be a representative way to test splitting susceptibility.

The number of radishes which split as a result of impact texture analysis was highly correlated with RWC ($P<0.001$). However, no relationship was observed between RWC and the number of split radishes which were recorded by G's or weather conditions and RWC. It should be remembered that RWC was only measured at Harper Adams and not at G's at the point of harvest. To determine definitively that there was no relationship between RWC and the splitting observed by G's or weather conditions, further assessment of the RWC of the radishes at harvest would need to be made at G's. Split radishes may lose water more rapidly than intact radishes due to the split surface which would not only have lost protection from the periderm but would also have an increased surface area. This could potentially cause boxes containing a large percentage of split radishes to have a lower RWC on arrival at HAU than boxes with relatively few split radishes. When the RWC of the radishes which were not split and were split on arrival were compared no significant difference was observed yet the water content was significantly different, with the radishes which split having greater water contents. This might suggest the radishes had, had high relative water contents at the point of splitting as they still maintained at high water content but they had lost more water than the non-split radishes in the time between splitting and measurement of the RWC, therefore resulting in a reduced RWC.

Weather conditions during growth and at harvest were significantly correlated with the amount of splitting which was recorded by the quality assessment team at G's and the model containing the correlated weather parameters accounted for 57.0 % of the variance in splitting observed at G's. Due to the nature of weather data all the parameters would have had interactions with each other and it is impossible to determine exactly which were having an effect on splitting. Further investigation under controlled conditions is required to determine exactly which factors during growth and at harvest have an effect on splitting.

The weather parameters were not significantly correlated with the amount of splitting observed on arrival at HAU or the number of radishes which split as a result of impact texture analysis. Weather conditions were unlikely to be correlated with the amount of splitting which was observed on arrival at HAU or as a result of impact texture analysis. This can be explained by the amount of time which had passed after harvest. The radishes were couriered on the day of harvest and did not arrive at HAU until the following day at least 24 hours later. The temperature and relative humidity during transport from G's to Harper Adams were not controlled by the couriers and would have been different to those experienced by the radishes during growth and at harvest and different again from the conditions which the radishes were exposed to during impact texture analysis which was done under ambient conditions. The postharvest conditions were likely to have had an effect on the relative water content and temperature of the hypocotyl during transport and then during texture analysis and calculation of RWC but as these were not measured the effects cannot be determined. Further investigations into the effects of RWC on post-harvest splitting susceptibility were conducted under controlled conditions at HAU.

Objective 2: To establish a key for the development stages of radish

Radish phenology is well represented by the BBCH root and stem scale with the exception of growth stage 41. The proposed scale with the suggested modifications is essential for research into splitting as growth stage 41 is an important phenological stage prior to which no splitting can occur.

A key was developed for the growth stages of radishes. Timings for the growth stages of the cultivar 'Rudi' were also established although further work is required to determine how available water content and other environmental factors affect growth rate and how variable these timings can be for different cultivars. Rates of growth had not plateaued at harvest and there had been no flowering showing radishes are not physiologically mature at commercial harvest.

Objective 3: To investigate the splitting susceptibility of different radish cultivars

In support of the hypothesis that choice of cultivar has a significant effect on splitting susceptibility, a significant difference in splitting between cultivars was observed. *Raphanus sativus* 'Rudi' was found to have significantly more (P=0.001) splits at harvest than 'Celesta' or 'Topsi'. An explanation for the differences in splitting susceptibility between cultivars is periderm thickness. Research into splitting in carrots has shown that removing the periderm makes them more resistant to splitting (Hartz *et al.* 2005). Similarly a thinner periderm in radishes may be more resistant to splitting possibly as a result of greater elasticity. No significant difference was found in periderm thickness between cultivars although the periderm thickness followed the pattern of splitting susceptibility with 'Rudi', which split the most readily, having the thickest periderm and 'Celesta', which split the least, having the thinnest periderm. These results support the hypothesis that periderm thickness has an effect on splitting susceptibility, although further work with greater replication is requited for statistical robustness. These findings are similar to research conducted in cherries where cuticle thickness of different cultivars has been shown to be negatively correlated with the splitting susceptibility of that cultivar meaning cherries with thicker cuticles are more prone to splitting (Demirsoy and Demirsoy 2004). Further research is required to determine if periderm thickness is under genetic control in radishes. Due to the small differences and high levels of heterogeneity in periderm thickness within cultivars, large numbers of replications would be required to establish representative values for periderm thickness for different breeding lines. However, if periderm thickness is under genetic control then splitting resistance in radishes has the potential to be improved through breeding.

A similar result was observed within cultivar types as the radish which split had a thicker periderm on average compared to radish of the same cultivar which did not split although this difference was only significant in the case of the cultivar ‘Rudi’. Two of the varieties; ‘Rudi’ and ‘Topsi’, used in this experiment have previously been described by Muminović (2005) as having a high degree of heterogeneity within cultivar. The replication for this work was quite low; therefore further work with greater replication is required as this may give more conclusive results.

Objective 4: To investigate the effects of water availability during growth on splitting

Objective 4a: To investigate the effect of substrate water content during growth on splitting

Growth rate was affected by substrate water content, the radishes which were grown under dry conditions took five days longer to achieve commercial diameter size than radishes grown under wet conditions. This finding is supported by previous research in which drought conditions were found to reduce or stop cellular division and cellular expansion in radishes (Joyce *et al.* 1983). Leaf growth was also reduced in the radishes grown under dry conditions. At harvest when the radishes grown under dry conditions had been grown for an additional five days the leaf area, number and fresh weight were all significantly less than the results for the radishes grown under wet conditions. Smaller leaves would have resulted in a reduced photosynthetic area and may explain in part the reduced growth rate of the radish hypocotyls. As leaves are removed from the majority of radishes prior to sale in the UK, it is not thought leaf size would be of great importance to the consumer.

The amount of splitting observed at harvest and postharvest splitting susceptibility were both lower in radishes grown under dry conditions. The radishes grown under dry conditions had lower water content at harvest ($P<0.001$) suggesting they may have had a lower turgor pressure and the cells were under less pressure making them less susceptible to splitting. However, as turgor pressure was not measured this theory cannot be tested. Differences in splitting susceptibility may have also been due as a result of difference in growth rate. A slower growth rate may have resulted in less stress within the hypocotyl. However, this would not explain the difference in postharvest splitting susceptibility. Difference in splitting during grown and in postharvest splitting susceptibility could also be due to differences in cellular composition. Joyce *et al.* (1983) suggested lignin synthesis may be reduced to a lesser extent by water deficit than cell division and expansion resulting in a build-up of cell wall material. Changes in the structure and strength of cell walls may affect splitting susceptibility both during growth and postharvest as splits have been shown to propagate through cells rupturing the cell walls.

Marketable yield was greater for radishes grown under dry conditions as there was no significant difference in fresh weight between the two treatment groups but there was significantly less splitting observed in radishes grown under dry conditions, therefore the weight of commercially viable radishes would have been greater for the dry group. In addition the radishes grown under dry conditions were rounder and potentially more attractive to the consumer. The mean width to length ratio for the radishes grown under dry condition was 0.90 compared to 0.77 for the radishes grown under wet conditions.

In conclusion the hypothesis that substrate water content and water availability during growth do have a significant effect on splitting in radishes as was previously suggested in the larger winter varieties (Kang and Wan 2005) was supported. Radishes split significantly more when grown under wet conditions compared to dry conditions.

Objective 4b: To determine the effects of water availability on splitting in three different cultivars

Similar to the results from Objective 4a, less rapid growth rates were observed for radishes which were grown under dry conditions were observed. The cultivar 'Rudi' had a five day difference in harvest time for the two irrigation treatments but 'Celesta' and 'Saxa 2' were effected to a greater extent by the treatments as they had a seven day difference in harvest time.

As in Objective 4a available water content affected splitting with radishes which were grown under dryer conditions splitting less. Possible explanations for this were discussed in Objective 4a.

The other results from Objective 4b were also very similar to those from Objective 4a. No significant effect of cultivar was found for any of the variables measured. This knowledge is of use to growers because it suggests results from irrigation studies for one cultivar can be extrapolated to other cultivars without the requirement for additional experiments.

The hypothesis that splitting is affected by substrate water content during growth was supported. The hypothesis that cultivar may have an interaction with the effects of substrate water content was refuted. In potato, cultivar has been shown to have a significant interaction with the effects of turgor on failure force (Bajema et al 1998). The results from this experiment suggest all cultivars are similarly affected by substrate water content.

Objective 4c: To investigate the effects of the timing of a period of drying down on splitting

Differences in minimum substrate VWC for the dry treatment during the first and second dry period were observed. Treatment D/W which was dry in the first half of the experiment reached a minimum VWC of 15.1% whereas treatment W/D which was dry for the second treatment period reached a minimum VWC of 6.6%. These results were expected and can be explained by greater transpiration of the more developed stage of plants in the second treatment period of the experiment. By the end of the second dry treatment period, W/D plants had 125% more leaves (data not shown) on average than the D/W plants did at the end of their earlier dry period.

The substrate VWC of the trays in the D/W treatment had not increased compared to the VWC of the W/W trays by the end of the experiment. This can be explained by both hysteresis and the high humus content of the substrate. Hysteresis can result in substrates at the same water potential having different soil moisture content depending on whether they are wetting or drying substrates. Wetting substrates have lower moisture than drying substrates at the same water potential which is in keeping with results from this experiment. John Innes No.2 has high humus content as it contains plant based material in the form of sphagnum moss peat. The initial dry treatment may have resulted in plasmolysis of some of the cells in the plant material reducing the water holding capacity of the substrate.

The amount of splitting in radishes at harvest was affected by the VWC of the substrate between day 7 and day 17, the time of the first treatment period. The splitting rate was not significantly different for the plants in W/W and W/D, the common factor between these treatments is a higher VWC during this period compared to the treatment D/W which split less and had a lower VWC at this time. Preliminary experiments have shown the majority of the hypocotyl expansion in radishes occurs after day 17. The radish hypocotyl diameter increased 10 fold after day 17 (data not shown). The increase in splitting which was observed in plants which are exposed to a high VWC before this point may be a consequence of high turgor pressure in a hypocotyl with a small area which does not have the capacity to expand as far as a larger hypocotyl would. The high water content of the growing medium may result in a greater uptake of water by the hypocotyl resulting in a greater turgor pressure in the hypocotyl; this coupled with the relatively small size of the hypocotyl may cause it to split.

The hypocotyl water content was affected by substrate water content in the final 10 days prior to harvest as treatments W/W and D/W had hypocotyl water contents which were not significantly different to each other yet were significantly greater than the hypocotyl water content of treatment W/D at harvest despite W/D having a similar mean VWC (47.3 %) to D/W (49.0 %). These results are as expected; reduced VWC of the growing medium would reduce the water availability to the plant. As the water content of the growing medium decreases the pressure required by the plant to extract the water increases.

In conclusion, the result from this experiment showed timing of available water content was crucial in predicting splitting. The substrate VWC on day 17, approximately growth stage 41, was really important and explained far more of the splitting than the mean substrate VWC.

Objective 4d: To investigate the effects of a period of drying down on plant responses

Differences were again observed in the number of splits at harvest. As with the previous experiment the treatment which received a 10 day period of drying down from day 8 had the fewest splits at harvest. However, in this experiment a far larger number of split radishes (W/W mean 7.7, D/W mean 6.5 per tray) were observed in both treatment groups than in the previous experiment (W/W mean 4.2, D/W mean 1.0 per tray). This may have been due to the higher water content of the compost at drilling, in this experiment the VWC was 59.6% compared to 47.0% in Objective 4c. The higher VWC from the onset could have resulted in a greater turgor pressure in the hypocotyls due to up take of water from the growing medium for a longer duration. The higher VWC at drilling retarded the growth of the plants compared to plants in the previous experiment. At harvest, plants from this experiment had a mean trimmed hypocotyl weight of only 47.5 g / tray compared to 105.9 g / tray for Objective 4c. The smaller plants, and consequently leaves, in the second experiment will not have transpired as much and therefore the VWC during the drying period only dropped to 20.2% compared to 15.1% in the previous experiment.

There were fewer radishes of commercial size for W/W than D/W. The radishes from W/W could have been grown longer to allow them to achieve a similar proportion of commercially sized radishes but this would have also increased the time they had to split. At harvest, W/W already had a greater proportion of split radishes than D/W and this would only have increased.

On day 18 a significant difference was observed in leaf temperature between treatment groups. However, there was no significant difference observed in leaf area or stomatal resistance. This difference could have been due to other factors such as increased hair on leaves in D/W plants and requires further investigation.

Objective 4e: To determine the factors in a period of drying down which result a reduction in splitting

Splitting was not due to the duration of the drying period during growth; it was principally affected by the substrate VWC around the time of growth stage 41 as the number of split radishes was found to be positively correlated with the VWC (%) on day 18 but there was only a slight negative correlation between splits and duration of dry period. Objective 2 established that day 18 is shortly after growth stage 41 when the hypocotyl begins to expand.

Both timing and duration of the dry period are important components of radish yield. A negative correlation was found between the duration of the dry period and the trimmed hypocotyl weight at harvest.

T2 which received a 10 day period of drying down from day 8 to day 17 had the highest marketable yield as a result of both a high proportion of radishes of a commercial size and a low proportion of split radishes which is in keeping with results from objective 3a and 3b.

The treatment with the longest period of drying down, T4 produced radishes which were proportionally longer in length than diameter than the other treatment groups ($P<0.001$) this may have been due to a lack of water preventing hypocotyl swelling or may have been due to taproot elongation in the search for water.

Objective 5: To investigate the effects of hypocotyl water content on post-harvest splitting susceptibility

Hypocotyls are more susceptible to damage from dropping, compression and puncture at high hypocotyl water contents. This is in accordance with work carried out on carrots and potatoes where increased water potential and turgor have been shown to be related to increases in splitting (McGarry 1993, 1995, Konstankiewicz and Zdunek 2001). It was concluded that an increase in tissue turgor pressure results in an increase in the tension of the cell walls. This experiment showed radish hypocotyls are more susceptible to damage from compression, puncture and dropping at high hypocotyl water content at ranges which may be experienced within the commercial supply chain. In this experiment the propensity of radish hypocotyls to split due to dropping was found to increase only at hypocotyl water contents over 96.5%. In experiments for Objective 4 hypocotyl water content at harvest often exceeded 95.6% which is above the threshold for splitting as a result of dropping at a height of 1.4 m. In addition, although a negative correlation was found between hypocotyl water content and puncture force it is uncertain if the radishes would be exposed to punctures of this magnitude during post-harvest handling, further work is also required in this area. Break force as a result of compression was found to not only be linked to water content but also to the size of the radish. Radishes with a larger diameter were found to be more resistant to splitting as a result of compression. These finding suggest that it may be possible to reduce splitting susceptibility by harvesting radishes when they are slightly larger.

A link between water content and hypocotyl pressure was found supporting the theory that increased water content makes radishes more susceptible to splitting as a result of increased pressure within the hypocotyl. However, only 18.4% of the variance in hypocotyl pressure was accounted for by water pressure suggesting other factors may also be involved.

Objective 6: To investigate the effects of hypocotyl temperature on post-harvest splitting susceptibility

In keeping with previous research into the effects of temperature on failure force (Bourne 1982), the splitting susceptibility of radishes following impact texture analysis was found to be negatively correlated with temperature, i.e. the lower the temperature, the more likely radishes are to split. Under commercially conditions, radishes are stored and packed between 2 and 5°C which is slightly lower than the lowest temperature, 5.6°C, used in this investigation. Using the model $S = -1.819T + 71.972$, which described the linear relationship between temperature (T) and percentage splitting (S) in this investigation, it is predicted that at 5°C, 62.9% of the radishes which were dropped would split, and at 2 °C, 68.3% of the radishes would split. Over the range studied a 1°C reduction in temperature leads to a 1.8% increase in splitting. These results suggest, in terms of reducing splitting susceptibility it may be preferably to store and process radishes at warmer temperatures and then to chill them after handling to limit the consequences on shelf life and respiration.

Conclusions

- Cultivars differ significantly in their splitting characteristics
- Water content during growth has similar effects on splitting in different cultivars
- Water content of the growing medium at growth stage 41 is correlated to the amount of splitting observed at harvest in the cultivar 'Rudi'
- Radishes tend to be more uniform in diameter if they have had a 10 day drying down period from day 8 to 17
- There is a negative correlation between hypocotyl water content and the force required to puncture the hypocotyl
- There is a positive correlation between the diameter of the hypocotyl and the force required to crush it
- There is a negative correlation between hypocotyl water content and the force required to crush it
- Radishes are more susceptible to splitting as a result of dropping at hypocotyl water contents above 96.5%

Knowledge and Technology Transfer

December 2011	Poster presented at HAU College Post-graduate Colloquium
December 2011	Oral presentation at HAU College Post-graduate Colloquium
July 2012	Poster presented at HDC Studentship Conference
September 2012	Oral presentation at PEPg Ecophysiology Workshop
November 2012	Poster presented at HAU Post-graduate Colloquium
September 2013	Oral presentation and paper submitted at international conference 'MQUIC 2013'
September 2013	Poster presented at HDC Studentship Conference
November 2013	Oral presentation at HAU Post-graduate Colloquium
November 2013	Poster presented at RHS Studentship Symposium
December 2013	Research seminar given at HAU
April 2014	Poster presented at UK PlantSci Conference 2014
May 2014	Meeting with G's representatives at Feltwell and presentation of work to date
May 2014	Oral presentation at UK Brassica Research Committee annual meeting
August 2014	Poster presented and paper submitted at International Horticultural Congress 2014
August 2014	Oral presentation and paper submitted at International Horticultural Congress 2014
September 2014	Oral presentation at British Tomato Growers Conference 2014
November 2014	Oral presentation at Leafy Salads Roadshow
November 2014	Oral presentation at RHS Studentship Symposium

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