Harper Adams University

A Thesis Submitted for the Degree of Doctor of Philosophy at

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HARPER ADAMS UNIVERSITY

Minimising post-harvest losses in radishes through an understanding of pre and post-harvest factors that influence root splitting

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

by

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1. Declaration

This thesis was composed by me and is a record of work carried out by me on an original line of research. All sources of information are shown in the text and listed in the references. None of this work has been presented/accepted for the award of any other degree or diploma at any University.

Rachel Lockley June 2016

2. Abstract

Radish splitting reduces marketable yield and increases production costs. Minimising losses may be possible through identification of factors affecting splitting.

Splitting occurs during growth (growth splits) and post-harvest (harvest splits). Growth splitting was shown to be affected by cultivar and periderm thickness, split radishes tended to have thicker periderms. Growth splitting was also affected by irrigation; greater volumetric water content resulted in more split radishes. This may have been due to increased turgor pressure from greater hypocotyl water uptake. Radishes were particularly sensitive to increased substrate VWC at Growth Stage 41, defined as the point when the periderm becomes the hypocotyl surface. Radish development stages were developed following the Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBCH) scale. This suggests irrigation management could reduce growth splits, however consistent control of soil moisture may be difficult in field-grown crops and manipulating post-harvest conditions may be more achievable. Radishes with different hypocotyl water content (WC) were tested for susceptibility to harvest splitting as a result of mechanical damage. Hypocotyl WC was negatively correlated with compression and puncture failure force and more radishes split as a result of impact at higher hypocotyl WC. Hypocotyl WC and relative WC were correlated with water pressure leading to the conclusion that greater turgor pressure within the hypocotyl resulted in the radishes being more susceptible to splitting. Radishes were also shown to be increasingly susceptible to splitting with decreasing temperature. Again, this was thought to be as a result of turgor pressure; the cytoplasm and cell wall may contract more than the vacuole at low temperatures resulting in increased pressure. The mode of failure was shown to be plasmoptysis, increasing turgor pressure increases susceptibility to plasmoptysis.

In conclusion, susceptibility to splitting could be reduced by decreasing post-harvest hypocotyl turgor pressure. Therefore, using current commercial production methods it is more feasible to minimise harvest splitting than growth splitting.

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4. Published work

Lockley, R., Grove, I. and Monaghan, J. (2015) 'Splitting in Radish – Does Preharvest Environment Influence the Response to Postharvest Handling?', *Proceedings of the Sixth International Conference on Managing Quality in Chains*, 1(1), pp. 231–237.

Lockley, R., Grove, I. and Monaghan, J. (*in press*) 'Minimising Losses in Radish (*Raphanus sativus*) at Harvest Due to Splitting by Manipulation of Water Availability During Growth', *Proceedings of the XXIX International Horticultural Congress: IHC2014*.

Lockley, R., Grove, I. and Monaghan, J. (*in press*) 'Investigating Factors Affecting Post-Harvest Splitting in Radish (*Raphanus sativus*)', *Proceedings of the XXIX International Horticultural Congress: IHC2014*.

5. Contents

6. List of tables

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9. List of abbreviations

%	Percent
°C	Degrees centigrade
µg g^{-1}	Micrograms per gram
ABA	Abscisic acid
ANOVA	Analysis of variance
BBCH	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie
BD	Bulk density
cm	Centimetre
cm^2	Centimetres squared
cv.	Cultivar
df	Degrees of freedom
dH_2O	De-ionised water
DNA	Deoxyribose nucleic acid
FC	Field capacity
g cm^{-3}	Grams per centimetre cubed
g	Gram
GWC	Gravimetric water content
HAU	Harper Adams University
IMS	Industrial methylated spirit
kg	Kilogram
kg/ha	Kilogram per hectare
kg/kg	Kilograms per kilogram
km	Kilometre
L	Litre
m	Metre
m^2 S mol^{-1}	Moles per metre squared per second
m^3/m^3	Metres cubed per metre cubed
mL	Millilitre

mm day^{-1}	millimetres per day
mm S^{-1}	millimetres per second
mm	Millimetre
MPa	Mega pascals
mRNA	Messenger ribose nucleic acid
mV	Millivolt
PWP	Permanent wiling point
QA	Quality analysis
RH	Relative humidity
RWC	Relative water content
UK	United Kingdom
V	Volt
v/v	Volume per volume
VWC	Volumetric water content
W	Watts
w/v	Weight per volume
WC	Water content

1. Literature review

1.1 Introduction

This research project has been carried out in response to the losses of marketable yield and increases in processing and packing expenditure being experienced by UK radish growers and packers due to splitting. Supermarkets will usually reject batches of radishes which contain more than 10% of radishes which do not meet commercial standards. This includes radishes which are split. As radish splitting rates can exceed 30%, batches can be rejected purely for excessive splitting. To prevent this, split radishes have to be removed by hand prior to packaging, a time-consuming, costly and wasteful procedure. This project aims to minimise splitting in radishes by understanding the pre and post-harvest factors which affect it.

Little research has been carried out into splitting in radishes, in particular the small red summer radishes which are predominantly grown in the UK. However, splitting is also a problem for other vegetable crops such as carrot, potato and kohlrabi and it is also a problem for fruit crops such as cherry, tomato and pepper and a greater quantity of research has been carried out into the causes of splitting in these crops. Research into splitting in a variety of crops which is considered relevant to radishes will be discussed in this literature review.

1.2 The radish crop

Radish (*Raphanus sativus*) is an economically important herbaceous plant which is cultivated worldwide as a vegetable, for animal fodder and for production of radish seed oil. Radishes grown as vegetables will be the focus of this work. Annual global production of radishes as a vegetable crop is estimated at 7 million tonnes which is about 2% of the total world production of vegetables (Schippers 2004).

Raphanus sativus is a cultigen which has been selectively bred since early civilization. There are inscriptions in the pyramids which suggest *Raphanus sativus* was used by the Egyptians around 2000 B.C., there is also evidence of its existence in Japan about 700

B.C. and in China roughly 500 B.C. (George & Evans 1981). It is believed there have been three separate domestication events of vegetable radishes, the black radish, Asian winter radishes and European summer radishes. European summer radishes are believed to have been bred either from a single wild species or through hybridization of wild species in the Mediterranean (Yamane *et al*. 2009). Salad varieties were developed in the 18th century and were originally white. Later a greater variety in shape and colour were developed (George & Evans 1981). Radish has a pungent peppery flavour as, like many cruciferous vegetables, it contains glucosinolates (Depree *et al*. 1998; Holst & Williamson 2004; Verkerk *et al*. 2009).

In general, radishes which are grown to be eaten as vegetables are divided into two distinct types, small summer radishes (Figure 1-1) and large winter radishes (Figure 1-2). The smaller quick growing summer radishes are popular as salad vegetables in Europe and North America whereas, the larger winter radishes have particular prevalence in Asia in countries such as China, Japan, Korea and India (Zaki *et al*. 2012; Ullah *et al*. 2011). Summer radishes are annuals and they have a rapid growth cycle, typically taking 3 to 6 weeks from planting to harvest (Van Andel 2009). Winter cultivars may be annual or biennial and require longer to mature, taking up to eight weeks to achieve harvest size (Abdel 2011). Winter radishes are often referred to as ‘daikon’ from the Japanese name or ‘mooli’ from the Hindi name. This project concentrates on the smaller globular summer radishes which are grown commercially as a salad crop in the UK.

Figure 1-1 Summer radish (G’s Fresh Ltd 2012)

Figure 1-2 Winter radish (JagsFresh 2014)

1.2.1 Taxonomy

The common name radish derives from the Latin for root, radix. The name *Raphanus* derives from the Greek ra, meaning quickly, and phainomai, meaning to appear, this refers to the rapid germination of the seeds.

Radishes belong to the class Equisetopside, subclass Magnoliidae, suborder Rosanae, order Brassicales, family Brassicaceae and genus *Raphanus*. Pistrick (1987) divided the cultivated forms of the *Raphanus* genus into three groups: Convar. *oleifera* which includes oilseed and fodder radishes, Convar. *caudatus* which is also known as var. mougri or rat tail radish and finally Convar. *sativus* which includes all forms of radish grown for the edible swollen hypocotyls including small and large varieties sometimes known as var. radicula, nigra, niger, sinensis, acanthiformis and longipinnatus (Pistrick, 1987 as cited in (Maroufi & Farahani 2011)). All varieties intercross freely and hybridise with wild *Raphanus* species.

1.2.2 Morphology

Although the entire radish plant is edible, typically, radish, like swede, celeriac, beetroot and turnip, is grown for the swollen hypocotyl and tap-root. Swollen radish hypocotyls and taproots can grow and develop into a variety of different shapes, sizes and colours (George & Evans 1981). The upper section of the fleshy part of the radish is usually devoid of lateral roots and consists of a swollen hypocotyl. Depending on the type of radish the lower section may be formed of an enlarged taproot and have lateral roots. The proportion of taproot is greater in long winter varieties as opposed to globular summer varieties which are the focus of this thesis (Figure 1-3). As may be expected differences in cell size and number at different locations in the radish hypocotyl and taproot have been shown to differ between long type and round type radishes (Zaki *et al*. 2012). More specifically the differences between long white and round red radishes have been shown to be due to differences in the growth of the upper part of the tap root (Ting & Wren 1980). It is thought the differences in shape have a genetic basis and 11 genes have been identified which are differentially expressed in two radish cultivars of different shape (Zaki

et al. 2012). For ease of reference the swollen hypocotyl and taproot will be referred to as the hypocotyl in this thesis.

Radishes have lobed leaves arranged in a rosette. Each leaf has a large single terminal lobe and smaller paired lateral lobes which are irregularly toothed (Figure 1-3). Radish flowers have four petals alternately arranged with four sepals. The petals range in colour from white to pale violet. The inflorescence is borne on an elongated raceme. Radishes are usually self-incompatible and are categorized as allogamous (George & Evans 1981). Radish flowers are insect pollinated (George & Evans 1981) however, commercial radishes grown as a salad vegetable are harvested prior to anthesis.

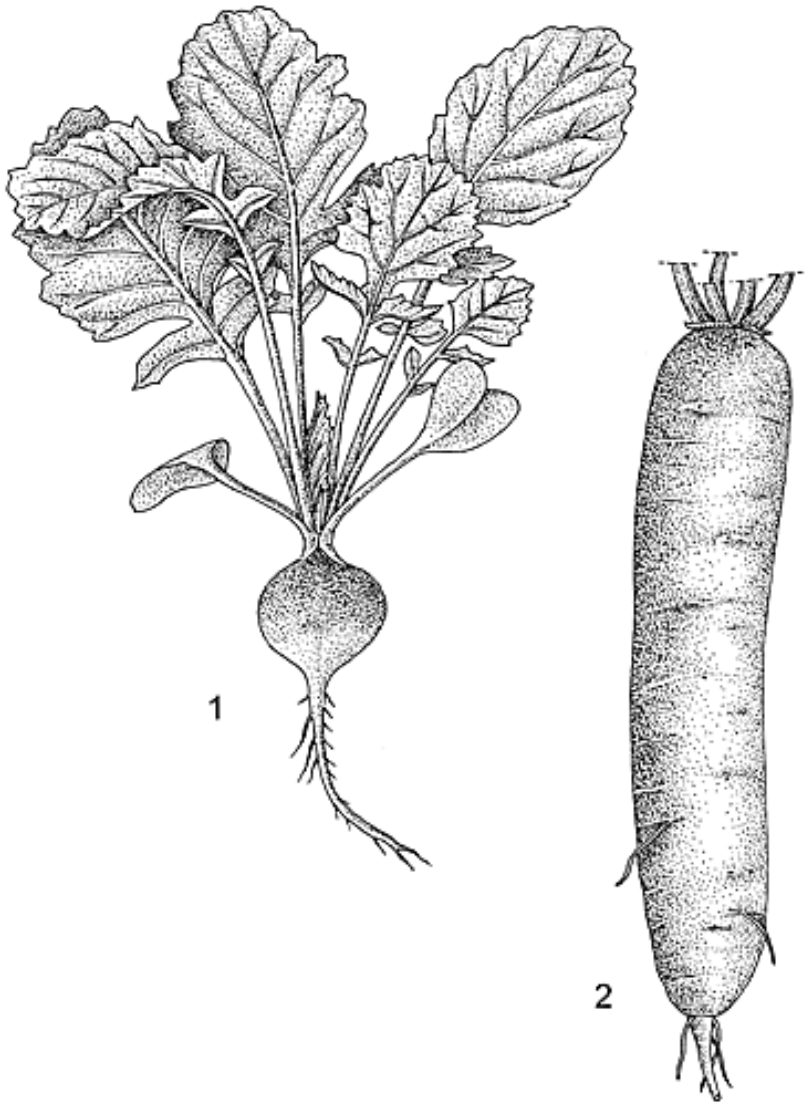


Figure 1-3 General radish morphology (Schippers *et al*. 2004). 1 = globular summer radish, 2 = long winter radish.

1.2.3 Growth and development

Standard codes for describing growth stages enable accurate scientific descriptions and comparisons to be made between plants at the same physiological age. Specific descriptions of growth stages are available for many crops including cereal (Zadoks *et al*. 1974), canola (Sylvester-Bradley 1985), potato (Jefferies & Lawson 1991) and peas (Knott 1987). There is a growth code specific to wild radish (*Raphanus raphanistrum*) which includes ten primary growth stages (Madafiglio *et al*. 1999). The growth stages of *Raphanus sativus* have not been uniquely described but are included in the Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBHC) identification keys under root and stem vegetables, which includes nine principal growth stages (Meier 2001) (Table 1-1). This key has been useful in defining growth stages for peer reviewed work for example it was used by Schreiner *et al*. (2002) in their investigation into seasonal effects on radish quality (Schreiner *et al*. 2002). However, the scale lacks plant specific descriptions particularly during the development of the harvestable vegetative parts and gives no indication of the timings for each of the stages.

Table 1-1 Biologische Bundesanstalt, Bundessortenamt and CHemical industry (BBHC) root and stem vegetable identification key (Meier 2001)

Principal growth stage 0: Germination	
00	Dry seed
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle emerged from seed
07	Hypocotyl with cotyledons breaking through seed coat
09	Emergence: cotyledons break through soil surface
Principal growth stage 1: Leaf development (Main shoot)	
10	Cotyledons completely unfolded; growing point or true leaf initial visible
11	First true leaf unfolded
12	2nd true leaf unfolded

13	3rd true leaf unfolded
1…	Stages continuous until . . .
19	9 or more true leaves unfolded
Principal growth stage 4: Development of harvestable vegetative plant parts	
41	Roots beginning to expand (diameter > 0,5 cm)
42	20% of the expected root diameter reached
43	30% of the expected root diameter reached
44	40% of the expected root diameter reached
45	50% of the expected root diameter reached
46	60% of the expected root diameter reached
47	70% of the expected root diameter reached
48	80% of the expected root diameter reached
49	Expansion complete; typical form and size of roots reached
Principal growth stage 5: Inflorescence emergence	
51	Main shoot begins to elongate
53	30% of the expected height of the main shoot reached
55	First individual flowers of main inflorescence visible (still closed)
57	First individual flowers of secondary inflorescences visible (still closed)
59	First flower petals visible; flowers still closed
Principal growth stage 6: Flowering	
60	First flowers open (sporadically)
61	Beginning of flowering: 10% of flowers open
62	20% of flowers open
63	30% of flowers open
64	40% of flowers open
65	Full flowering: 50% of flowers open
67	Flowering finishing: majority of petals fallen or dry
69	End of flowering
Principal growth stage 7: Development of fruit	

71	First fruits formed
72	20% of fruits have reached typical size
73	30% of fruits have reached typical size
74	40% of fruits have reached typical size
75	50% of fruits have reached typical size
76	60% of fruits have reached typical size
77	70% of fruits have reached typical size
78	80% of fruits have reached typical size
79	Fruits have reached typical size
	Principal growth stage 8: Ripening of fruit and seed
81	Beginning of ripening: 10% of fruits ripe, or 10% of seeds of typical colour, dry and hard
85	50% of the fruits ripe, or 50% of seeds of typical colour, dry and hard
89	Fully ripe: seeds on the whole plant of typical colour and hard
	Principal growth stage 9: Senescence
92	Leaves and shoots beginning to discolour
95	50% of leaves yellow or dead
97	Plants or above ground parts dead
99	Harvested product (seeds)

1.2.4 Commercial production

Radish crops can be grown commercially both in the field and in the glasshouse; there are advantages and disadvantages to both methods, the primary deterrent being cost of glasshouse production and the primary benefit being environmental control (Stannard, T. 2012. Pers. Comm. G's Marketing). In the UK radish is available to buy all year around. The typical UK radish season runs from April to October being produced commercially predominantly in the East of England. From October to April, when UK produce is not

available, field grown radish is imported from areas such as Morocco and Senegal and from glasshouse production in Holland (Stannard, T., 2012 Pers. Comm. G's Marketing)

1.2.4.1 Soil type and irrigation

Radishes grow well on a variety of soil types with the exception of those with a heavy texture and high clay content which are prone to waterlogging. Soils with a light sandy texture which are well drained provide easy lifting of the radishes and soils which have a high organic or peat content are thought to give a good periderm colour (Red Tractor Farm Assurance 2010). When radishes are grown on soil with a light texture, irrigation is often required to assist germination and may be necessary throughout growth. Irrigation is thought to be of particular importance when the hypocotyl begins to swell especially if scab is likely to be a problem (Red Tractor Farm Assurance 2010).

1.2.4.2 Nutrition

The soil should be sampled prior to drilling for major elements such as phosphate, potassium and magnesium. Typical nutrient requirements are given in Table 1-2, in addition to this, further top dressings of nitrogen may be required for following crops on the same land. The amount of nitrogen which should be applied will vary between 40 and 75 kg/ha depending on the soil type and time of year. Excessive nitrogen application is a problem and only the minimum requirements should be applied, particularly to bunched radish. Unnecessary nitrogen may result in too much top growth and a greater susceptibility to certain diseases, such as downy mildew (Red Tractor Farm Assurance 2010). Over application of lime is also a problem for radish production as it is thought to encourage scab (Red Tractor Farm Assurance 2010).

Table 1-2 Typical nutrient requirements for radishes (Red Tractor Farm Assurance 2010)

	Soil index					
Nutrient (kg/ha)	**0**	**1**	**2**	**3**	**4**	**4+**
Nitrogen	110	60	20	a	a	a
Phosphate (P_2O_5)	175	125	75	25	nil	nil
Potash (K_2O)	250	200	100 to 150	50	nil	nil
Magnesium (MgO)	150	100	nil	nil	nil	nil

a = a small amount of nitrogen may be needed if levels are low in the top 10-30 cm of soil.

1.2.4.3 Harvest and storage

Radishes are harvested when they are of a commercial size; this is usually 18 to 32 mm in width for pre-packed radishes (Figure 1-4). Pre-packed radishes are harvested either by machine or by hand. Bunched radishes (Figure 1-5) are harvested purely by hand (Red Tractor Farm Assurance 2010). Both radishes harvested by hand and by machine benefit from rapid transport from the field with minimal dropping and should not be exposed to hot sun to avoid deterioration. Shelf life is thought to be optimised by harvesting early in the morning when conditions are cooler (Red Tractor Farm Assurance 2010).

Figure 1-4 Commercially grown radishes which are ready for harvest

Figure 1-5 Commercially grown bunched *Raphanus sativus* ‘French Breakfast’

Pre-packed radishes have their leaves removed, a processed referred to as being ‘topped’, shortly prior to harvest (Figure 1-6, Figure 1-7). They are then lifted (Figure 1-8) and dropped into a trailer (Figure 1-9) from heights of up to 1.4 m using a modified potato harvester. After harvest the radishes are washed (Figure 1-10) in potable water and the leaf petioles and roots are removed. Once trimmed the radishes are graded and sorted by hand for the first time removing non-marketable produce (Figure 1-11). They are then put into large containers and transported (Figure 1-12) to storage (Figure 1-13). Here the temperature is between 2 and 5°C and the radishes acclimatise to this naturally. Radishes

can be cooled more rapidly (roughly 1 hour) using vacuum cooling but this is not usually used as it is believed within the industry to increase harvest splitting. The radishes can be stored for several days prior to packing and usually for no less than 1 to 2 days as it is thought this is the period when the radishes are most likely to split. After storage the radishes are graded and sorted by hand for a second time (Figure 1-14), still under chilled conditions, removing split and unmarketable produce and packed ready for sale (Figure 1-15). They are then transported to supermarkets remaining under climate controlled environments.

Figure 1-6 Commercially grown radishes being topped

Figure 1-7 Commercially grown topped radishes

Figure 1-8 Commercially grown radishes being lifted

Figure 1-9 Commercially grown lifted radishes in trailer

Figure 1-10 Commercially grown radishes being washed out of trailer

Figure 1-11 Commercially grown radishes being graded and sorted by hand for the first time prior to storage

Figure 1-12 Commercially grown radishes being transported to storage in curtain sided trailers

Figure 1-13 Commercial radishes stored in Dolavs

Figure 1-14 Commercial radishes being sorted and graded for a second time prior to packing

Figure 1-15 Commercial packed radishes ready for transport to the supermarket

1.2.4.4 Commercial standards

There are standards which must be adhered to before many vegetables, including radishes, can be sold or exported for sale. Part A of Annex I to Commission Regulation 1221/2008 sets a general marketing standard for all fresh fruits and vegetables which

specifies minimum quality, maturity and labelling requirements. In reality the European standards are arbitrary as they are further superseded by supermarkets that impose their own specific requirements for labelling, size, shape and condition of the radish. These requirements are typically far more stringent than the legal requirements. In The UK radishes are normally required by supermarkets to be between 18 and 32 mm in width, be uniform and round in shape, have less than 10% of the produce split, glassy, diseased or scuffed, and less than 5% of the produce should have pest damage, signs of disease or dehydration.

1.2.4.5 Pests

Radish crops in the UK will typically undergo 3 year crop rotation cycles to prevent the build-up of pests and disease in a particular field (Watson, S. 2012. Pers. Comm.).

The worst pest of radishes in the UK is cabbage root fly (*Delia brassicae*). The larvae of the root fly burrow into the radish hypocotyl resulting in it being unsaleable, even light infestations can be seriously detrimental to the marketable yield. There can be several generations of cabbage root fly in a year typically starting in May and continuing into autumn. As the UK radish season typically starts in April and ends in October the cabbage root fly is a problem for the majority of the growing season. Treatment involves the use of traps for the adult flies used in conjunction with fine mesh netting and firm consolidation of the seedbed in an attempt to prevent eggs being laid. The timing of generations can be predicted using computer models to enable more accurate use of traps and nets (Red Tractor Farm Assurance 2010).

Another occasional and local pest which affects radishes is cabbage stem weevil. The larvae of this pest tunnel into the leaf stalks and heavy infestations may reduce yield by causing deformation in the radish plants. The rise in incidence over recent years has been attributed to the increased area of oilseed rape which is being grown. One preventative measure which is suggested to growers is to avoid growing radishes adjacent to oilseed rape crops (Red Tractor Farm Assurance 2010).

Aphids and flea beetles are pests which are considered to be more serious for bunched radish than pre-packed radish crops as they affect the quality of the leaves. Flea beetles tend to infest radishes during very dry periods and cause small holes in the leaves which become more obvious as the leaves expand. Growers are advised to keep the crop moist and use fine mesh thrip netting to reduce the incidence of this pest. Although various species of aphids can infect radishes, they are only an occasional problem. Light infestations can usually be controlled using natural predators and parasites such as parasitic wasps (Red Tractor Farm Assurance 2010).

1.2.4.6 Diseases

There are several diseases which can affect radishes in the UK and unfortunately unlike many crops there is not a particularly good choice of disease resistant cultivars of radish (Red Tractor Farm Assurance 2010).

Scab (*Streptomyces scabies*) causes circular white lesions 5 to 15 mm in diameter with raised edges and sunken centres which appear on the surface of hypocotyl close to the time of harvest (Levick *et al*. 1985). The spots expand and the radish can become soft and rotten. Scab is a soil borne fungus. Both radish and some types of potato streptomycetes are pathogenic to radishes (Levick *et al*. 1985). Scab is more prevalent in soils with a high pH therefore creating alkaline conditions by over liming the soil should be avoided, as should soils with a history of the disease. Irrigation and soil moisture is important in the management of scab. Radishes grown in a relatively wet un-drained plot (soil moisture above -0.15 MPa) had significantly (P=0.01) less scab (mean 4% radishes infected) than radish grown in a relatively dry (soil moisture below -0.3 MPa) soil (mean 50% radishes infected). Incidence has been shown to be significantly reduced in plants which are irrigated regularly, 2 to 3 day irrigation intervals compared to ambient or no rainfall. Incidence has also shown to be significantly lower in plants irrigated for 2 to 3 weeks after drilling when compared to plants irrigated for only 1 week (Levick *et al*. 1985).

Downy Mildew (*Peronospora parasitica*) can be very prevalent during favourable cool humid conditions. This disease causes small yellow spots on the leaf surface and white

fluffy mould under the surface of the leaves. Spores wash down from infected leaves and infect the hypocotyl. The hypocotyl can then be disfigured by black spots which makes it unmarketable. There are no resistant varieties to this disease but some control can be achieved using cultural methods by avoiding watering during still, warm periods as this can encourage the disease.

White blister (*Albugo candida*) is a disease which causes raised white blisters on the leaves of radish plants and in severe cases can result in distortion of all the leaves. It is therefore of particular relevance to bunched radishes. It is a disease which spreads rapidly, there are no cultural control methods available for treatment or prevention but good hygiene after harvest is thought to help control the spread of the disease (Red Tractor Farm Assurance 2010).

Two diseases where there are resistant radish cultivars available are club root (*Plasmodiophora brassicae*) and fusarium (*Fusarium oxysporum*). Club root affects the root and base of the hypocotyl by infecting the root hairs of the developing root. Infection results in distorted swellings and knobs on the hypocotyl. Heavily infected plants may become stunted and wilted due to compromised root function. Disease transmission occurs through infected soil and fungus spores can remain viable in the soil for at least seven years. Therefore, if possible, it is best not to grow radishes in soil which has a history of the disease (Rowe 1980). Low pH and excessive moisture also favour infection (Rowe 1980). Fusarium is predominantly a problem for glasshouse grown radishes although it can affect field grown radishes in hot summers. It can survive in the soil for several years. The disease causes the lower leaves to yellow and the infected leaves drop off. In severe cases all the leaves may be lost (Red Tractor Farm Assurance 2010).

1.2.4.7 Weeds

Weeds are seldom a problem with radish production; it is a quick growing crop and usually outgrows the competition. In the UK there can be as many as 5 harvests per annum from a single field of radish (Watson, S. 2012. Pers. Comm.). Ensuring the seedbed is clean before drilling usually negates the requirement to use herbicides (Watson, S. 2012. Pers.

Comm. General Manager Feltwell Growers G's). No contact post crop emergent or residual herbicides are approved for use in radish production but contact herbicides with the active ingredients; diquat, glufosinate ammonium and glyphosate are available to use pre-drilling (Red Tractor, 2010).

1.3 Splitting

Splitting in fruit and vegetables is an unsightly problem which reduces marketability and exposes internal tissue to the external environment and potential pathogens (Gracie 2004). Radish growers can experience splitting rates up to 30% yet supermarket tolerance to hypocotyl splitting in radishes is typically less than 10% (pers comms Scott Watson G's Growers). Excessive splitting not only reduces the marketable yield but it also results in batches of radishes having to be processed by hand to remove the split produce. This process is both time consuming and costly for the grower and packaging companies. Despite these problems, little is known about the environmental and physiological causes of splitting particularly in summer radishes. Previous research into splitting has tended to focus on fruit crops such as apple, cherry and tomato. Due to the differences between the physiology of fruit and vegetable tissue and the effects this has on mode of splitting this review will concentrate predominantly on splitting in vegetable crops as this is more relevant to radishes. Comparisons will be made with splitting in fruit where appropriate and when similarities are present. The majority of research into splitting in vegetables has been carried out into carrots. Splitting is a major problem for this crop as in excess of 10% of a carrot harvest can be lost due to splitting (McGarry 1995). There are some similarities between the physiology of radishes and carrots suggesting research into splitting in carrot may be relevant to radishes. The edible portion of both vegetables is produced by the expansion of the storage root and hypocotyl. Although radish is predominantly hypocotyl and carrot is chiefly storage root, resulting in a greater proportion of the carrot being under the soil surface during growth.

Splitting occurs when mechanical stress exceeds the ability of the tissue to withstand it (Hole *et al.* 1999). There are two types of splitting, cellular debonding where the cells

remain intact but pull away from each other and plasmoptysis where the cells rupture. The mode of failure depends on the relative strengths of the intercellular bonds and cell walls (Lin & Pitt 1986). In many vegetable crops, root and tuber splitting is thought to occur predominantly due to plasmoptysis as opposed to cellular debonding. McGarry (1993) found splits occurred in carrots by cell wall breakage and Lippert (1999) investigated cracking in kohlrabi tubers and found ruptured cell walls indicating intracellular fractures. This is unlike cracking in some fruits which often show cracks along cell walls without damage to the cell and cell walls (Khan 1989 as cited in (Lippert 1999)). The difference is thought to be due to the limited amount of intercellular or apoplast volume in some vegetables, less than 5% v/v in kohlrabi (Lippert 1999) and ranging between 6 and 15% v/v in carrot decreasing with crop age (McGarry 1995), compared to 20-25% v/v in apples (Lippert 1999). Although not discussed in the paper the pictures of cross-sections by Skok (1941) of radishes which have split during growth (Figure 1-16) appear to show a similar trend with little observable intercellular space and splits propagating through the cells (Skok 1941). These results suggest splitting susceptibility in these vegetable crops must be determined to some extent by cell wall strength and composition.

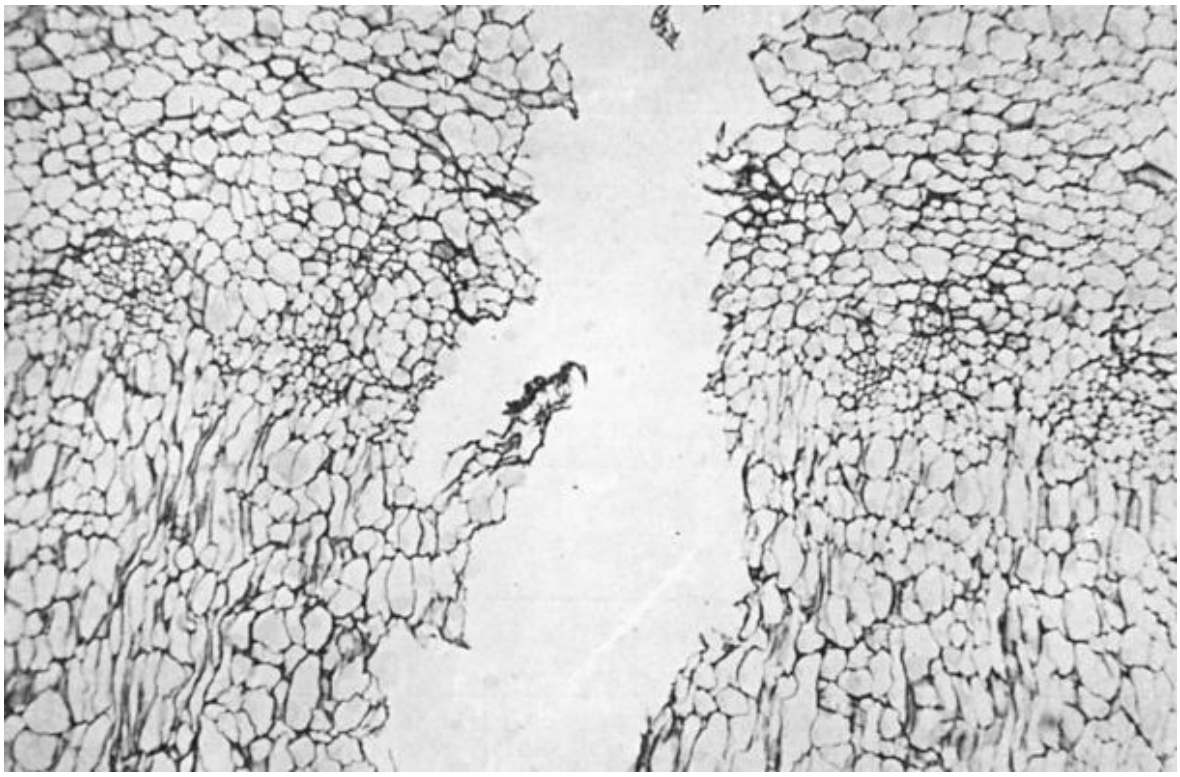


Figure 1-16 Split in the cambium region of radish (Skok 1941). Fracture appears to involve cellular rupture.

1.3.1 Differences in splitting susceptibility between cultivars

Splitting susceptibility is likely to depend on genotype-environment interactions. Therefore, the choice of cultivar is thought to have an effect on the amount of splitting which occurs. Some investigations have found effects of cultivar selection on splitting susceptibility in different crops. In some papers physiological differences between the cultivars have been correlated with splitting susceptibility and have been proposed to explain the differences. These are summarised in Table 1-3.

Table 1-3 Dissimilarities between cultivars of different crops which have shown differences in splitting susceptibility as reported in published scientific papers

Factor	Crop	Correlation with splitting	Reference
Cuticle thickness	Sweet cherry	Negative	Demirsoy (2004)
Round irregularly shaped sub-epidermal cells	Sweet cherry	Positive	Demirsoy (2004)
Epicarp thickness	Tomato	Not described	Dorais *et al.* (2004)
Rate of growth	Kohlrabi	Positive	Lippert (1999)
Cell area	Carrot	Positive	Hole (1999)

In fruit such as cherry (Demirsoy & Demirsoy 2004) and tomato (Dorais *et al.* 2004) and vegetables such as kohlrabi (Lippert 1999) and carrot (Hartz *et al.* 2005; Hole *et al*. 1999) 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 can explain some of the genotypic difference 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. Dorais *et al.* (2004) state however, that as many genes are likely to be involved in splitting susceptibility, breeding for resistance is difficult.

McGarry (1993, 1995) found although water status was an important factor in determining splitting susceptibility within cultivar it did not explain the differences in splitting susceptibility between carrot cultivars, this suggests other genetically determined factors must also be responsible for determining splitting susceptibility. Hole *et al.* (1999) also observed genetic variation in susceptibility to splitting in carrot. In this paper it was suggested anatomical structure was likely to be associated with susceptibility to splitting as the two cultivars which split the most readily also had the largest cross-sectional cell area. Another anatomical explanation for genetic differences in splitting susceptibility expressed by Khan (1989) as cited in Lippert (1999) is differences in the arrangement of cells and presence of air spaces may act to interrupt the progression of cracks. The pattern of cell organisation may have a genetic basis and hence vary between cultivar and some cultivars could have more irregularly arranged cells and more air spaces than others (Lippert 1999). In contrast to this theory Demisoy (2004) found there was more cracking in sweet cherry cultivars which had irregularly arranged round sub-epidermal cells compared to cultivars which had flatter and regularly ordered cells.

The importance of the genetic basis of splitting compared to the effects of environment are questioned by Hole *et al.* (1999) because the variation in spitting between carrot cultivars was smaller in their investigation than the differences observed due to environment. In conclusions similar to those made by Dorais *et al.* (2004) for tomato, Hole *et al.* (1999) considered little control over splitting could be achieved by breeding for strength.

1.3.2 Anisotropy

Splitting in both carrot (McGarry 1993) and kohlrabi (Lippert 1999) is anisotropic, with splitting tending to follow a similar pattern. This would indicate the tissue is not equally

susceptible to splitting in all directions suggesting cells in the more susceptible areas are under more stress or have a lower resistance to failure. Similarly the tissue of a potato tuber is not uniform in splitting susceptibility with differences in the mechanical properties of the inner and outer cores being shown by Konstankiewicz and Zdunek (2000). In this investigation, inner core tissue had a greater compressive strength but lower resistance to micro-damage compared to outer core tissue. They proposed these differences may have been due to differences in cell size with inner core tissue having smaller cells which are more resistant to compression but more susceptible to micro-damage (Konstankiewicz & Zdunek 2001). Similar results have been found with carrots, Hole *et al.* (1999) found carrot cultivars which split most readily had cells with the largest cross-sectional area.

Further explanations for differences in splitting susceptibility as a result of tissue heterogeneity are expressed by Wan and Kang (2005). They suggest splitting observed in winter radishes was due to differences in expansion rate between the internal tissues and the periderm. They propose the internal tissues expand more rapidly than the periderm putting pressure on it and causing it to split. In addition Sorenson (2000) found rings of tissue within carrot were under different strains and Hartz (2005) found carrots were less susceptible to splitting if the periderm was removed.

It has been suggested splitting in apples may also be due to inequalities in pressure within tissues. Skene (1980) observed splitting in apples did not start until the apples reached a minimum size. It was suggested the apples needed to achieve this size before a sufficient imbalance in growth between inner and outer tissues was achieved and stress could develop. The hypodermal tissues of the apples were also observed to develop thicker cell walls at this point possibly affecting the elastic stress within the apples. Skene (1980) also provided evidence that the pattern of splitting susceptibility in apples changes during growth. During early growth, elastic strain was found to be greatest in the longitudinal direction with cuts made in a transverse direction gaping more than longitudinal cuts. Later in the season, after mid-July, this pattern reverses and greater strain in the transverse direction was observed. As the direction of growth at the surface of the apples was always greatest in the longitudinal direction, this cannot explain the change in the

direction of stress. Skene (1980) suggested the difference may be due to changes in shape of the fruit or to changes in the mechanical properties of the tissues within the fruit.

1.3.3 Growth splits

Splitting in radishes and other crops can occur both during growth (growth splits) and post-harvest during the harvesting, storage and packaging procedures (harvest splits). As it is beneficial for growers to know the best practices to reduce splitting during both of these periods, research has been carried out into both the pre and post-harvest factors which affect splitting susceptibility. In this section factors affecting splitting during growth will be discussed.

Much of the research into the causes of splitting during growth in other crops is of an empirical nature, investigating the environmental conditions and agronomic practices which correlate with levels of splitting. Several researchers have observed susceptibility to splitting varies during ontogeny and also diurnally. Previous research which has observed differences in the pattern of splitting throughout growth and the day is summarised in Table 1-4.

Table 1-4 Effects of growth stage and rate on splitting in different crops as reported in published scientific papers

Factor	Crop	Effect	Reference
Growth stage	Carrot	More splitting later in development	Gracie (2004)
Growth stage	Apple	Increased at a specific size	Skene (1980)
Time of day	Carrot	More splitting at end of day	Gracie (2002)
Time of day	Tomato	More splitting at end of day	Dorais (2004)
Growth rate	Radish	Faster growth more splitting	Latimer (1991)

There is evidence to suggest growth splitting is affected by growth stage. Splitting in carrots has been shown to be affected by crop maturity with splitting mainly occurring later in crop development (Gracie & Brown 2004). In apples, the pattern of stress within the fruit

changes during growth (Skene 1980). Throughout early growth, stress appears to be influenced by fruit size. Stress develops when the fruits reach a particular size in different years irrespective of differing weather conditions. During later growth, stress was found to be influenced to a greater extent by weather as large strains were often associated with periods of heavy rain. However, not every period of heavy rain was associated with a large strain during later growth, suggesting stress was not exclusively caused by rainfall (Skene 1980).

Splitting susceptibility is thought to not only change over the lifetime of the crop but also over the course of the day. Diurnal fluctuations in splitting susceptibility have been observed in carrots (Gracie & Brown 2004). The highest rates of splitting occur at sunrise, splitting rates then decrease throughout the morning and early afternoon before increasing again later in the day. This matches the diurnal pattern of radial growth in the taproot. It has been suggested the reason for increased splitting susceptibility in carrots at the ends of the day is due to the increased rate of growth at these times (Gracie & Brown 2004). Similar diurnal patterns in splitting have been observed in tomato but these have been attributed to changes in tissue water status throughout the day (Dorais *et al.* 2004). Growth rate has also been suggested to affect splitting in radishes. In an experiment, the leaves of radishes were brushed twice a day for 13 days during growth to impose mechanical stress and this was found to result in a significant ($P=0.05$) decrease in splitting, 25% of the radishes which were brushed split compared to 39% of the radishes which were not brushed. In this investigation the decrease in splitting was observed alongside a reduction in hypocotyl growth rate. At 24 days after sowing the radishes which were brushed were 9% smaller in diameter compared to the radishes which were not brushed ($P<0.05$). The reduction in growth rate was suggested as a possible cause of the reduction in splitting (Latimer 1991). However, Dowker and Jackson (1977) found carrots which had the slowest growth rate, with the longest duration from drilling until harvest, split the most (Dowker & Jackson 1977). However, as these carrots had been planted in different months at different densities there are a number of confounding factors which may have resulted in the observed differences in splitting.

1.3.3.1 Water

Water is thought to be one of the principal factors affecting growth splitting. Some of the research in this area is summarised in Table 1-5.

Table 1-5 Effects of water on growth splitting in different crops as reported in published scientific papers

Factor	Crop	Effect on splitting	Reference
Rainfall	Sweet cherry	Increase	Sekse (1995)
Increased crop water status	Tomato and pepper	Increase	Dorais *et al.* (2004)
Irrigation frequency	Winter radish	3 days optimal to minimise splitting	Wan and Kang (2005)
Soil water potentials	Winter radish	-0.035 MPa optimal to minimise splitting	Kang and Wan (2005)
Timing of water availability	Carrot	Increase if dry during mid-growth and then rain prior to harvest	Salter (1967)
Drought stress	Carrot	Increase if early drought stress Decrease if drought during mid-growth	Sorenson (1997)
Increased tissue water potential	Carrot	Increase	McGarry (1995)
Soil moisture (maximum or mean)	Carrot	No effect	Hartz (2005)
Soil texture	Carrot	Soil sand content negatively correlated to splitting	Hartz (2005)

The predominant environmental factor which is thought to affect growth splitting is water. This can be in the form of rainfall or irrigation. In sweet cherry fruit, rain induced growth splitting is thought to be caused by a combination of two mechanisms, firstly by rain water entering through the skin and degrading the dermal cell walls of the fruit and secondly by an increase in pressure on the skin from within the fruit as a result of water uptake by the vascular system (Sekse 1995). In carrot (Gracie & Brown 2004), tomato and pepper (Dorais *et al.* 2004) plants cracking has a diurnal pattern with higher incidence of cracking early in the morning and at the end of the afternoon. This is thought to be due to swelling and shrinking which occur as a result of changes in water status in the crop. Fruit shrinkage can be observed at times of increased water demand for instance at midday under conditions which cause high rates of transpiration such as high solar radiation and low relative humidity and after the night to day transition when there is a rapid increase in transpiration. It is thought fruit swelling and shrinkage are causes of cuticle cracking in these crops (Dorais *et al.* 2004).

The effects of water availability during growth are also thought to affect splitting susceptibility in radishes and other vegetable crops. In the large winter varieties of radishes, fluctuations in soil water potential during growth have been shown to affect splitting (Wan & Kang 2005). It was found radishes irrigated once every three days had the lowest cracking rate and well developed hypocotyls when compared to radishes irrigated daily, once every two days, once every four days, once every six days and once every eight days. In this experiment soil matric potential increased with increasing irrigation frequency. Frequent irrigation during growth resulted in high levels of splitting as did large fluctuations in soil water potential. No difference was observed in the growth rates of the hypocotyls between treatments. Wan and Kang (2005) hypothesised high soil water potentials in the frequent irrigation treatments may have caused rapid rates of expansion in the parenchyma cells but not of the periderm causing tissue stress and splitting. They also suggest splitting in the infrequently irrigated treatments may have been due to cyclic water stresses on the hypocotyl similar to the swelling and shrinkage observed in tomato and pepper fruit. As the least splitting occurred between two other

frequencies which are close together results from this experiment might indicated how sensitive radishes are to differences in soil matric potential.

In an additional experiment Kang and Wan (2005) also investigated the effects of soil water potential on the large winter type of radish. They grew radishes at five different soil water potentials, -0.015 MPa, -0.025 MPa, -0.035 MPa, -0.045 MPa and -0.055 MPa. The irrigation treatments had no effect on growth rate or yield of the radish crop but there was an effect on splitting ($P<0.05$). The highest level of splitting was observed under the wettest conditions, -0.015 MPa, where 18.9% of the radishes split (n=72) and the lowest number of radishes, 1.4%, split at -0.035 MPa (n=74) (Kang & Wan 2005). These results suggest it is not rate of growth which causes splitting in radishes but no explanation was proposed in the paper for potential causes of splitting as a result of soil water potential. As in the previous experiment, it was again a treatment in the middle of a series of similar treatments which resulted in the least amount of splitting. Again, this could be considered an indication of how sensitive radishes are to differences in water availability. However, it should be noted there are only small differences in the water potential between the treatments used in this experiment and none of the treatments were likely to have resulted in a water deficit for the plants. Possible inconsistency with the results from this experiment come from Hartz *et al.* (2005), who found no correlation between cracking susceptibility in carrot and soil moisture in terms of mean soil moisture potential or maximum soil moisture potential during growth. However, splitting susceptibility was found to correlate with turgor potential within the carrots and soil texture. The greatest amount of splitting occurred in soil with the least amount of sand and the least amount of splitting occurred in soil with the greatest sand content (Hartz *et al.* 2005). As water drains more freely in soil with a high sand content, this suggests available water content may have had an effect on splitting in this investigation but was not detected by the equipment used in the experiment. The available water content for the field was measured with three resistance sensors (Watermark blocks, Irrometer Co., Riverside, California) which may not have been adequate replication. There is considerable heterogeneity within soil and estimating soil water potential using just three sensors may not have been representative

of the field as a whole (Jones 2007). If water did drain from the soil with a higher sand content more rapidly the results from this experiment would suggest higher levels of splitting were associated with wetter growing conditions which is in keeping with results from other investigations.

There is evidence that timing of suitable 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 (Salter & Goode 1967). Similarly, Sørensen (1997) 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. Sørensen (1997) attributed the differences in timing of water stress to differences in the type of growth occurring at each development stage (Sørensen *et al.* 1997). During the early period of drought stress which failed to reduce splitting, carrot growth is characterised by cell division whereas during mid-growth when a period of drought stress reduced splitting, carrot growth is created by rapid radial root expansion caused by cell enlargement. As splitting is thought be affected by cell wall strength and composition, factors which affect this may affect splitting susceptibility. Sørensen (1997) suggested the decrease in splitting may have been due to a decrease in the rate of expansion during this period. 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).

By using a texture analyser to drive a wedge into blocks of phloem parenchymal tissue from carrots at different stages during ontogeny, McGarry (1995) showed fracture toughness in carrot was broadly correlated with tissue water potential during growth. However, the correlation coefficients between water potential and fracture toughness were not high, the correlation gradients varied between treatments and there were two periods of tissue strengthening which occurred irrespective of water status indicating other factors were also affecting splitting susceptibility. Criticism of the method employed by McGarry (1995) was presented by Hole *et al.* (1999) who observed no relationship between

susceptibility to splitting damage and tissue fracture toughness on blocks of tissue suggesting this method may not be the most accurate method for measuring splitting susceptibility in carrot.

1.3.3.2 Other factors affecting growth splitting

Other factors are also thought to effect growth splitting. These have been summarised in Table 1-6.

Table 1-6 Factors affecting growth splitting in different crops as reported in published scientific papers

Factor	Crop	Effect on splitting	Reference
Autumn planting	Kohlrabi	Increase	Lippert (1999)
High air temperature 30 days prior to harvest	Carrot	Increase	Hartz *et al.* (2005)
Boron deficiency	Radish	Increase	Skok (1941) Proctor (1987)
Boron application	Tomato	Decrease	Dorais *et al.* (2004)
High nitrogen application	Carrot	Increase	Hartz *et al.* (2005)

Other environmental factors which have been suggested to affect splitting in vegetable crops are season and temperature although the results are less than conclusive. Lippert (1999) observed a higher rate of cracking in kohlrabi which was planted in the autumn compared to earlier in the year. This may have been as a result of many factors and interactions of factors which occur as the seasons change, for example, differences in light, rainfall or temperature, none of which were measured in this study. Hartz *et al.* (2005) found air temperature in the final 30 days prior to harvest was positively correlated with growth splitting in carrot suggesting cooler temperatures prior to harvest may result in less splitting. However, in contrast to this, McGarry (1995) observed an increase in field

grown carrot strength during the later period of growth as did Hole *et al*. in 1995. These differences were not observed in the following 1996 season or by decreasing temperature in a controlled environment experiment (Hole *et al.* 1999) suggesting differences in splitting were not in fact due to season or temperature but another factor not measured in these papers.

Boron deficiency has been shown to affect splitting in radishes (Skok 1941; Shelp *et al.* 1987) and the application of boron through spraying on radishes (Shelp *et al.* 1987) and other crops such as tomato (Dorais *et al.* 2004) has been shown to decrease splitting. As a dicotyledonous plant, their optimal leaf boron concentration is between 20 and 80 μg g^{-1} of dry matter (Sedlacek 2001). Boron is required for the formation of cell walls and membranes therefore it may have an effect on splitting as a result of plasmoptysis by affecting cell wall strength. Plants absorb boron in its water-soluble form boric acid. The availability of boric acid can be affected by pH, temperature, soil texture and soil moisture (Sedlacek 2001). Both soil moisture which is too high and soil moisture which is too low can result in boron deficiency. If the soil moisture is too low plants tend to extract water from greater depths and boron content in the subsoil is lower than the topsoil. If the soil moisture is too high then boron may be leached leading to deficiency (Sedlacek 2001).

Increased incidence of splitting has been linked to high nitrogen application in carrot (Hartz *et al*. 2005). In this study it was suggested over application of nitrogen fertiliser affected periderm strength and consequently splitting susceptibility. However, high levels of variability were observed in this study and no correlation was observed between nitrogen in the carrot tissue suggesting other factors may have also been affecting splitting susceptibility.

1.3.4 Harvest splits

Splitting can happen during the harvest, storage and packing processes as produce is exposed to changes in environmental conditions and mechanical stresses. The principle post-harvest changes to the crop which are thought to effect susceptibility to splitting as a result of mechanical damage are changes in tissue water status and temperature.

Previous research into the causes of harvest splitting has focused on these two areas. These have been summarised in Table 1-7 and Table 1-8.

1.3.4.1 Tissue water status

Table 1-7 Summary of the effects of tissue water status on harvest splitting in different crops as reported in published scientific papers

Factor	Crop	Effect on splitting	Reference
Increased turgor	Carrot	Increased residual stress and strain	Kokkoras (1995)
Decreased turgor by partial lifting	Carrot	Decrease	Gracie (2004)
Increased turgor	Potato	Increase	Konstankiewicz and Zdunek (2000)
			Bajema *et al.* (1998)
Increased water potential and turgor	Carrot	Decrease in failure force	McGarry (1993)
Increased turgor	Several (review paper)	Reduction in resistance to damage	Galindo *et al.* (2004)
Increased water potential	Carrot and radish	Increase in cut force	Herppich *et al.* (2004)

Postharvest tissue water status is an important factor affecting splitting susceptibility. It is thought to affect tissue mechanics and splitting susceptibility through turgor pressure. At high turgidity plant cell walls are believed to already be stretched and as a consequence are more easily ruptured (Kokkoras 1995).

An indication of water status having an effect on splitting comes from cherry varietal tests for splitting. These are performed by placing cherries into water and recording the percentage which split. Although this test does not fully replicate *in situ* splitting, correlations have been observed with results from the field (Measham 2011). Evidence of

a reduction in turgor reducing splitting susceptibility is provided by Gracie (2004). Gracie (2004) found a reduction in turgor pressure caused by partially-lifting carrots reduced splitting susceptibility. The carrots were partially-lifted to sever the fibrous root system then left in the soil over night before harvesting the following morning. These carrots with reduced turgor had a greatly diminished splitting susceptibility and could not be induced to split using a penetrometer. Further evidence to support turgor pressure affecting splitting susceptibility is provided by the investigation by Konstankiewicz and Zdunek (2001). In this investigation the turgor pressure of potato tuber tissue was manipulated by immersion in solutions of mannitol at different concentrations. They found the compressive strength of the tissue samples decreased with increasing turgor pressure suggesting potato tubers are less susceptible to splitting when they are more turgid (Konstankiewicz & Zdunek 2001). However, the use of mannitol has been criticised as it can directly influence tissue mechanical properties, it has been suggested by Bajema *et al.* (1998) that the effects of water relations should be investigated using fresh samples at different turgor levels achieved by dehydration in air (Bajema *et al.* 1998). Despite this, the results obtained by Konstankiewicz and Zdunek (2001) are similar to those of Bajema *et al.* (1998) who found potatoes with lower turgor, as a result of dehydration in air, had higher compressive strength than more turgid potatoes. McGarry (1993) also found failure force was negatively correlated with both water potential and turgor pressure. McGarry (1993) measured the failure strain of phloem parenchyma tissue by driving a wedge into blocks of tissue with a texture analyser. However, turgor pressure was only negatively correlated with failure strain within cultivar. The turgor pressure of the splitting susceptible cultivar used in the experiment was lower than the split resistant cultivar suggesting differences in splitting susceptibility can only be partially attributed to turgor pressure. In their review paper into factors affecting the postharvest quality of vegetables, Galindo *et al.* (2004) concluded that although higher turgor had been shown to correlate with lower resistance to damage it did not explain all the variation. Galindo *et al*. (2004) considered other important factors in determining splitting susceptibility may be the strength of superficial tissues, cell packing, adhesion and cell wall composition (Galindo *et al*. 2004).

Further confusion into the effects of tissue water potential on tissue strength comes from Herppich *et al*. (2004), who showed water potential was positively correlated with cutting force in carrots and radishes. They acknowledged their results contradict those of previous researchers and suggested the discrepancy may be due to differences in the mode of failure induced by the different testing methods (Herppich *et al.* 2004). Herppich *et al.* (2004) used cutting force, whereas McGarry (1993) used a wedge to induce fractures. The effects of turgor are thought to depend on the type of splitting which is occurring. When the mode of failure is plasmoptysis higher turgor pressure has been shown to reduce tissue strength but if splitting occurs as a result of cell debonding the opposite is true (Lin & Pitt 1986). In support of this McGarry (1993) found failure force of phloem parenchyma occurred due to cell rupture (plasmoptysis), as would be expected from an increase in splitting susceptibility at higher turgor pressure. The mode of failure was not recorded by Herppich *et al.* (2004).

1.3.4.2 Temperature

Table 1-8 Summary of the effects of temperature on harvest splitting in different crops as reported in published scientific papers

Factor	Crop	Effect	Reference
Decreased temperature	Several (review paper)	Increased firmness	Bourne (1982)
Decreased temperature	Potato	Increased splitting	Bajema *et al.* (1998)
Decreased temperature	Carrot	Increased stress	Kokkoras (1995)
Decreased temperature	Carrot	Decreased cut force	Herppich *et al.* (2002)
Decreased temperature	Radish	No effect	Herppich *et al.* (2002)

There is evidence within the literature that temperature affects splitting susceptibility. In a review of the effects of temperature on a range of fruits and vegetables, Bourne (1982) showed for the majority of crops tested, increased temperature was associated with decreasing firmness, 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 led the investigators to conclude that the same mechanism must explain both the effects of temperature and turgor. Kokkoras (1995) also found an effect of temperature on tissue stress within carrots but found no effect of temperature on tissue strain. Tissue strain was established by cutting discs of carrot tissue and measuring the resulting deformation. The value for stress was then calculated from this. Stress was calculated by multiplying the values of strain by the modulus of elasticity. The modulus of elasticity was measured by tensile tests with a texture analyser. By affecting the modulus of elasticity, temperature affects the stress but not the strain within the carrot tissue. Kokkoras (1995) concluded low temperatures cause an increase in cellular turgidity by causing differences in contraction between the vacuole content, which is predominantly water, and the cytoplasm and cell wall. It is thought the cytoplasm and cell wall may contract to a greater extent than the vacuole at low temperatures causing an increase in turgor pressure. Therefore, low temperatures increase damageability because at low temperatures tissue becomes more turgid (Kokkoras 1995).

Contradictory results were found by Herppich *et al.* (2002), who found the force required to cut a carrot was negatively correlated with temperature with the highest forces being required cut the carrots at 5°C. When testing the force required for cutting radishes no relationship was found between temperature and cutting force. In the same investigation Herppich *et al.* (2002) investigated the effects of water status on cutting force and found a relationship for both carrot and radish. This led Herppich *et al.* (2002) to conclude differences in the force required to cut carrot at different temperatures must not be turgor

specific but must be tissue or species specific. It was speculated that the differences may be due to the ability of carrots to undergo cold acclimation, changing their cell wall properties and tissue stiffness within hours of transfer to cold conditions. They concluded the differences in cutting force at different temperatures were due to changes in the cell walls of carrots (Herppich *et al.* 2002). The differing results of Herppich *et al.* (2002) may have been due to the method of texture analysis. Herppich *et al.* (2002) used cut force whereas Bajema *et al.* (1998) used compression failure force and Kokkoras (1995) used tissue deformation. This highlights the need to design experiments so they measure the correct type of tissue failure for splitting susceptibility.

1.4 Water

As discussed in pervious sections of this literature review, water is thought to be a key factor affecting growth and harvest splitting susceptibility in a variety of crops. The available water content of the soil during growth is thought to affect growth splitting and post-harvest, the tissue water content is thought to be a significant factor. In order to understand how to design, conduct and interpret experiments which investigate the effects of water on growth and harvests splitting it is important to have an understanding of the behaviour of water within the soil and the plant. This area of research has been investigated extensively and the relevant information has been summarised for this review.

1.4.1 Soil water content

The amount of water in the soil is constantly changing. Water is lost through evapotranspiration and replaced by irrigation or rainfall. The net amount of water which is lost due to evapotranspiration can be predicted using modifications of the Penman-Monteith equation (Monteith 1965) such as the FAO Irrigation and Drainage Paper No. 56 ‘Crop evapotranspiration - Guidelines for computing crop water requirements’ (Allen *et al.* 1998) which superseded the earlier FAO Irrigation and Drainage Paper No. 24 'Crop

Water Requirements' (Doorenbos & Pruitt 1977) which was found to either overestimate or give inconsistent results for levels of evapotranspiration.

In the soil, water moves by bulk flow through interconnected pore spaces. Water is held in the soil in pores and films by adhesive and cohesive forces. It is classified as being gravitational, capillary or hygroscopic based on the tension with which it is held in the soil. Capillary pore spaces are small pores (30 – 60 µm or less) which retain water against gravity (Kramer 1983). As the rate of capillary movement is determined by pore size the size and distribution of capillary pore spaces within a soil will govern the movement of water within it. The texture of the soil, which is determined by the relative proportions of sand, silt and clay, will determine the pore size distribution within it (Kramer 1983), though organic matter content will modify this to some degree. The finer the particle size of the soil the smaller the pore size will be and the greater the capillary movement will be. Sandy soil has a large proportion of medium to large sized capillary pores and the capillary movement is rapid but the ultimate height to which water will be drawn is limited. Clay soils have a high proportion of fine capillary pores and the ultimate height of capillary movement is greater than that of sand but the rate of progress is slower due to frictional forces and the tortuous nature of the pore distribution. The rate and height of capillary movement in loam soils is intermediate of that of sand and clay (Iwata *et al.* 1988).

Different soil types have different proportions of capillary and non-capillary pore space within the soil structure meaning soil type will affect the drainage and aeration of the soil. In the UK, soils are classified as sands, loams, or clays depending on the proportions of particles sizes they contain. It should be noted soil texture depends on the country and there will be notable differences in the classification and particle sizes in different countries. As this thesis is concentrating on radishes grown in the UK, this literature review has focused on UK soil types and classification. Particles sizes are defined as large (>2000 to 20 µm), intermediate (20 to 2 µm), and fine (<2 µm) (Kramer 1983). Soil pore size is an important factor in the amount of water available to the roots. A pore size of 30 µm or above has a water potential of >-0.01 MPa and contains water which is freely available under gravity to the plant. This pore size is important to allow expansion of the

roots. Generally speaking, plants can also absorb water from a pore size of 0.3 to 30 µm (-0.01 MPa to -1.0 MPa), this pore size is important in times of drought as water is retained in smaller pores more strongly (Kramer & Boyer 1995). The PWP for most plants is broadly considered to be -1.5 MPa as plants are able to extract water from soil colloids to this point.

Organic matter can also affect the water holding capacity of soil. Soil organic matter is comprised of plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms. Arable topsoil typically contains between 1 and 6% organic matter. Soil from low-lying wet areas can contain up to 90% organic matter. Humus is soil organic matter which has long been decayed and therefore is stable and no longer recognisable as plant material. Humus can hold the equivalent of 80 to 90% of its weight in moisture. It is the combined effects of pore size and organic matter content which determine the field capacity of a soil (Kramer & Boyer 1995).

1.4.1.1 Measuring soil water content

Soil water content is defined as the water lost from the soil upon drying to a constant mass at 105°C. It can be both expressed gravimetrically as mass of water per unit of dry soil (kg/kg) or volumetrically and measured in units of volume (m^3/m^3). It should be remembered the total volume will consist of air, water and soil whereas total mass will consist of only soil and water. Bulk density is a measure of a soils mass per unit volume, a decrease in pore space increases bulk density. By multiplying the gravimetric water content (GWC) by the bulk density it can be used to convert the GWC into volumetric water content (VWC).

Gravimetric and VWC measurements will only have meaning in combination with a moisture release curve as the percentage water content for different soil textures will represent different available water contents to the plant. The same water content may describe a fully saturated sandy soil with a large amount of available water or a dry clay with little available water (Jones 2007). The soil water retention curve shows the

relationship between water potential and the water content, the curve is therefore different for each soil. Alternatively the water content of the soil can be measured as pressure or tension, this has the benefit of enabling comparisons of the plant water availability between soils. Soil water potential determines the rate and direction of flow of water in soil and plants. Soil water potential is comprised of, solute potential, pressure potential, gravimetric potential, humidity potential and matric potential. Soil water potential which is optimal will meet the physiological needs of a plant and will allow both water and nutrition uptake. Soil water potential which is too low will negatively impact plant growth, as will a soil water potential which is too high. Low soil water potential can impede plant growth by resulting in increased soil strength with can impede root penetration, increase stomatal resistance and limit photosynthesis. Soil water potential which is excessively high may negatively impact plant growth by causing leaching of nutrients and limitation of oxygen diffusion through the soil to the roots (Kang & Wan 2005).

The available water content in the soil to the plant is the range between the field capacity and the permanent wilting point (Kirkham 2005). Field capacity is the maximum amount of water the soil can retain against gravity. Capillary pore spaces are small pores (30 to 60 µm or less) which retain water against gravity. It is these pores which determine the field capacity of a soil. Non-capillary pore space is the fraction of soil volume from which water drains by gravity and provides the air space essential for aeration of roots (Kirkham 2005). Field capacity is not a soil constant, but depends on the conditions under which it is measured. For simplification field capacity is usually determined as the amount of water in the soil at -0.005 to -0.010 MPa (White 2006). Permanent wilting point (PWP) is the soil content at which plants remain wilted overnight or in a humid chamber unless they are re-watered. Briggs and Shantz (1912) (as cited in Kirkham (2005)) found plants of many species wilted at approximately the same water content in a given soil therefore, -1.5 MPa is commonly taken as the water potential at the point of permanent wilting. It should be remembered that although the value -1.5 MPa is used to conveniently define the permanent wilting point, it like field capacity is not a sharply defined value (White 2006), in addition for some plants the lower threshold can be much greater than -1.5 MPa, for

example some plants, mainly xerophytes, are able to extract water to -3.1 MPa. The water content at any given water potential is higher during drying or desorption than during wetting or sorption, this phenomenon is called hysteresis. This is due to the menisci, swelling and air entrapment in wetting soil and rate of filling and emptying of pore spaces (White 2006). It is important to take into consideration hysteresis when taking soil moisture measurements in research as it influences the readings and may lead to inappropriate conclusions.

1.4.2 Plants and water

1.4.2.1 Movement of water from the soil into and around the plant

Plant water status is determined by the interrelationships of soil, plant and atmospheric factors; a simplified model of this is the soil-plant-atmosphere-continuum (SPAC) which describes the pathway of water as it travels from the soil, through the plant to the atmosphere (Kramer & Boyer 1995). Water can move by passive movement, mass flow, diffusion, osmosis and active uptake.

To access water in the soil the roots must be in close proximity to it. Nye (1994) reported that main roots (1 mm diameter) will lose full contact with the soil due to root shrinkage at a soil matric tension of 0.02 MPa while finer roots (0.1 mm diameter) will lose full contact at about 0.07 MPa and root hairs (0.01 mm diameter) at about 0.23 MPa (Nye 1994). When roots are in contact with the soil Poiseuille's law can be used to calculate the velocity of water uptake by the roots. Poiseuille's law describes the velocity of flow of liquids through a cylindrical capillary tube (Kirkham 2005). The gradient in water potential between the soil and the root xylem is the force which drives water from the soil into the plant. When there is more negative water potential in the roots compared to the soil water moves by osmosis from the soil along the concentration gradient into the root. Roots typically have a water potential of about -1.0 MPa. The negative pressure in the xylem draws water from the soil to replace the water which is lost through transpiration, decreases in water potential in the roots increases their water extraction capacity from the soil (Kramer 1983).

Short distance transport of water initially into the roots and then between cells in the plant occurs via one of three alternative pathways, namely the; appoplastic, symplastic or transcellular pathways. When entering the plant through the apoplastic pathway water and solutes diffuse along permeable cells walls into the root cortex. Before water and solutes are able to pass into the stele they are forced to enter the symplastic pathway by the Casparian strip. The Casparian strip is a hydrophobic barrier comprised of suberin deposited in bands on the radial walls of cells (Kramer 1983). Water and solutes entering the root via the symplastic pathway immediately cross the cell membrane and move from cell to cell through plasmodesmata which are cytoplasmic tunnels. Apoplastic transport of water and solutes occurs freely by diffusion through the extracellular space. Symplastic transport of water and solutes across the plasma membrane occurs by active transport enabling movement and accumulation of solutes against a concentration gradient. Transcellular transport is a cell to cell transport mechanism that involves crossing both the apoplast and symplast and requires activity of transporters or channels to cross the plasma membrane (Robert & Friml 2009). Water in the plants is often described as existing in two pathways, apoplastic water is found in the cell walls, intercellular space and xylem vessels and symplastic water is found in the cell cytoplasm (Kramer 1983).

Within the plant there are two vascular networks for long distance transport of water and solutes, the phloem and the xylem. These have opposite directions of flow. Xylem transports and stores water, nutrients and hormones (including ABA and cytokinins) from the roots to the above ground tissues. Phloem distributes the products of photosynthesis (mainly carbohydrates) and proteins, mRNAs and hormones (ABA, auxin, cytokinins) from source tissues to sink tissues. The water in the vascular tissue moves as a column due to the adhesive and cohesive properties of water; any break in this column discontinues water movement through the system (Kirkham 2005). Driven by evapotranspiration, which results in a pressure differential between the plant and atmosphere, this continuous column of water moves from the soil into the plant and out to the atmosphere.

1.4.2.2 Roles of water in the plant

Water is very important to plants as most cellular processes depend on cell water status. Water potential influences many physiological aspects of the plant; ABA and solute accumulation increase under mild water stress, photosynthesis and stomatal conductance decrease in plants under dry conditions and protein synthesis, cell wall synthesis and cell expansion all decrease under mild water stress (Griffiths et al. 2002). Limited water can therefore limit or cause malfunction in numerous metabolic processes.

Abundant water in the soil results in turgid plant cells as water moves by osmosis from the soil along the concentration gradient into the root. Turgor potential (positive) is the difference between water potential (negative) and osmotic potential (negative) (Kirkham *et al.* 1972) (Katerji *et al.* 1997). Cell enlargement is generated by water diffusing into cells, and turgor pressure is the result of the increasing volume of vacuolar sap (Kirkham 2005). Cell expansion and turgor are both caused by the inward diffusion of water resulting from a difference in water potential between the interior and exterior of cells. The turgor of a plant cell is determined by water potential, hydrostatic pressure and osmotic pressure. Höfler diagrams can be used to show the RWC or turgor at different water potentials, hydrostatic pressures and osmotic pressures (Kirkham 2005). It should be noted there is a much bigger decrease in pressure potential and water potential for a given change in cell volume and water content for cells with rigid walls than for cells with elastic walls (Kramer 1983). The internal pressure of cells affects the mechanical properties of tissues. Lin & Pitt (1986) argue that turgid cells cause the cell wall to be stressed (Lin & Pitt 1986). Tissues containing turgid cells are crisper, stiffer and more easily fractured than flaccid tissues containing low turgor-pressure cells (Hiller, S. Bruce, D.M. Jeronimidis 1996).

1.4.2.3 Water stress

Water stress refers to situations where normal functioning is disturbed by reductions in water potential and turgor. Water stress can vary in intensity from small decreases in water potential, to temporary midday wilting, to permanent wilting and finally to death by desiccation. Decreasing water content causes loss of turgor and wilting, cessation of cell

division and enlargement, closure of stomata, reduction in photosynthesis and interference with many metabolic processes (Kramer 1983).

Water stress should be considered in relation to ontogeny as the degree of damage will depend on the growth stage. Seeds are often tolerant to dehydration and can remain viable in dry conditions for years yet seedling germination and establishment are often inhibited by soil water deficits. Vegetative growth can be severely inhibited by moderate water stress due to the effects on cell division and enlargement. Reduction in vegetative growth due to water stress may result in loss of yield and be detrimental to growers. Abdel (2007) found irregular watering of radish plants decreased both the yield and yield quality. Kirkham (1972) found a positive turgor pressure above 0.5 MPa was found to produce the greatest cell division and a positive turgor pressure above 0.3 MPa was found to have the greatest increase in cell expansion in radish cotyledons. By measurement of DNA in radish cotyledons Gardner and Nieman (1964) found cell division reduced at -0.1 MPa but continued at low but measureable levels to a water potential of -1.6 MPa. Kirkham *et al.* (1962) found a disparity in the turgor which affected cell division and cell enlargement with cell division in radish cotyledons being inhibited at a higher turgor than cell enlargement. Joyce (1983) found although radish cellular expansion and division was reduced at times of water stress the effect was not permanent and after 9 days of a relief of the water stress expansion of the hypocotyl was at a rate comparable to that of plants which had not been stressed. However, plants with a history of water deficit were not able to recover to the same size as non-stressed radish in the same time period. Commercially this could result in growers either having a reduced yield or a longer duration to harvest which might reduce the quantity of harvests per growing season.

If water stress develops slowly plants may be able to continue growth at lower water potentials by adjusting their osmotic potential sufficiently. Leaf enlargement is often very sensitive to water stress and is often reduced or stopped before photosynthesis is reduced (Boyer, 1970). When plants undergo water stress the rate of photosynthesis may be drastically reduced due to both a reduction in rate as a result of closure of stomata and a reduction in photosynthetic area. The photosynthetic area may be reduced due to old

leaves senescing more rapidly and new leaves developing more slowly. Under water stress root to shoot ratios are generally increased. Root weight usually decreases but not as much as shoot weight (Kramer 1983).

1.4.2.4 Measuring plant water content

Measuring plant water content can be done directly by measuring tissue water content or indirectly by measuring plant physiological responses to water status (Jones 2004).

1.4.2.5 Measuring tissue water content

Water content can be expressed as a percentage of fresh weight, dry weight or turgid weight. Water content as a percentage of fresh or dry weight after oven drying at 60 to 85°C is commonly used to express water content. The method of using turgid weight is termed RWC in the literature. The method is similar to the fresh weight and dry weight methods but has an added step where the tissue is saturated to attain the turgid mass. It is expressed by:

$$RWC\ (\%) = [FM - DM)/(TM - DM)] * 100$$

Where, FM, DM and TM are the fresh mass, dry mass and turgid mass respectively. Methods for achieving turgid weight vary and can cause complications. Achieving saturation of entire leaves or other plant parts can take a considerable amount of time so small discs of tissue are often used as an alternative. These are either floated on water or placed on a wet surface in a moist chamber. When saturating plant material it can be difficult to determine when the tissue is fully turgid and can take between 4 and 48 hours. Water uptake can be divided into two periods, the first being the elimination of the water deficit and the second being associated with growth. The first period is of interest when calculating RWC but the second period is not and would result in incorrect results. Unfortunately it is not always easy to differentiate between the two. As a result of these problems there is no standard protocol for saturation as the best method will vary with

each plant and tissue type therefore the method should be refined and determined for each tissue (Kramer 1983).

An alternative method to measure water status is turgor pressure. Turgor pressure pushes the plasma membrane against the cell wall in plants. This pressure is caused by the osmotic flow of water from an area of low solute concentration outside the cell into the cell's vacuole, which has a higher solute concentration. Healthy plant cells are turgid and plants rely on turgidity to maintain rigidity. Turgor pressure is important with regards to cell enlargement, guard cell movements and other processes reliant on changes in cell volume and permeability to water and ions. Turgor pressure is usually described as the difference between the water potential and osmotic potential in a cell. In a flaccid cell the turgor pressure is zero and in a fully turgid cell the turgor pressure is equal to the osmotic potential. Water potential can be measured using thermocouple psychrometers or a Scholander pressure chamber. Osmotic potential of sap can be measured cryoscopically by extracting sap from frozen tissue, with an osmometer or by using liquid equilibrium (the Shardakov dye method is an adaptation of this method). All of these methods for measuring osmolarity measure sap from a mixture of both the symplastic and apoplastic pathways. Direct measurements of turgor pressure in large cells can also be made using a micromonometer attached to a capillary tube inserted into a cell (Kramer 1983).

1.4.2.6 Measuring physiological responses

Methods of measuring physiological responses include stomatal conductance (porometer, thermal sensing) and growth rate. Hsiao (1973) reported that cell growth is very sensitive to tissue water deficit responding to water deficits of less than 0.1 MPa. Stomatal conductance can be a useful measure of water stress in the short term but during long term water stress plants may adapt by decreasing leaf area with the consequence that stomatal conductance and photosynthesis may be comparatively constant per unit area as the soil dries (Jones 2004). Growth rate can be measured using dendrometers. Plants have diurnal fluctuations in diameter as a result of fluctuations in water content. The

magnitude of expansion or shrinkage over time can be used to indicate water status but it is important measurements are made at the same time of day (Kramer 1983).

1.5 Conclusions from the literature review

Previous research into other crops has found splitting is caused by a complex interaction of both genotypic and environmental factors. It is thought a number of genes are likely to be involved in affecting splitting susceptibility hence breeding for resistance is expected to be too complex. Therefore, the majority of this thesis will concentrate on ways to manipulate the environment during growth and post-harvest to minimise splitting. However, a comparison between different cultivars of radishes will be made in an attempt to identify which cultivars have greater resistance to splitting and the potential physiological reasons for this will be investigated.

An attempt will be made to understand the physiological mechanisms behind splitting. This has been identified as a gap in the existing research as previous research has tended to halt at the correlation of factors associated with lower levels of splitting rather than continuing the investigation to provide a potential explanation for the reduction. This has made interpretation of some of these investigations confusing especially when the results appear to be contradictory. To facilitate this, the growth stages of radishes will be categorised to enable treatments to be applied at defined physiological ages. Different environmental conditions may affect growth rate therefore it would be inappropriate to apply treatments at certain time points as the physiological age of the plant may differ. The type of splitting, cellular debonding or plasmoptysis, which occurs in radishes, will also be identified as this will assist with the interpretation of results.

This literature review showed the majority of research into environmental causes of both growth and harvest splitting in other crops has focused on the effects of water. This has been in terms of rainfall and irrigation during growth and postharvest tissue water content. As part of this literature review the effects of water on splitting in radishes has been identified as an area where there is a gap in the current scientific knowledge. There is very limited research in this area and the investigations which have been performed were predominantly conducted on slower growing winter radishes with little difference between experimental treatments. Therefore, an investigation into the effects of water availability

during growth and post-harvest tissue water content on splitting susceptibility of radishes will form the bulk of this thesis.

This literature review found some investigations have been carried out into the effects of temperature on post-harvest splitting in other crops suggesting this has an effect in splitting susceptibility. As during post-harvest processes radishes are kept in a controlled temperature environment manipulating this may be a way growers can reduce harvest splitting. The effects of post-harvest temperature on radish susceptibility to splitting will also be investigated as part of this thesis.

1.5.1 Objectives of the research

After conducting this literature review the following areas where further investigation is required were identified:

- What are the growth stages of *Raphanus sativus*?
- Are there differences in splitting susceptibility between different cultivars of *Radish*? If there are differences are there any physiological explanations for the differences?
- What is the mode of failure during splitting in radishes?
- Does growth rate correlate with susceptibility to growth splitting?
- Does water availability during growth have an effect on growth rate?
- Does water availability during growth have an effect on growth splitting in radishes?
- Does water availability during growth affect the hypocotyl WC, RWC or water pressure?
- Does hypocotyl WC, RWC or water pressure affect susceptibility to growth splitting?
- Does hypocotyl WC, RWC or water pressure affect susceptibility to harvest splitting?
- Does post-harvest temperature affect harvest splitting in radishes?

1.6 Thesis map

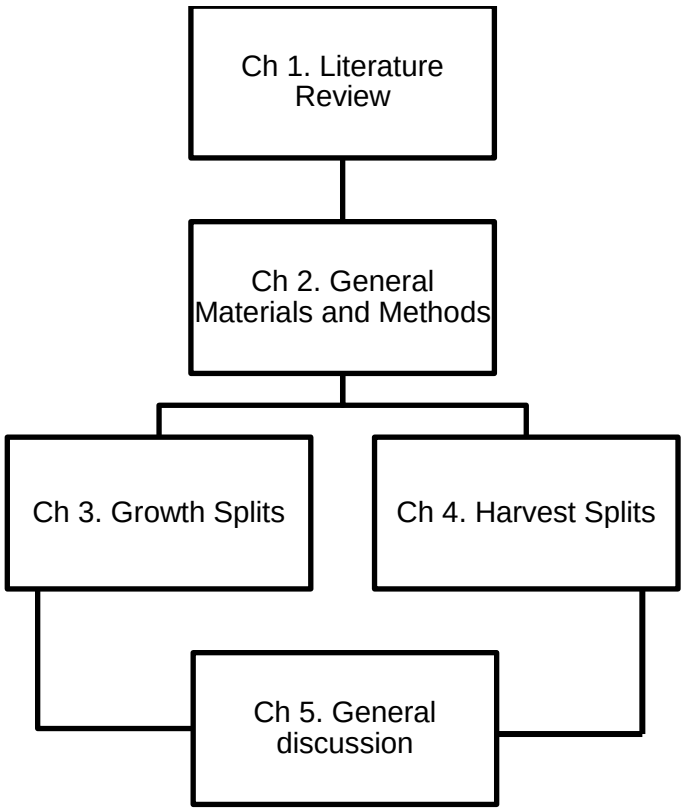


Figure 1-17 Whole thesis map

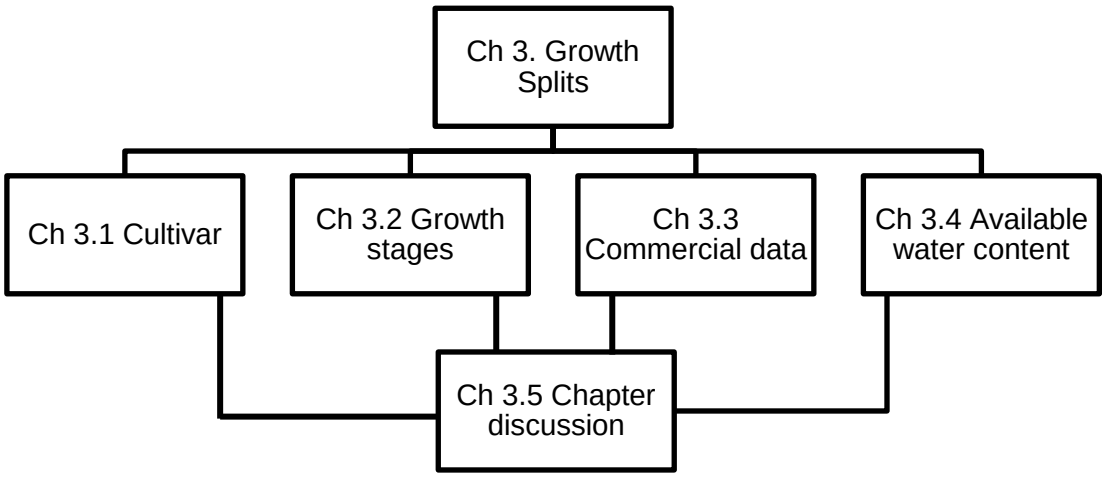


Figure 1-18 Map for Chapter 3: Growth Splits

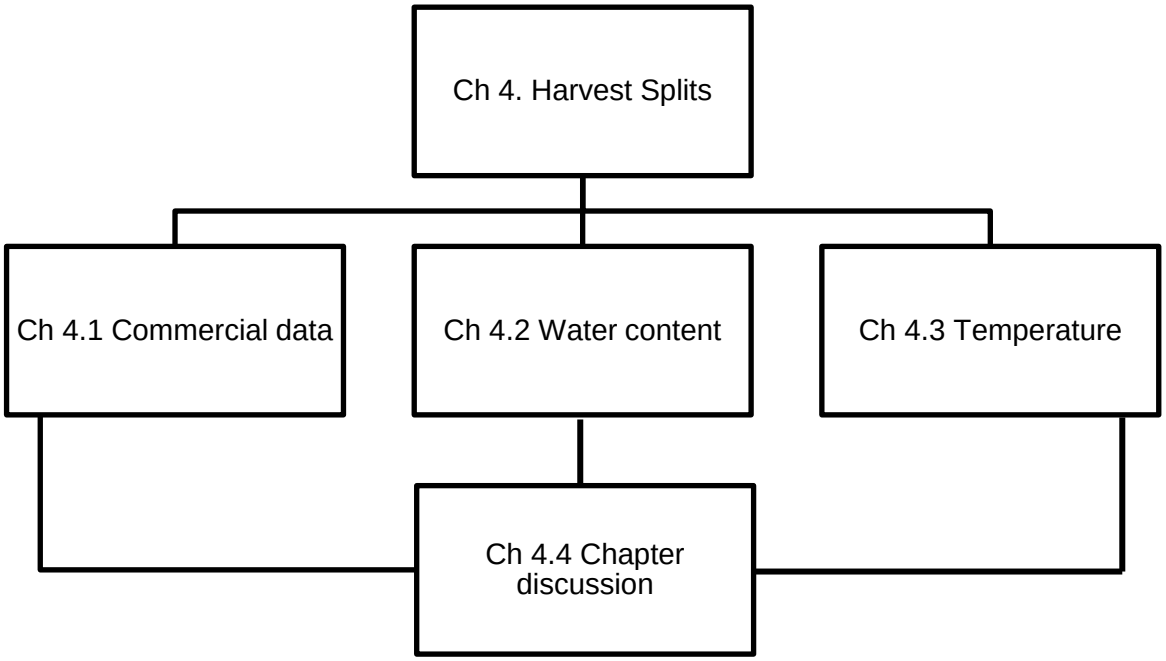


Figure 1-19 Map for Chapter 4: Harvest splits

2. General materials and methods

2.1 Growing radishes

Unless stated otherwise, for each experiment 1.75 L 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, 170 mm in width and 60 mm in depth. All trays were filled level with the rim of the pot, to a weight of 1.5 kg, 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.

The plant trays were arranged in a randomised block design on the glasshouse bench and twenty *Raphanus sativus* 'Rudi' seeds, provided by G's Growers, were planted at a depth of approximately 7 mm (commercial practice) into them. The seeds were planted in pairs in two rows of five with a spacing of 40 mm between the pairs of seeds and the edge of the pot and 90 mm between the two rows of plants. The commercial planting distance between radish seeds in a row is 41 mm. The plant trays were irrigated using capillary irrigation. All trays were irrigated for the initial seven days after the seeds were planted for even establishment of seedlings. The bench irrigation was 17 mm day^{-1} dispensed over three periods of five minutes a day.

After seven days the seedlings which had germinated from the initial 20 seeds were thinned to 10, leaving the most evenly sized plants. The final arrangement was the same as the initial planting of seeds, two parallel rows of five plants 90 mm apart, a spacing of 40 mm between plants in each row and 40 mm between the rows and the edge of the pot (Figure 2-1), obviously these were the initial measurements and the distances decreased as the hypocotyls expanded. Following plant thinning, irrigation and drying-down treatments were imposed.

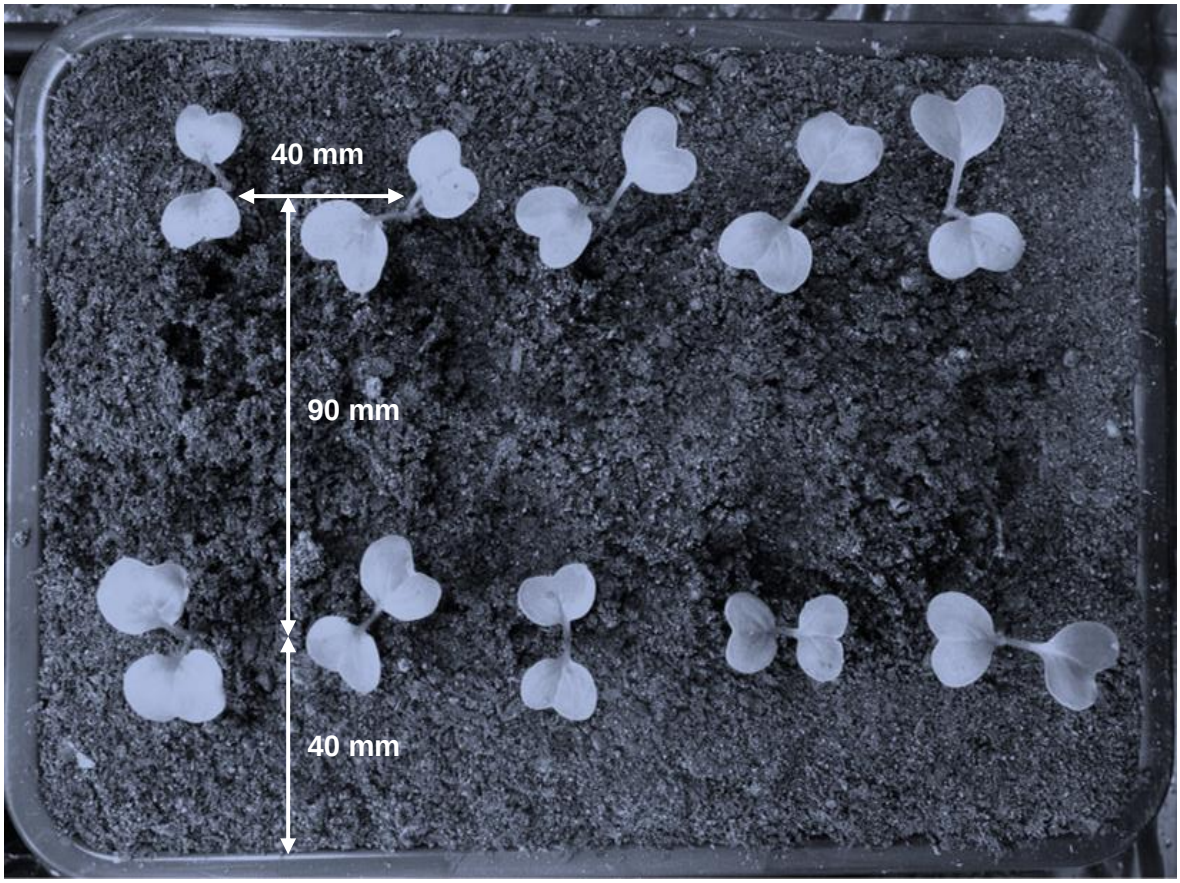


Figure 2-1 Plant pot containing 10 radish seedlings after radish plants had been thinned from 20 seedlings planted in pairs

Glasshouse temperature was set to 20/5°C day/night, the relative humidity and temperature were logged in the glasshouse using TGP 4500 TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK). The loggers were raised off the bench to a similar height as the radish plants and they were shaded from direct sunlight.

From October to March supplementary lighting from 400W SON/T lamps (Thermoforce Ltd., Cumbria, UK) was provided in the glasshouse for 16 hours a day.

2.2 Determining pot capacity

The volumetric water content (VWC) of John Innes No. 2 compost at pot capacity was determined by the gravimetric method (Hall *et al.* 1977). Three 1.75 L G18B half sized seed trays (Garland Products Ltd., Kingswinford, UK) were filled level with the rim of the pot, to a weight of 1.5 kg, 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. The pots of compost were then watered to saturation. The pots were considered to be saturated when water applied to the surface of the pot caused water to immediately flow out of the bottom of the pot. After saturation the pots were covered to prevent surface evaporation and raised up on small saucers to allow the gravimetric water to flow from the holes at the base of the plant pot.

The pots were repeatedly weighed using a FKB 16K 0.1 balance (Kern and Sohn GmbH, Balingen, Germany) until there was little change in weight over a 48 hour period, this was determined as the weight at pot capacity (WW). The trays of compost were then dried to a constant weight at 105°C (DW).

The gravimetric water content (GWC) on a dry weight basis was found to be 110.8 %, determined by the equation:

Equation 1: GWC

$$\mathrm{GWC} = \frac{(\mathrm{WW} - \mathrm{DW})}{\mathrm{DW}} \times 100$$

To calculate the VWC, the GWC was multiplied by the bulk density (BD) (cm^{-3}):

Equation 2: VWC

$$\mathrm{VWC} = \mathrm{GWC} \times \mathrm{BD}$$

Bulk density (BD) was calculated by dividing DW (1042 g) by the volume (V) of the tray (1750 cm^3) and was found to be 0.6 g cm^{-3} giving the VWC at pot capacity of 65.9 %:

Equation 3: BD

$$\mathrm{BD} = \frac{\mathrm{DW}}{\mathrm{V}}$$

2.3 Imposing water availability treatments

Two methods for manipulating water availability to the radish plants during growth were employed. These were, irrigating by hand to particular VWC or preventing irrigation resulting in a period of drying.

2.3.1 Irrigating to particular water content

It is possible to water to certain VWC by watering the pots to a particular weight. It is known the dry weight of the compost is approximately 1042 g and the bulk density (BD) is 0.6 g cm^{-3}. Therefore, by combining Equation 1, Equation 2 and Equation 3 the wet weight (WW) (g) for certain VWC can be determined:

Equation 4: WW

$$\mathrm{WW} = \left(1042\left(\frac{\mathrm{VWC}\ \times 0.6}{100}\right)\right) + 1042$$

When using this method the weight of the plant tray (107 g) and the saucer (108 g) was taken into consideration as was the increasing weight of the radish plants. Destructive harvests were made, each time the plants were watered, of a minimum of three plants which had been exposed to the same treatments. A mean weight of the radish plants which were destructively harvested for each treatment was calculated, multiplied by 10 to give an approximate weight for all of the plants in the tray and added to the weight of the plant tray and the WW giving the weight which the tray should be watered to.

The trays were watered by hand using a squeezable water bottle with a fine nozzle to ensure an even distribution of water over the surface without damaging seedlings. They were watered to the required weight on a FKB 16K 0.1 balance (Kern and Sohn GmbH, Balingen, Germany).

Figure 2-2 Pots containing radish plants were surface irrigated using a water bottle with a fine nozzle to ensure even distribution of water over the surface without damaging the seedlings

2.3.2 Drying down

Drying down was achieved by lifting the plant trays off the surface of the bench and placing them on upturned saucers so they were not in contact with the capillary matting and therefore did not receive any irrigation (Figure 2-3). All trays were lifted off the bench so any differences observed were as a result of the irrigation treatments and not from lifting and moving the trays around. Plant trays which were to continue receiving irrigation were placed immediately back onto the matting.

Figure 2-3 Plant trays containing radish plants were raised on upturned saucers to prevent irrigation from the capillary matting

2.4 Measuring water content during experiments

Changes in VWC were measured using a ML2x ThetaProbe (Delta T Devices, Cambridge, UK) connected to a HH2 moisture meter (Delta T Devices, Cambridge, UK). ThetaProbes measure volumetric soil water content by determination of the apparent dielectric constant. This is possible because there is a linear relationship between the square root of the dielectric constant $\sqrt{\varepsilon}$ and volumetric water content (Miller & Gaskin 1996). The ThetaProbe rods measured 60 mm long, the same as the depth of the pot (Figure 2-4).

Figure 2-4 The VWC of the compost in a plant pot was measured using a Theta Probe (Delta T Devices, Cambridge, UK). In this picture the prongs of the Theta Probe are fully inserted in the compost.

The moisture content was measured in millivolts (mV), converted to volts (V) then converted to VWC using the equation:

Equation 5: VWC

$$\mathrm{VWC} = \frac{(1.07 + 6.4\,\mathrm{V} - 6.4\,\mathrm{V}^2 + 4.7\,\mathrm{V}^3) - \mathrm{a}_0)}{\mathrm{a}_1}$$

This is the equation provided by Delta T Devices for use with the ML2x when accurate soil specific moisture readings are required. The terms a_0 and a_1 depend on the soil properties and should be determined using the method described in the user manual for the equipment. For John Innes No. 2 a_0 was found to be 1.3 and a_1 was 7.1 giving the equation:

Equation 6: VWC

$$\mathrm{VWC} = \frac{(1.07 + 6.4\,\mathrm{V} - 6.4\,\mathrm{V}^2 + 4.7\,\mathrm{V}^3) - 1.3)}{7.1}$$

Despite using the compost specific equation, when this equation was calibrated with compost at a range of water contents a slight discrepancy between the VWC calculated from the GWC (VWC_G) and the VWC calculated from the Theta Probe (VWC_{TP}) was observed (Figure 2-5). The difference between the two measurements being expressed by the equation:

Equation 7: VWC_G

$$\mathrm{VWC_G} = 1.008\ (\mathrm{VWC_{TP}}) - 0.642$$

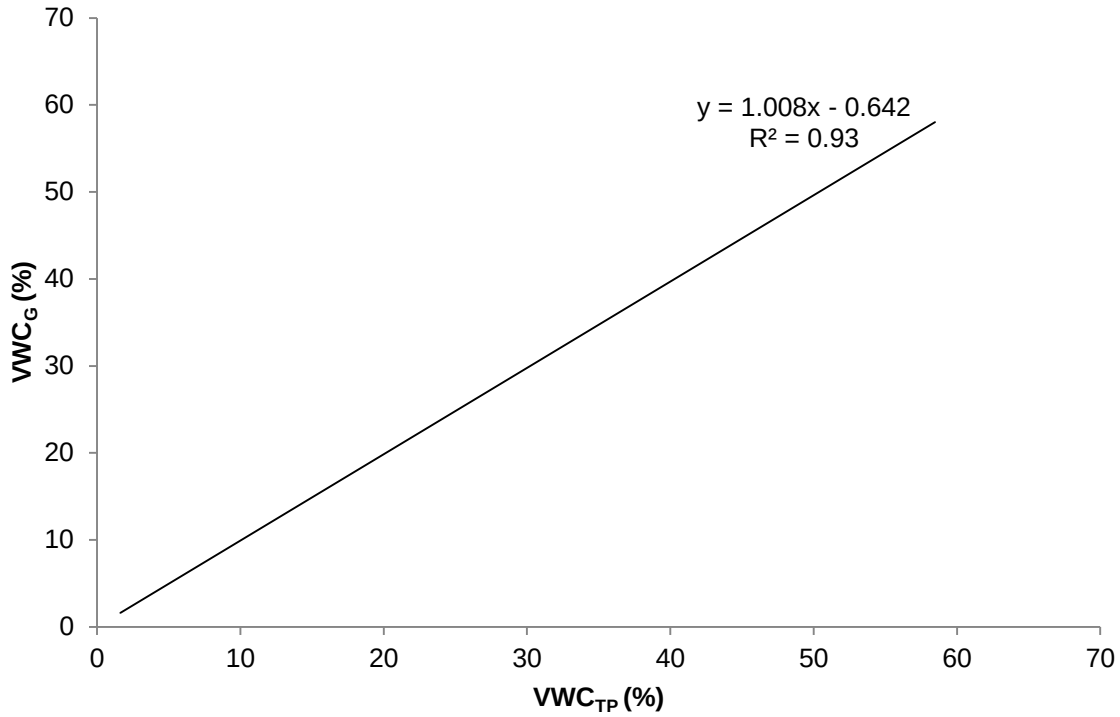


Figure 2-5 Calibrating the Theta Probe by comparing VWC calculated from the GWC (VWC_G) with VWC calculated form the compost specific equation for the Theta Probe (VWC_{TP}).

Therefore, the equation for converting the readings from the Theta Probe to VWC is:

Equation 8: VWC

$$\mathrm{VWC} = 1.008\left(\frac{(1.07 + 6.4\,\mathrm{V} - 6.4\,\mathrm{V}^2 + 4.7\,\mathrm{V}^3) - 1.3)}{7.1}\right) - 0.642$$

It should be remembered that the Theta Probe display shows millivolts (mV) not volts (V) so the readings will need to be converted before using the equation above.

2.5 Water retention (pF) curve

The water retention curve of John Innes No. 2 (Keith Singletons Horticultural products, Cumbria, UK) was determined using pressure membrane apparatus (Soil Moisture Equipment Corp. Santa Barbara, USA). Five samples of compost were placed in rings on the ceramic plate supplied with the equipment. The rings of compost were saturated by

soaking in water for 48 hours whilst covered with tin foil to prevent surface evaporation. Once saturated the covered samples on the ceramic plate were placed inside the pressure chamber. The outflow pipe for the water was connected, the lid of the chamber was sealed and the pressure applied. Once water ceased to be released from the outflow tube at a particular pressure, sections of the compost were removed and immediately weighed using a PCB 2500-2 balance (Kern and Sohn GmbH, Balingen, Germany), this was the wet weight (WW). These sections were then dried at 105°C to a constant weight, this was the dry weight (DW). The GWC was calculated on a dry weight basis using the equation:

Equation 9: GWC

$$\mathrm{GWC} = \frac{(\mathrm{WW} - \mathrm{DW})}{\mathrm{DW}} \times 100$$

The GWC was then converted to VWC by multiplying by the bulk density (0.6 g cm^{-3}).

The remaining compost was returned to the chamber, recovered with tinfoil and the pressure inside the chamber was increased and the process repeated. The water content was determined at 0.04, 0.06, 0.08, 0.102, 0.151, 0.204, 0.253, 0.303, 0.351, 0.401 and 0.429 MPa. To define the water retention curves for John Innes No. 2, the applied pressures (MPa) were plotted against the VWC (%) on a log scale producing a curve (Figure 2-6) with the equation:

Equation 10: VWC (%)

$$\mathrm{VWC}\ (\%) = -8.28 \ln\big(\mathrm{matric\ potential(MPa)}\big) + 5.084$$

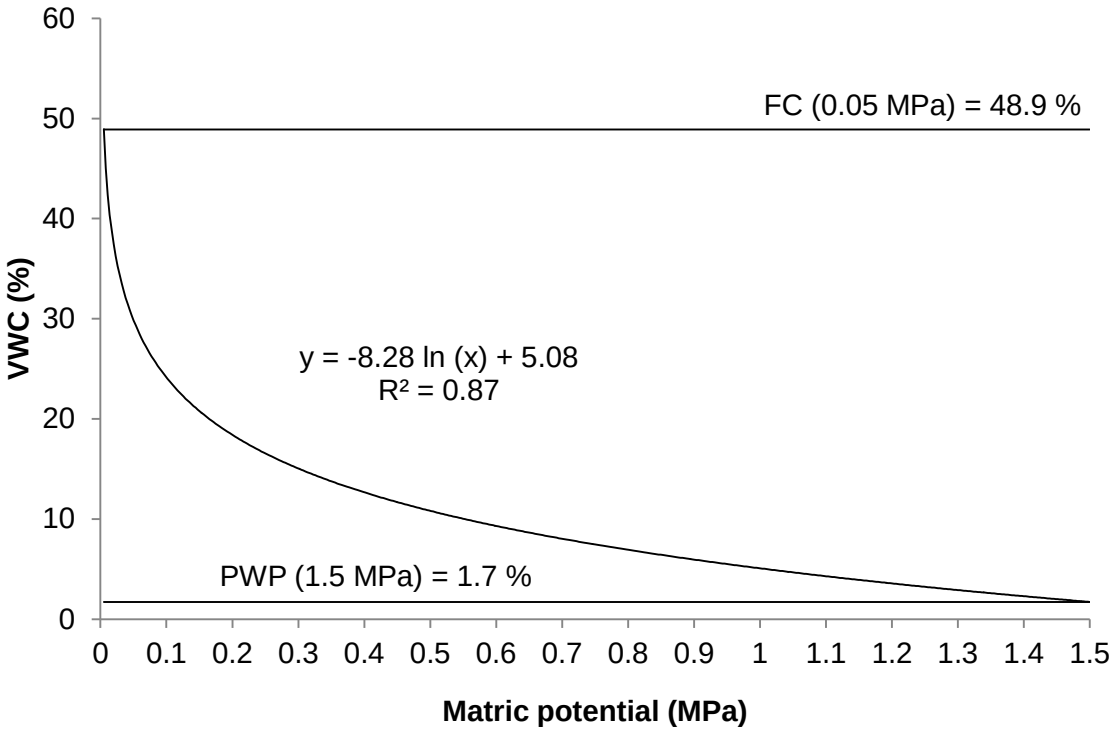


Figure 2-6 Moisture release (pF) curve for John Innes No. 2 produced using pressure membrane apparatus (Soil Moisture Equipment Corp. Santa Barbara, USA)

The moisture content at field capacity (FC) 1.5 MPa is 48.9 % and permanent wilting point (PWP) 0.05 MPa is 1.7%. Available water is the water retained between field capacity (FC) (0.005 MPa) and permanent wilting point (PWP) (1.5 MPa) (Hall *et al.* 1977). Using the water retention curve, it can be determined the VWC of John Innes No. 2 at FC is 48.9% and at PWP is 1.7%.

2.6 Hypocotyl size

Uniformity in radish diameter is desirable as supermarkets usually require radishes which are between 18 mm and 32 mm in width, anything outside of this range is too small or too large for commercial sale in the UK.

During growth the widest part of the hypocotyl visible above the surface of the compost was measured (Figure 2-7). At harvest the maximum hypocotyl length and diameter were measured. All measurements were made using digital callipers (Draper Expert 46610, Draper Tools Ltd., Hampshire, UK).

Figure 2-7 The radish hypocotyl protruding from the compost surface was measured with digital callipers

2.7 Leaf area

Total plant leaf area (cm^2) was measured using Li-3000A leaf area meter (Li-Cor Lincoln, NE, USA). This was done by removing the leaves from the hypocotyl with scissors. It was found, as a result of the close planting of the radish plants within a tray, the smaller radish leaves could not be measured without removing them from the hypocotyl.

2.8 Leaf temperature

Leaf temperature of the youngest fully opened non-shaded leaf was measured using a 66 infrared thermometer (Fluke, WA, USA).

2.9 Stomatal conductance

Stomatal resistance (m^2 s mol^{-1}) of the youngest fully opened leaf was measured using an AP4 porometer (Delta-T Devices, Cambridge, UK). The porometer was calibrated using the calibration plate provided prior to use. Readings were taken in the morning between 8 am and 12 pm.

2.10 Harvest

At harvest the leaves from all plants in a tray were removed using scissors. These were immediately weighed for their fresh weight. The radish hypocotyls from the tray were then removed from the compost and briefly washed in tap water at ambient temperature to remove the compost. They were weighed using a FKB 16K0.1 balance (Kern and Sohn GmbH, Balingen, Germany) with the leaves for total plant fresh weight then the roots were removed with scissors and the hypocotyls were weighed alone for hypocotyl fresh weight.

2.11 Dry matter content

After the hypocotyls and leaves had been weighed to obtain the fresh weight (FW) they were separately put into a labelled perforated polypropylene bags (300 x 450 mm) (Abpac Ltd., Wincanton, UK) which had been already been weighed empty. The bags containing the leaves and the hypocotyls were then placed into a drying oven at 105°C until the contents had reached a constant weight using a FKB 16K0.1 balance (Kern and Sohn GmbH, Balingen, Germany). This minus the weight of the bag was determined as the dry weight (DW). The dry matter content (DM) (%) was calculated using the equation:

Equation 11: DM

$$\mathrm{DM} = \frac{\mathrm{DW}}{\mathrm{FW}} \times 100$$

2.12 Hypocotyl water pressure

The water potential (bar) of radish hypocotyls was measured using a digital pressure bomb (SKPM-1400, Skye Instruments Ltd, Powys, UK) (Figure 2-8).

Figure 2-8 Digital pressure bomb (SKPM-1400, Skye Instruments Ltd, Powys, UK)

The hypocotyl of the radish was placed inside the chamber with the severed petiole protruding (Figure 2-9).

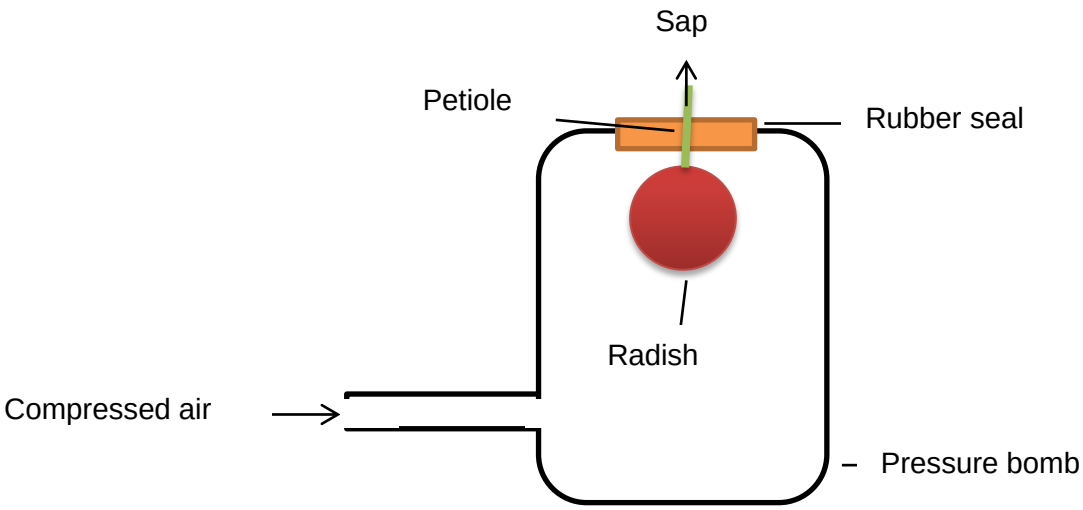


Figure 2-9 Diagram showing how the radish hypocotyl was positioned inside the digital pressure bomb (SKPM-1400, Skye Instruments Ltd, Powys, UK)

2.13 Hypocotyl relative water content (RWC)

Hypocotyl relative water content (RWC) was measured by weighing the whole radish hypocotyl to ascertain the fresh weight (FW). The hypocotyl was then sliced into eight sections with a knife. The sections were placed into a container (100 mL polypropylene cup with cap, Sarstedt, Nümbrecht, Germany) with approximately 100 mL dH_2O. The containers were closed with a lid and placed into cold storage at 4°C for 48 hours. The temperature in the cold storage was logged using TGP 4500 TinyTag logger (Gemini Data Loggers (UK) Ltd., Chichester, UK). After 48 hours the pieces of radish were removed from the water, patted dry using kitchen towel and weighed using a PBB 2500-2 balance (Kern and Sohn GmbH, Balingen, Germany), this was the turgid weight (TW). The sections were then placed in a drying oven at 105°C and dried to a constant weight, this was the dry weight (DW). The RWC could then be calculated using the equation (Smart & Bingham 1974):

Equation 12: RWC

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

The number of sections and time to saturation were decided after conducting a preliminary experiment. For this experiment radishes were left whole, cut into halves, quarters or eighths. They were then put into container of approximately 100 mL of dH_2O at 4°C. At intervals radishes were removed from the water, patted dry with kitchen towel and weighed. It was found radishes which were cut into sections of eight took up water more rapidly and more in total than whole radishes or radishes which were cut into halves of quarters. All radish weights had begun to plateau at 48 hours (Figure 2-10).

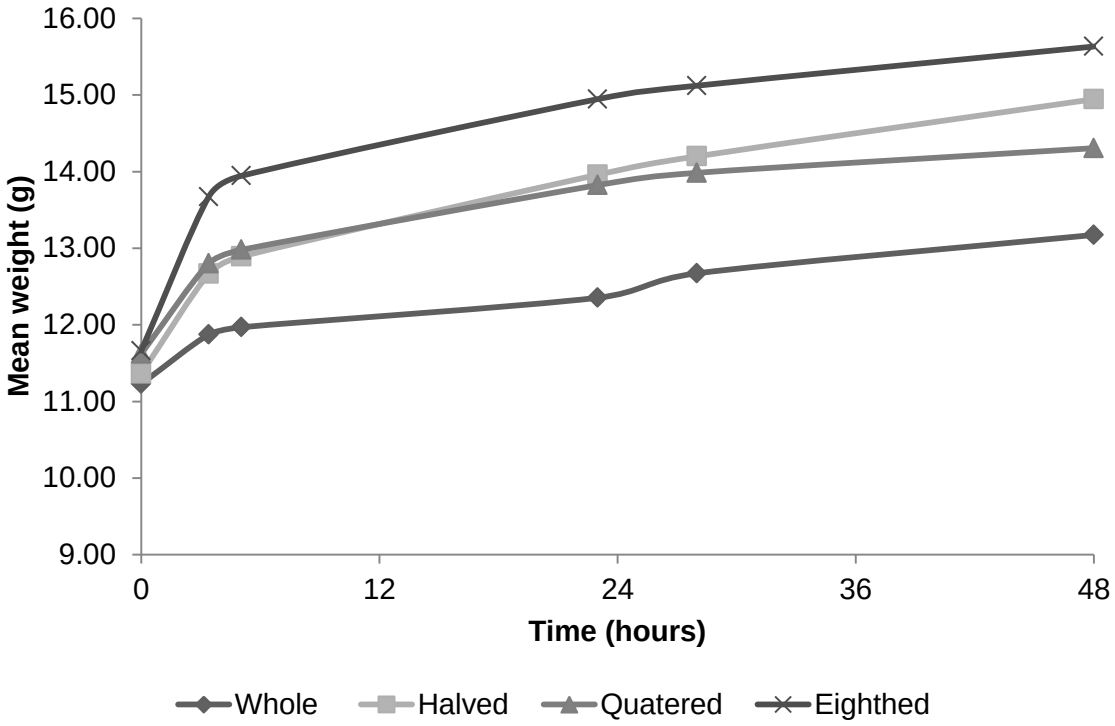


Figure 2-10 Rate of saturation of whole, halved, quartered and eighthed radishes at 4°C.

2.14 Sectioning

Radishes were placed into individual G3 grip seal bags measuring 75 x 80 mm (Weller Packaging, Lichfield, UK) and were sent for professional sectioning and staining (Finn Pathologists, Norfolk, UK). Non-split radishes were sectioned through the widest point of the hypocotyl and split radishes were sectioned through the splits. Sections were along the radial latitudinal axis.

On arrival at Finn Pathologists a segment through the widest or split part of the radish was taken and placed into a cassette. They were then placed in 70% industrial methylated spirit (IMS) for 24 hours. The segments were cleared in a gradient of IMS, followed by xylene and embedded in wax (Table 2-1).

Table 2-1 Step-by-step procedures for clearing and embedding radish hypocotyl sections prior to sectioning

Step	Reagent	Time (mins)	Temperature (°C)
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 10 µM sections were made which were stained with 1% w/v toluidine blue made up in 50% v/v isopropanol and dH_2O (Table 2-2).

Table 2-2 Step-by-step procedure for staining radish hypocotyl sections with 1% Toluidine Blue prior to image analysis

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 from Finn Pathogists to HAU 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.

2.15 Texture analysis

Radishes from a G's Growers (Norfolk) were couriered on the day of harvest to arrive at HAU (Shropshire), the following morning. 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, the commercially grown radishes were briefly washed in tap water to remove soil residue and trimmed using a knife to remove any remaining leaf petioles and fibrous roots.

Before texture analysis the maximum diameter of the radishes was measured using digital callipers (Draper Expert 46610, Draper Tools Ltd., Hampshire, UK) and they were weighed using a PCB 2500-2 balance (Kern and Sohn GmbH, Balingen, Germany).

2.15.1 Puncture

Puncture tests were performed using a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England) (Figure 2-11). The texture analyser was fitted with a P/2 cylindrical probe (Figure 2-12), the test speed was 2 mmS^{-1} and the test distance was 160 mm. Radishes were positioned on their side and force was applied to the widest point of the hypocotyl. During the experiment a curve was plotted of the force (kg) as a factor of distance (mm). The point at which the periderm of the radish was punctured could be observed on the plotted curve as abrupt decrease in force.

Figure 2-11 Texture analysis of a radish using TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England).

Figure 2-12 Puncture texture analysis of a radish using a P/2 (Stable Micro Systems, Surrey, England) cylindrical probe

2.15.2 Compression

Uniaxial compression tests were performed using a P/75 probe fitted to a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England) (Figure 2-13). Radishes were placed on their side and compression force was applied perpendicular to the lateral line. The test speed was 2 mmS^{-1} and the test distance was 250 mm. As with the puncture tests, a curve was again 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 split force.

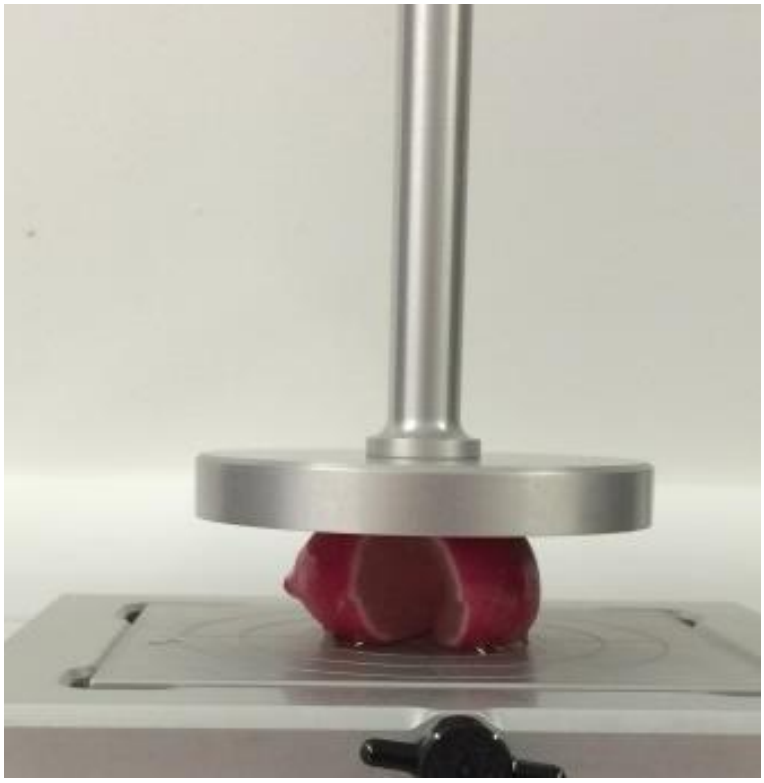


Figure 2-13 Compression texture analysis of a radish hypocotyl with a P/75 probe fitted to a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England)

2.15.3 Impact

Impact tests were performed using the method described by (Hartz *et al.* 2005) with a slight modification, in this experiment the drop height was increased from 1 m to 1.4 m 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.

Figure 2-14 Impact texture analysis of a radish hypocotyl from a height of 1.4 m onto a metal plate (a) resulting in a split radish (b)

2.16 QA of commercial radishes

In commercial production, after radishes have been harvested, washed and trimmed, they are placed into large containers called dolavs. Quality analysis (QA) of the produce is conducted at this point. One hundred 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 the unique batch number. From the batch number, the drilling date, harvest date and field where the radishes have been grown can be determined. There are approximately 10,000 batches of radishes harvested and quality

assessed each year providing an extensive database to search for correlations between weather conditions during growth and splitting.

2.17 QA of commercially grown radishes at HAU

Throughout the 2014 growing season field grown radishes were couriered from G's Growers to HAU as described in section 2.15 with the addition that the batch number was written on the box and this was recorded upon arrival at HAU. This enabled the weather conditions during growth to be determined as the batch number can be used to find the drilling date and harvest dates.

One hundred radishes were taken from the box and briefly washed in tap water to remove soil remaining from the field. The radishes were then trimmed to remove the petioles and roots.

2.17.1 Split on arrival

The 100 radishes were assessed for splitting and any split radishes were weighed and the diameter was measured. These were then dropped from a height of 1.4 m onto a metal plate as described in section 2.15.3. If the existing splits extended or if new splits were produced this was recorded. All of these radishes (or up to 25 if there were in excess of 25) were then saturated and dried to calculate the RWC as described in Section 2.13. The RWC was correlated with the number of split radishes using linear regression.

2.17.2 Impact texture analysis

To determine splitting susceptibility 100 commercially viable radishes were tested for splitting susceptibility using impact texture analysis as described in Section 2.15.3. Commercially viable radishes were determined as radishes which were free from pest, disease and splitting damage and were between 18 and 32 mm in diameter.

The 100 commercially viable radishes were briefly washed in tap water and trimmed. Each radish was weighed and the diameter was measured before dropping from a height of 1.4 m onto a metal plate. If the radish split or not was recorded. The first 25 radishes

which did not split and the first 25 radishes which did split were saturated and dried to calculate the RWC as described in Section 2.13. The RWC was correlated with the number of split radishes using linear regression.

2.18 Weather data

Weather data for RAF Marham, which is approximately 14 km from where the radishes were grown, was provided by British Atmospheric Data Centre (BADC) and the Met Office. Total daily rainfall and mean daily temperature were calculated from this data. As drilling and harvesting dates were known from the batch number the mean temperature during growth and the accumulated precipitation during growth could be calculated for each batch of radishes. This weather data could then be correlated with splitting using simple and multiple linear regression.

2.19 Statistical analysis

All data was analysed using GenStat for Windows 15th Edition (VSN International 2011). The tests used have been described in the materials and methods section for each experiment.

3. Growth Splits

3.1 Chapter 3 Growth splits: Introduction

Hypocotyl splitting in radish can occur during growth, harvest or post-harvest. Splits which happen prior to harvest are called 'growth splits' and splits which materialize during harvest and post-harvest are called 'harvest splits'. Identification of the factors governing splitting susceptibility at each of these stages may allow the development of field production, harvesting and handling practices which can minimise damage. Research is required to investigate the environmental conditions during commercial production which correlate with splitting in radishes as knowledge in this area is limited.

This chapter begins with a glasshouse experiment designed to determine if there are differences in the susceptibility to growth splits among three different cultivars (Experiment 3.1). Following this, the growth stages of *Raphanus sativus* 'Rudi' are defined (Experiment 3.2) and the growth rates established for five different cultivars grown under glasshouse conditions (Experiment 3.3). This information was used to facilitate the planning of the subsequent glasshouse experiments conducted in this chapter. The chapter continues with an experiment correlating commercial splitting rates with rainfall, temperature and relative humidity during growth for the years 2012, 2013 and 2014 (Experiment 3.4). The chapter then concludes with a series of nine glasshouse experiments (Experiment 3.5 to 3.12) into the effects of volumetric water content (VWC) during growth on splitting.

The main objective of work carried out in this chapter is to identify some of the factors which affect susceptibility to growth splits in radish.

3.2 Experiment 3.1: The effects of radish cultivar on susceptibility to growth splits

3.2.1 Experiment 3.1: Introduction

Splitting susceptibility has been shown to vary for different cultivars of potato (Bajema *et al.* 1998), cherry (Demirsoy & Demirsoy 2004), tomato (Dorais *et al.* 2004) kohlrabi (Lippert 1999), and carrot (Hartz *et al.* 2005; Hole *et al.* 1999). In some papers physiological differences, for example skin thickness, which differ between types of cultivar have been correlated with splitting susceptibility and have been proposed to explain the differences (Demirsoy & Demirsoy 2004; Emmons & Scott 1998). The susceptibility of a radish to splitting during growth may also depend on genotype x environment interactions but there is a lack of research in this area to provide any evidence for this. This experiment aims to investigate if different cultivars of radishes have different susceptibility to splits and then to investigate possible physiological explanations for these differences.

Effect of growth rate: Different cultivars of radish may have different growth rates and growth rate has been suggested to affect splitting in radishes. In an experiment conducted by Latimer (1991) the leaves of radishes were brushed during growth to impose mechanical stress and this was observed to decreased splitting. In this investigation the decrease in splitting was seen alongside a reduction in hypocotyl growth rate. The reduction in growth rate was suggested as a possible cause of the reduction in splitting (Latimer 1991). Dowker and Jackson (1977) also found a correlation between growth rate and splitting in carrots. They found carrots which had the slowest growth rate, with the longest duration from drilling until harvest, split the most (Dowker & Jackson 1977). However, as these carrots had been planted in different months at different densities there are a number of confounding factors which may have resulted in the observed differences in splitting.

Differences in growth rate have also been proposed as an explanation for differences in splitting between cultivars of kohlrabi. Of the two cultivars of kohlrabi which were investigated by Lippert (1999) the crack resistant cultivar ‘Noriko’ was found to grow

slower than the crack susceptible cultivar ‘Express Forcer’, ‘Noriko’ had a tuber:leaf ratio of 2:1 compared to ‘Express Forcer’ which had a radio of 9:1. The paper is unclear as to the unit measurements for leaf and tuber. In the final year of the experiment it is reported ‘Express Forcer’ cracked twice as often as ‘Noriko’. Unfortunately no statistical analysis was performed on the data, this is a comparison of percentage cracked kohlrabi of each cultivar, and therefore it is difficult to draw meaningful conclusions from this. From these results, Lippert (1999) determined genotypic differences in crack resistance were due to the expansion rate of the involved plant organ. These conclusions are based on limited information and require further investigation in addition to statistical analysis as there were no reported measurements of growth rate other than the ratio of leaf and tuber to enable quantification of the differences. The point at which the kohlrabi cracked was recorded and it would have been logical to see if points at which there were high rates of cracking coincided with periods of rapid growth.

No investigations have yet been conducted to determine if different cultivars of radishes have different growth rates and if the growth rates correlate with splitting during growth.

Radish roundness: Shape may also be a factor under some genetic control which affects susceptibility to splitting. Iwata *et al* (2004) showed Japanese radish shape was under both genetic and environmental control. In this experiment different soil types were used to vary environmental growing conditions. The results showed there were significant differences in shape for all varieties and for all soil types and there was no interaction between soil type and variety for most shape characteristics (Iwata *et al.* 2004) suggesting soil type had similar effects on all varieties tested and therefore neither genetics nor environment alone can explain shape. As stress is more uniformly spread in globes than shapes which deviate from this (Emmons & Scott 1998) shape is likely to be an important factor in determining splitting susceptibility. Shell theory can be applied to whole organs if their tissues are physically constrained by a membrane which is thinner than about one-tenth of the tissue radius (Considine & Brown 1981) which is the case for radishes. Shell theory shows changes in the profile of stress distribution from equator to pole for shapes which are elongated or compressed globes (Considine & Brown 1981). Differences in

stress within the tissue may cause it to split depending on the degree of stress and the mechanical strength of the tissue. Splitting occurs when mechanical stress exceeds the ability of the tissue to withstand it (Hole *et al.* 1999).

Mode of failure: Failure can occur by cellular debonding and plasmoptysis The mode of failure depends on the relative strengths of the intercellular bonds and cell walls (Lin & Pitt 1986). In many vegetable crops, root and tuber splitting is thought to occur predominantly due to plasmoptysis as opposed to cellular debonding. McGarry (1993) found splits occurred in carrots by cell wall breakage and Lippert (1999) investigated cracking in kohlrabi tubers and found ruptured cell walls indicating intracellular fractures. These results suggest splitting susceptibility in these vegetable crops must be determined to some extent by cell wall strength and composition. The mode of splitting which occurs within radishes has not been recorded, it is likely to be by cellular fracture similar to other vegetables but this needs to be demonstrated.

Periderm thickness: In fruit skin thickness has been correlated with splitting susceptibility and it is thought this may explain some of the genotypic differences which have been observed as different cultivars are thought to have different skin thicknesses. In tomato, cuticle and epidermal (epicarp) thickness has been shown to be negatively correlated with splitting susceptibility. Cultigens which were resistant to splitting had combined epicarp layers which were significantly thicker than susceptible cultigens (Emmons & Scott 1998). In a similarly study Demirsoy & Demirsoy (2004) showed cherry cuticle thickness of different cultivars was also negatively correlated with splitting susceptibility, the cultivars which had the thickest cuticle had the lowest cracking index. From the results of these studies it would appear, a thicker skin is correlated with an increased resistance to splitting. Research into the correlation between the splitting susceptibility of different cultivars and periderm thickness have not been conducted for radishes, it is not known if splitting susceptibility in radishes is correlated with a thicker periderm.

Effect of tissue water content: Tissue water status is an important factor affecting splitting susceptibility. It is thought to affect tissue mechanics and splitting susceptibility through turgor pressure. At high turgidity plant cell walls are believed to already be

stretched and as a consequence are more easily ruptured (Kokkoras 1995). In sweet cherry fruit, rain induced growth splitting is thought in part to be caused by an increase in pressure on the skin from within the fruit as a result of water uptake by the vascular system (Sekse 1995).

This chapter reports a series of experiments to determine if there are differences in splitting susceptibility between radish cultivars and study the relationship between periderm thickness and splitting. Measurements of roundness were made to identify if different cultivars differed in shape and if roundness was correlated with splitting susceptibility. Size at harvest was measured to determine if growth rate correlates with splitting, the larger the radishes were at harvest, the faster they grew. Furthermore, as part of this investigation, sections were taken of split tissue to determine the type of split which occurs in radishes, i.e. cellular debonding or plasmoptysis.

Aims: The aims of this experiment were to determine:

- If there are any differences in splitting susceptibility between three cultivars of commercially grown radishes
- If growth rate correlates with splitting
- If hypocotyl roundness correlates with splitting
- The mode of hypocotyl tissue failure
- If cultivar periderm thickness is correlated with splitting
- If periderm thickness is different between the split and non-split radishes
- If hypocotyl water content is correlated with splitting

Null hypotheses:

1. No difference will be observed between cultivars and their susceptibility to splitting
2. No correlation will be observed between growth rate and splitting
3. No correlation between hypocotyl roundness and growth splitting will be observed
4. Cultivar periderm thickness will not be correlated with splitting
5. There will be no difference in periderm thickness between the split and non-split radishes
6. Hypocotyl water content (WC) will not be correlated with splitting

3.2.2 Experiment 3.1: Materials and Methods

Cultivar selection: This experiment investigated the splitting susceptibility of three different radish cultivars, 'Rudi', 'Celesta' and 'Topsi'. 'Celesta' is the variety grown most commercially and it is considered by growers to be more split resistant than other varieties, 'Rudi' is a cultivar which is grown commercially and was previously the predominant variety although is currently 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.

Replication: There were six plants per pot in a total of eight experiment pots and three destructive harvest pots for each treatment, giving 11 pots per treatment, 33 pots in total containing a total of 198 radish plants, 144 of which were plants used for analysis at the end of the experiment. Pots were arranged in a random block design generated by GenStat for Windows 15th Edition (VSN International 2011), destructive pots were in a block of their own on the same bench.

Growth summary: Seeds were planted on 26.10.2012, seedlings were thinned on Day 7 (02.11.2012), plants were harvested and moved to storage on Day 29 (23.11.2012), and radishes were removed from storage 10 days later (03.12.2012).

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% with a range of 90.9% to 22.1%.

Growing conditions: Radishes were grown in 4.2 L 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 John Innes No. 2 growing medium (Keith Singletons Horticultural products, Cumbria, UK). The pots were filled to the rim of the pot. Once the pot was full, the compost was consolidated and smoothed level with the rim of the pot using a wooded pot tamper.

Pot preparation: In each pot 12 seeds were planted in six evenly spaced pairs 25 mm from the rim of the plant pot at a depth of approximately 7 mm, 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 3-1).

Figure 3-1 The experimental unit was one pot of six evenly spaced radish seedlings, positioned 25 mm from the edge of the pot

Irrigation: All pots were given the same irrigation regime for the duration of the experiment. They were watered three times a week by hand to the weight at 103% pot capacity. 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. 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 2-2). 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.

Measuring substrate VWC: VWC was measured using two methods. Firstly 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 60 mm long and were inserted into the surface of the compost. Therefore the Theta Probe only measured the VWC for the surface 60 mm.

The second method was pot VWC. This was calculated from the gravimetric water content (GWC) of the pot by multiplying the GWC 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 0.49 g cm^{-3} (this is different to the bulk density which was used for the shallower trays in other experiments).

Harvest: The mean temperature in the glasshouse during harvest was 21.6°C.

Roundness: At harvest, the maximum diameter and length of the hypocotyl to the first root hair was measured to enable a rough calculation of how spherical the radishes were to be made. This was done by dividing length by width, the closer the result was to 1 the more spherical the radish was considered to be.

Storage: The radishes were stored for 10 days after harvest. For this, the radish were put into a labelled cryovac bag to simulate commercial packaging and moved to a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd., Japan) again to mimic commercial storage conditions which are typically 4°C and 95% relative humidity.

The environmental test chamber achieved an average temperature of 3.1°C with a range between 5.9°C and 3.0°C. The mean relative humidity was 98.3% with a range between 99.3% and 92.6%.

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 80 mm (Weller Packaging, Lichfield, UK). Six 'Rudi' radishes with splits and four 'Topsi' with splits were also sealed in separate grip seal bags. There were only four split 'Topsi' and no 'Celesta' with splits which were suitable for sectioning. 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. Initially 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 hours. The segments were cleared in a gradient of IMS, followed by xylene and embedded in Histowax. Once embedded in Histowax 10 µM 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). The sections were then photographed and the pictures were analysed. The image was calibrated using a 1 mm graticule slide (Figure 3-2).

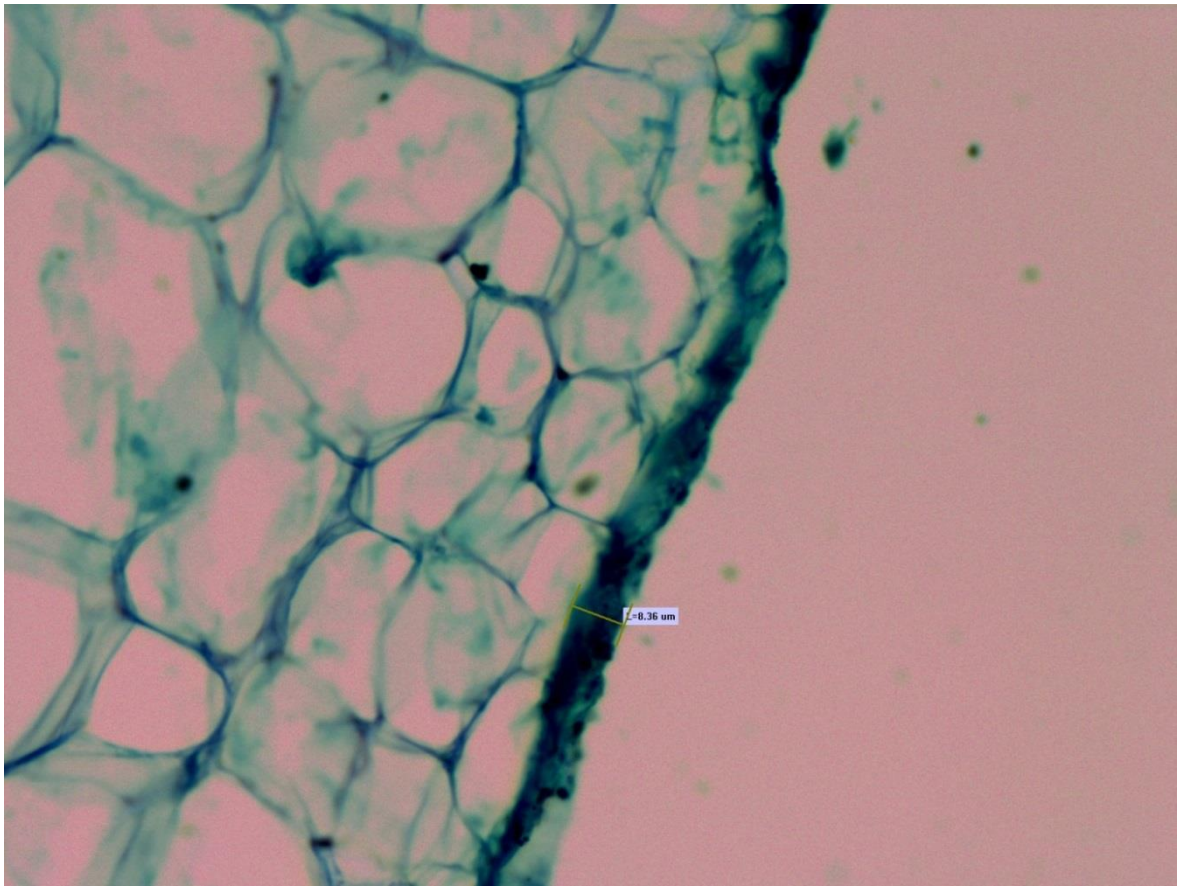


Figure 3-2 Measuring periderm thickness of *Raphanus sativus* 'Celesta' using infinity capture software. The section is 10 µM thick and stained with 10% w/v toluidine blue.

Hypocotyl water content: As an indicator of tissue water status the hypocotyl water content of the remaining radishes which were not sent for sectioning was calculated on a wet weight basis post-storage by drying all radishes at 105°C to a constant weight.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

The mean water content of split and non-split Rudi hypocotyls after 10 days of storage was analysed using an unpaired 2 tailed t-test.

All other data was analysed using analysis of variance (ANOVA). When a P value of less than 0.05 was observed a Tukey test was used to determine which results were different from each other. VWC during growth, measurements of growth rate, roundness, growth splitting and number of growth splits were analysed using cultivar and block as factors. Periderm thickness was analysed with cultivar as a factor and also as a multi-factorial ANOVA with cultivar and split/not split as factors. Hypocotyl water content after storage

was analysed with cultivar and block as factors. There were fewer blocks for water hypocotyl water content after storage than for measurements taken at harvest as some radishes had been removed for sectioning.

Table 3-1 Skeleton ANOVA for VWC during growth, radish growth rate, hypocotyl roundness and growth splits

Source of variation	d.f.
Block	7
Cultivar	2
Residual	14
Total	23

Table 3-2 Skeleton ANOVA for periderm thickness between radish cultivars

Source of variation	d.f.
Cultivar	2
Residual	26
Total	28

Table 3-3 Skeleton ANOVA for periderm thickness of split and non-split radishes for each cultivar

Source of variation	d.f.
Split or non-split	4
Residual	24
Total	28

Table 3-4 Skeleton ANOVA for radish hypocotyl water content after storage

Source of variation	d.f.
Block	6
Cultivar	2
Residual	12
Total	20

3.2.3 Experiment 3.1: Results

VWC: Each cultivar group was exposed to similar ranges in VWC (Table 3-5); there were no significant differences between the mean water content at the surface (P=0.883) or for the whole pot (P=0.994) for the different cultivar types.

Table 3-5 Maximum, minimum and mean VWC at the surface and for the whole pot during growth for each of the three cultivars of radishes grown (n=8).

Cultivar	Maximum VWC (%)		Minimum VWC (%)		Mean VWC (%)	
	Surface	Pot	Surface	Pot	Surface	Pot
Rudi	24.77	25.13	22.56	25.02	23.70	25.07
Celesta	24.86	25.20	23.01	24.98	23.93	25.07
Topsi	24.81	25.27	22.84	24.99	23.85	25.10
P	0.984	0.843	0.640	0.989	0.883	0.994
LSD (5%)	1.038	0.474	0.947	0.586	0.935	0.541

As would be expected due to gravity and surface evaporation, the compost at the surface had a lower VWC than the pot as a whole for the duration of the experiment.

Growth 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 (43.8%) on average per pot than ‘Topsi’ (8.3%) or ‘Celesta’ (2.1%) which did not have significantly different numbers of splits from each other (Table 3-6).

Table 3-6 The mean percentage of split hypocotyls per pot for three different cultivars of radish. Each pot contained 6 radish plants (n=8).

Cultivar	Split (%)
Topsi	8.33a[1]
Celesta	2.08a
Rudi	43.75b
P	<0.001
LSD (5%)	12.56

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Split healing: A proportion of the split radishes observed had healed at the time of harvest (Figure 3-3) and these were recorded separately as healed splits, the number of fresh splits was significantly different between cultivars (P=0.006) as were the number of healed splits (P=0.002). The healed radish hypocotyls appeared to have split and then subsequently a red scar had formed over the split (Figure 3-3).

Figure 3-3 Radish hypocotyl with healed split

Some of the splits which were observed appeared to have begun the healing process and were partially healed over, these radish were recorded as ‘fresh’ splits as the white tissue below the red epidermis was still visible at the point of harvest (Figure 3-4).

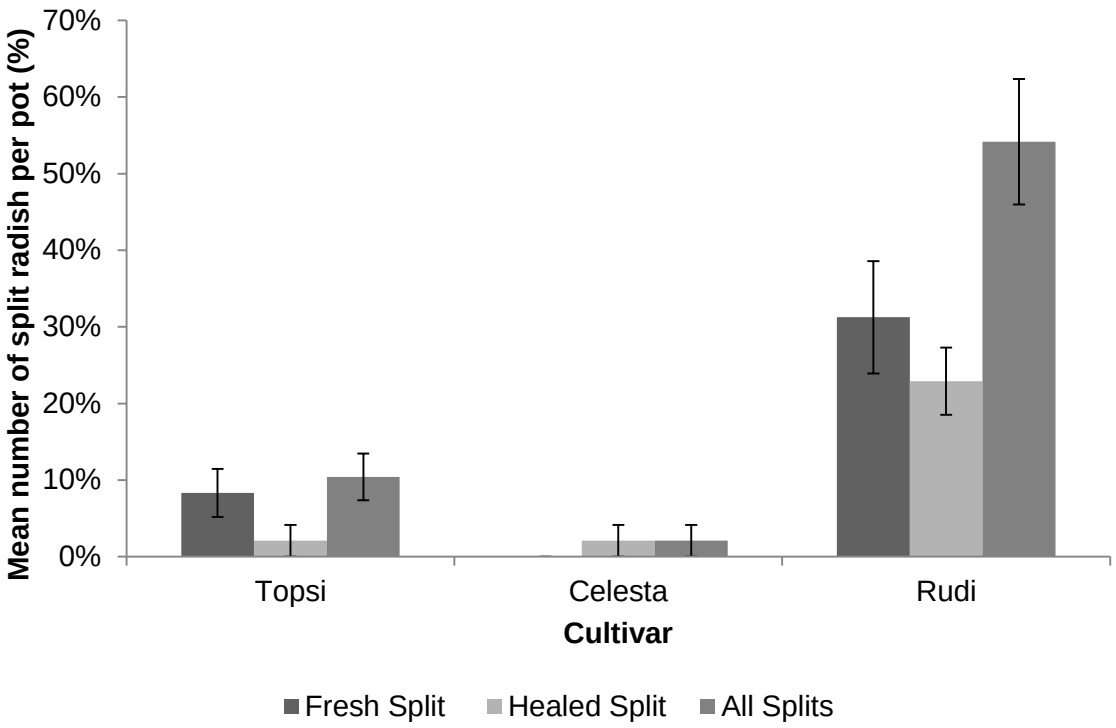


Figure 3-4 Mean number of fresh (P=0.006), healed (P=0.002) and total (P=0.001) split radish per pot of six plants at harvest (n=8). Bars represent standard error for each treatment

Growth rate: It was observed that 'Celesta' grew significantly faster than 'Rudi' for all factors measured and grew significantly faster than 'Topsi' for all factors measured except leaf weight (Table 3-7). 'Rudi' and 'Topsi' did not have any significant differences in growth rate for any of the factors measured.

Table 3-7 Significant cultivar effects on radish size at harvest (Day 29 for all cultivars) (n=8).

Cultivar	No. Leaves	Plant weight (g)	Hypocotyl weight (g)	Leaf weight (g)	Hypocotyl length (mm)	Hypocotyl width (mm)
'Topsi'	4.44a[1]	20.10a	12.07a	8.03ab	42.92a	27.46a
'Celesta'	5.10b	24.26b	15.07b	9.19b	47.74b	29.34b
'Rudi'	4.58a	19.91a	12.29a	7.63a	41.98a	27.51a
P	<0.001	<0.001	<0.001	0.015	<0.001	<0.001
LSD (5%)	0.292	1.648	1.040	1.028	2.499	0.909

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Roundness: There was no difference (P=0.213) in hypocotyl roundness, measured by dividing hypocotyl length (L) by width (W), between the cultivars (Table 3-8).

Table 3-8 Mean roundness, calculated by dividing length between poles (mm) by equatorial width (mm) for three radish cultivars (n=8)

Cultivar	Roundness (L/W)
Topsi	1.58
Celesta	1.64
Rudi	1.56
P	0.21
LSD (5%)	0.102

Harvest splitting: No additional splits formed during storage for any cultivar.

Mode of tissue failure: The sections which were taken of the split radishes showed broken cells therefore, it appears the radish splits are propagated by cell wall breakage as opposed to tissue separation along the middle lamella (Figure 3-5, Figure 3-6, Figure 3-7 and Figure 3-8).

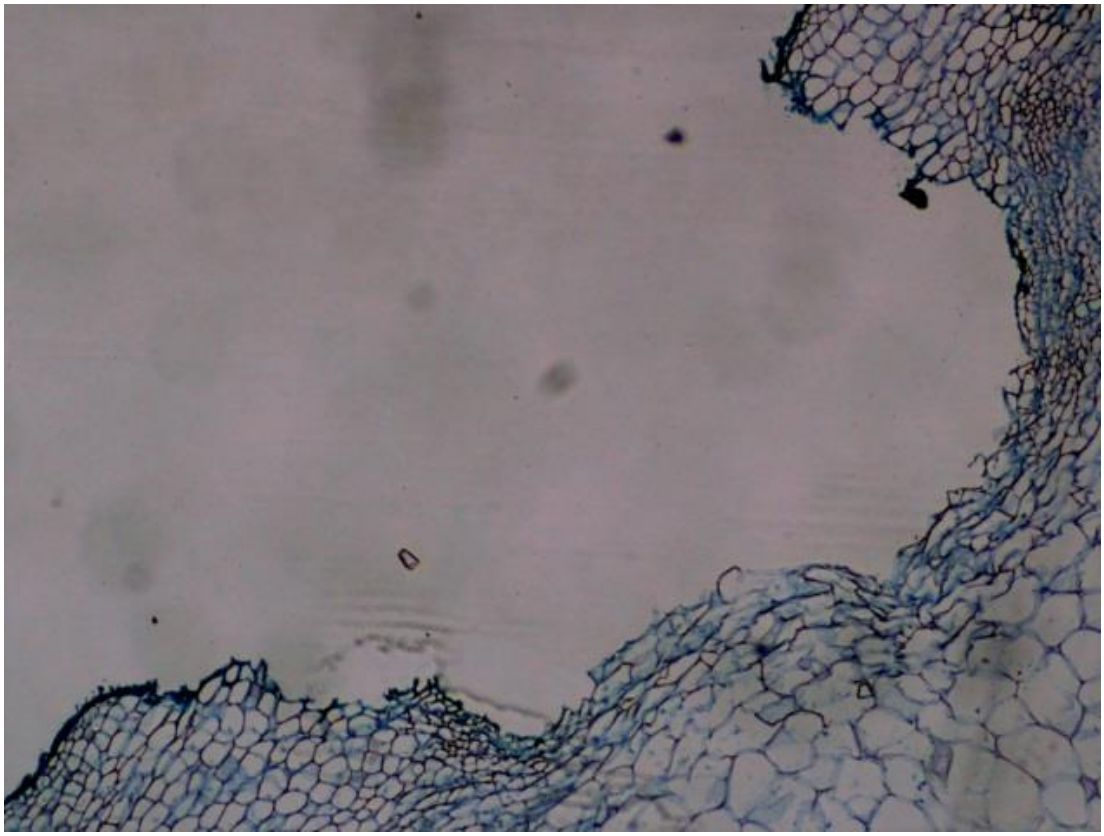


Figure 3-5 Microscope image, x40 magnification of split surface of *Raphanus sativus* 'Rudi' hypocotyls. Sections are 10 μM thick and stained with 10 % w/v toluidine blue. Broken cells can be observed suggesting the mode of failure was plasmoptysis

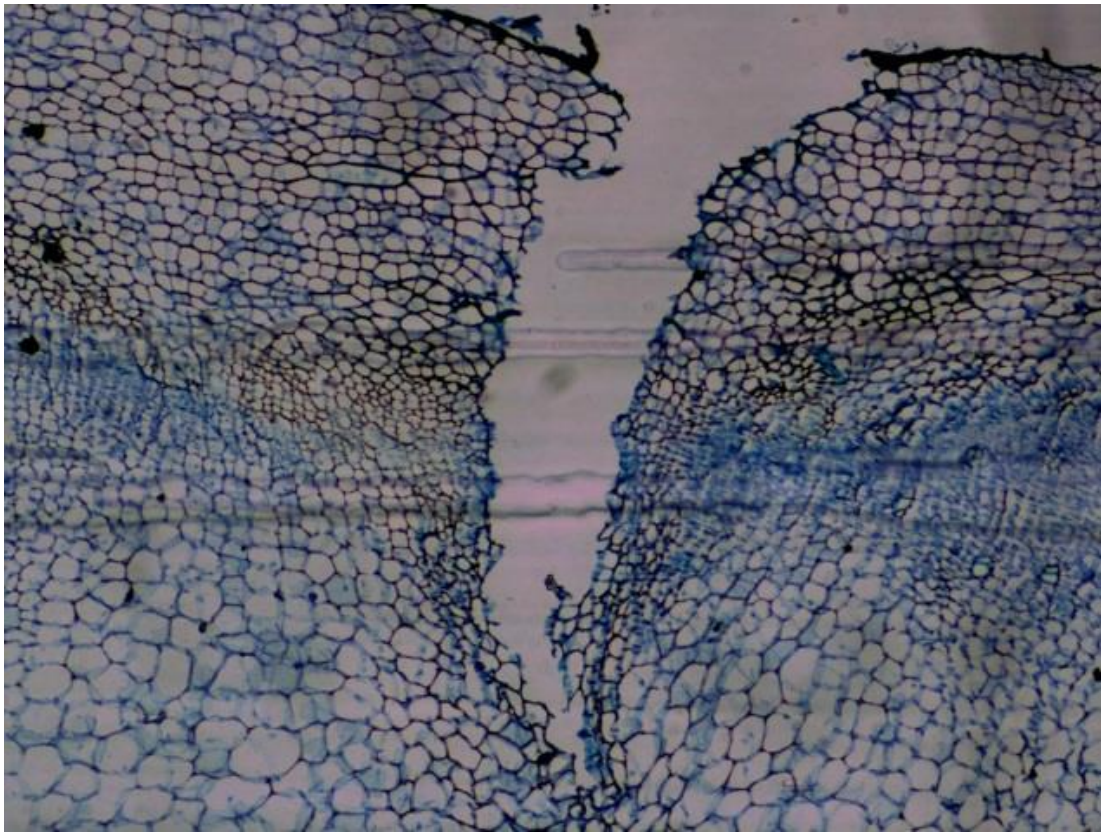


Figure 3-6 Microscope image, x40 magnification of split surface of *Raphanus sativus* 'Rudi' hypocotyls. Sections are 10 μM thick and stained with 10 % w/v toluidine blue. Broken cells can be observed suggesting the mode of failure was plasmoptysis

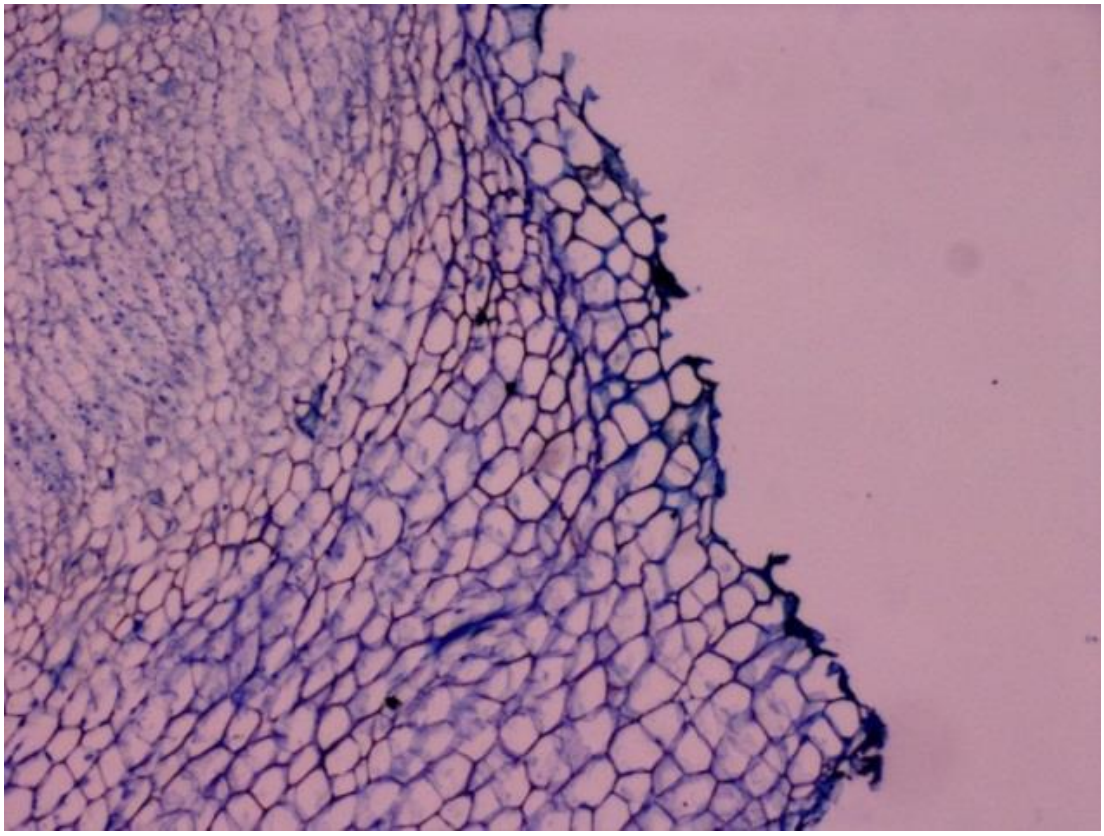


Figure 3-7 Microscope image, x100 magnification of split surface of *Raphanus sativus* 'Topsi' hypocotyl. Section is 10 µM thick and stained with 10 % w/v toluidine blue. Broken cells can be observed suggesting the mode of failure was plasmoptysis

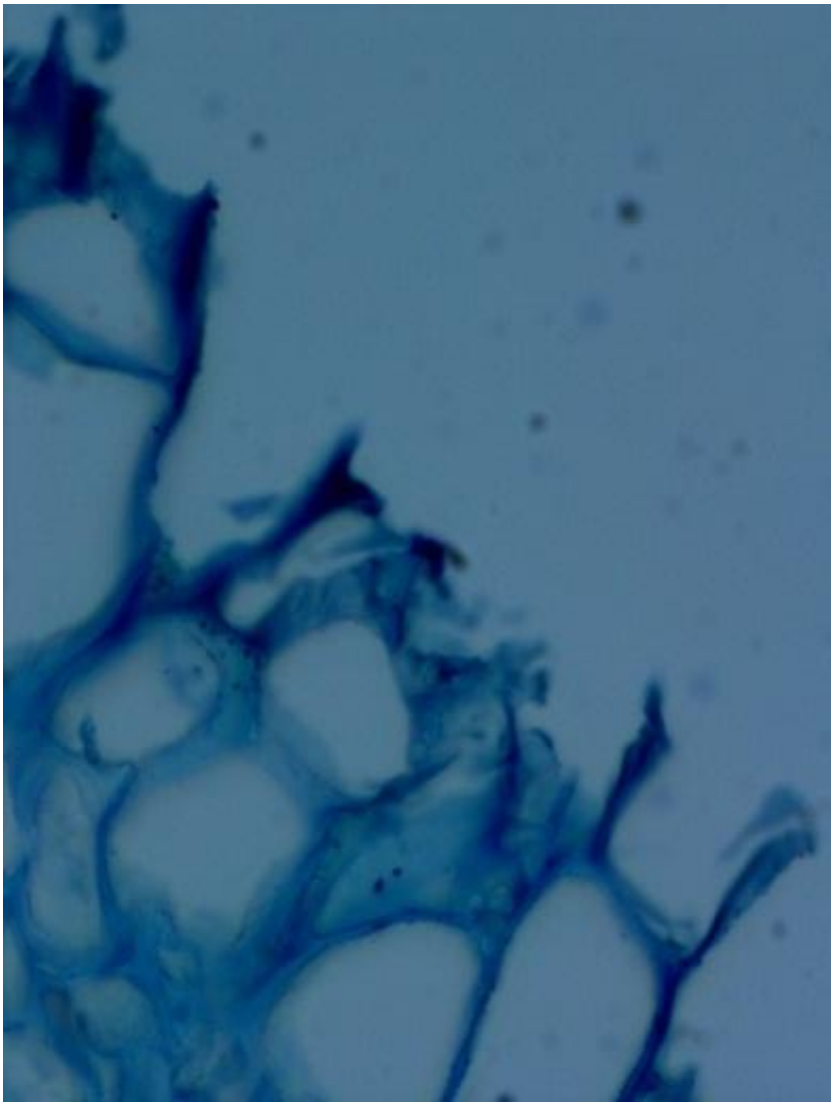


Figure 3-8 Microscope image, x400 magnification of split surface of radish, *Raphanus sativus* 'Rudi' hypocotyl. Section is 10 µM thick and stained with 1% w/v toluidine blue. Broken cells can be observed suggesting the mode of failure was plasmoptysis

Periderm thickness: After 10 days of storage there was no significant difference (P=0.674) in the mean thickness of the periderm between cultivars (Figure 3-9).

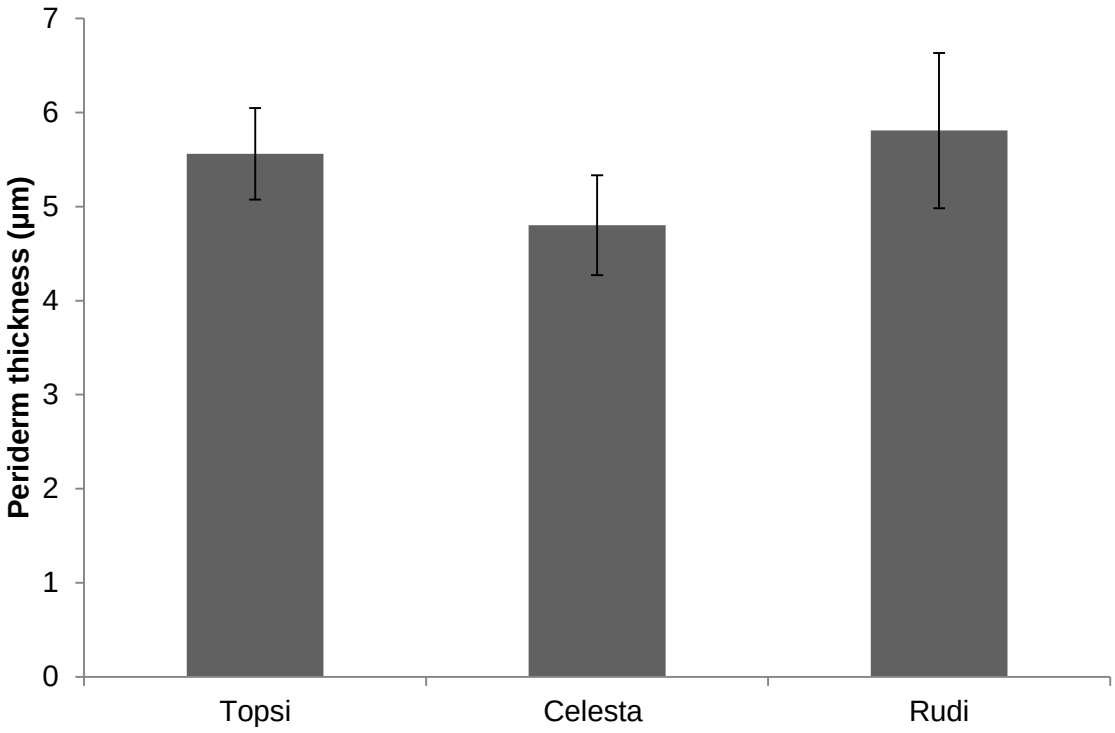


Figure 3-9 The mean periderm thickness of radish of different cultivars. For 'Topsi': n=10, for 'Rudi': n=12, for 'Celesta': n=6. Bars represent ± the standard error of the mean for each cultivar, P=0.674

In general, it was observed the thickness of the periderm tended (P=0.045) to be greater for the split radishes than the non-split radishes of the 'Topsi' and 'Rudi' cultivars (Figure 3-10), there were not enough split radishes of the cultivar 'Celesta' to measure. However, despite an overall trend for a greater periderm thickness for split radishes, significant differences between the periderm of split and non-split radish was only observed for the cultivar 'Rudi' (Figure 3-10).

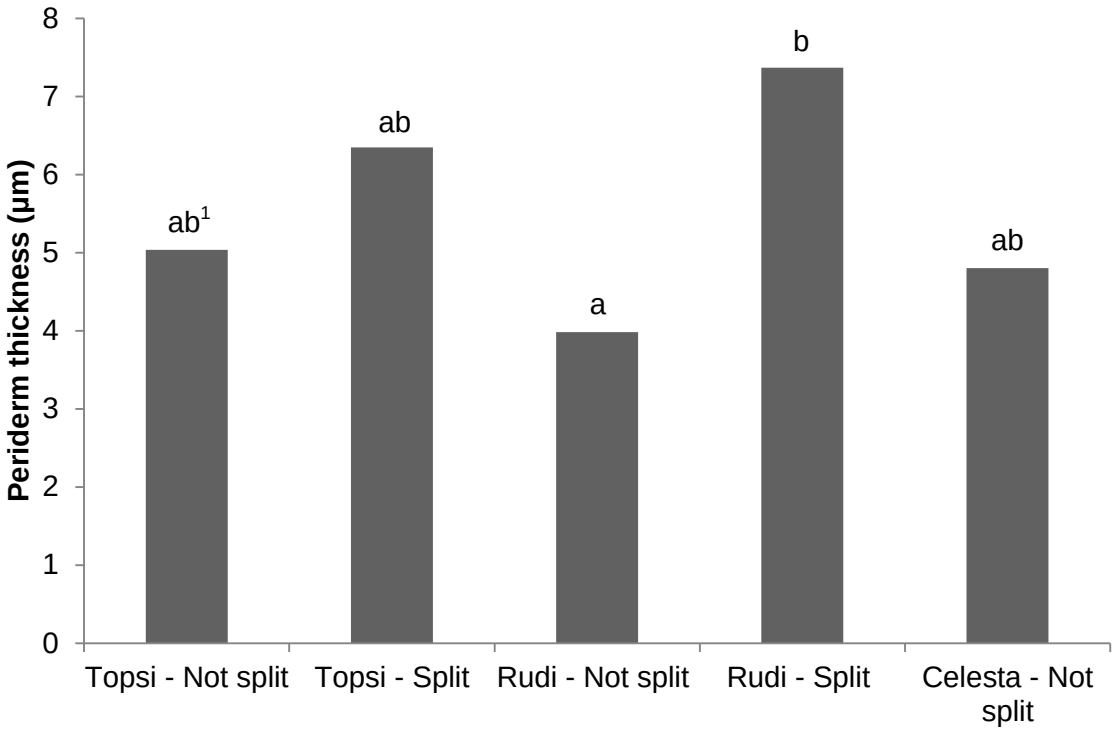


Figure 3-10 The average periderm thickness for fresh split and non-split radish of different cultivars. For non-split 'Topsi': n=6, for non-split 'Rudi': n=6, for non-split 'Celesta': n=6. For split 'Topsi': n= 4 for split 'Rudi' n=6. Max. rep. LSD = 2.193, min. rep. LSD = 2.901.There were not enough split 'Celesta' radishes for analysis. Bars represent ±the standard error of the mean. P=0.045.[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Water content after storage: After storage 'Celesta' had the greatest wet weight, water content and dry biomass with an average of 95.41%, 14.30 g and 0.66 g respectively (Table 3-9).

Table 3-9 Effects of cultivar on radish size and composition after 10 days of cold storage (n=8).

Cultivar	Water content (%)	Wet weight (g)	Dry weight (g)
'Topsi'	95.33b	11.46a	0.51a
'Celesta'	95.41b	14.30b	0.66b
'Rudi'	95.10a[1]	11.61a	0.56a
P	0.006	<0.001	<0.001
LSD (5%)	0.174	0.916	0.047

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

There was no significant difference (P=0.49) between the hypocotyl water content of the split and non-split radishes of the cultivar 'Rudi' (Figure 3-11). There were not enough split radishes of the cultivars 'Celesta' or 'Topsi' to compare the hypocotyl water content of the split and non- split radishes.

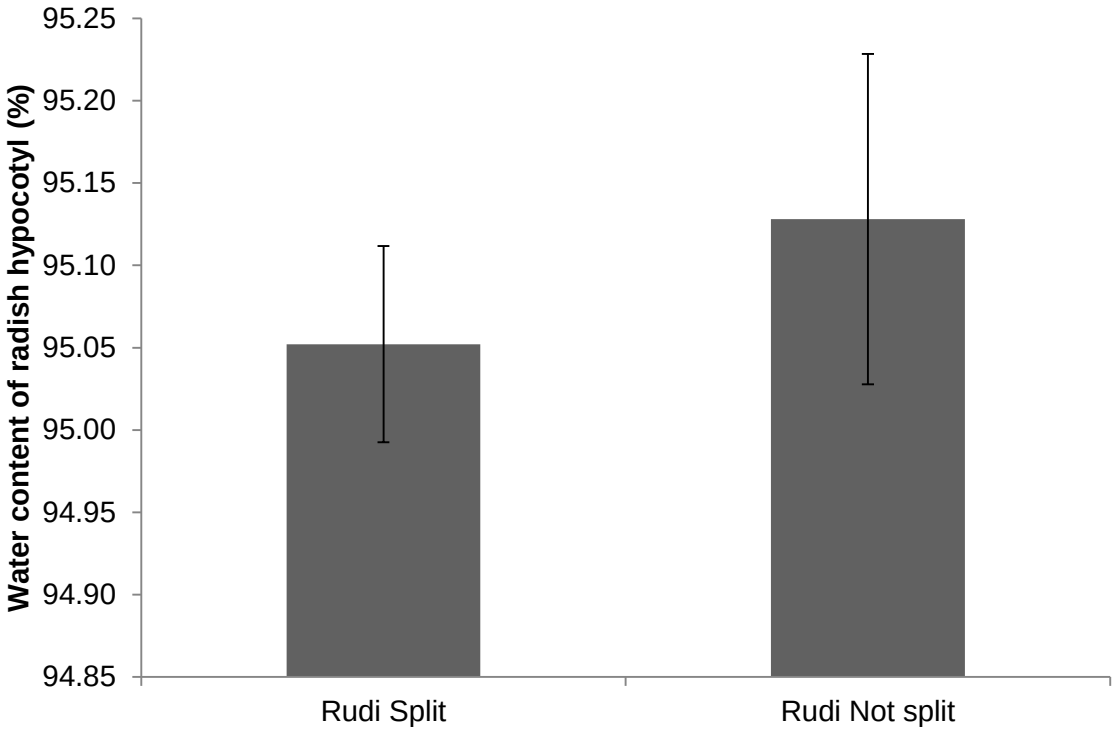


Figure 3-11 Mean water content of split and non-split Rudi hypocotyls after 10 days of storage. For non-split n=17 for split n=18. Bars represent the standard error for each group P=0.49.

3.2.4 Experiment 3.1: Discussion

Splits: The first null hypothesis was rejected as choice of cultivar was shown to have 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'.

At harvest the cultivar 'Celesta' had the lowest number of splits (not statistically different from 'Topsi'), the greatest yield in terms of trimmed weight, the greatest number of leaves, the greatest leaf mass (not statistically different from 'Topsi'), the greatest hypocotyl length and the greatest hypocotyl width.

Split healing: Many of the splits observed during this experiment had healed leaving a red scar as a remnant of the wound. When discussing splitting in kohlrabi Lippert (1999) stated how splitting in vegetative organs, such as roots, stems and tubers, is different to splitting in fruit because the cracks in vegetative organs grow wider as growth continues. Lippert (1999) concluded the splits in fruits such as apples, cherries and tomato are related to rapid expansion rates of the parenchymatous tissue which can occur during a wet period which follows a dry period. Lippert (1999) said the fractures in vegetative organs such as kohlrabi, carrots and swede differ from these and enlarge due to continuing growth processes. The paper concluded that genotypic differences in cracking susceptibility are due to expansion rates of the involved plant organ. The healed cracks in this experiment would appear to dispute the conclusions of Lippert (1999) about vegetative organs and would suggest splitting in radish is more complicated than being simply as a result of growth.

VWC: As the VWC of the compost in the pots was similar for all cultivars the differences observed in splitting susceptibility between cultivars were not likely to have been as a result of differences in available water content to the plants.

Rate of growth: Based on the comparison of cracking rates in two cultivars of kohlrabi, Lippert (1999) stated that cracking in vegetative organs was due to rapid growth. Lippert (1999) found the rapidly growing cultivar 'Express Forcer' was twice more likely to crack than the slower growing cultivar 'Noriko' although no evidence was given to quantify the

differing growth rates. However, results from this experiment provide evidence to the contrary as ‘Celesta’ split the least (P=0.001) of the three cultivars examined but grew the most rapidly. At harvest ‘Celesta’ had significantly larger whole weight (P<0.001), trimmed weight (P<0.001), plant biomass (P<0.001), number of leaves (P<0.001) and length (P<0.001) and width of the hypocotyl (P=0.008). As seedling emergence was comparable for all cultivars and all the cultivars were grown for the same amount of time ‘Celesta’ must have grown the most rapidly to reach this larger size. The larger leaf area of ‘Celesta’ would have resulted in a greater area for photosynthesis; this in turn may explain the greater size, wet weight and dry biomass of the ‘Celesta’ hypocotyls, as leaf area is responsible for dry matter production.

Despite ‘Celesta’ growing the most rapidly and splitting the least, the second null hypothesis was supported as no correlation was found between rate of growth and splitting in this experiment where all cultivars were harvested by date as opposed to size of the radish hypocotyl. ‘Celesta’ grew faster than ‘Topsi’ yet there were no significant differences in splitting between ‘Celesta’ and ‘Topsi’. There were no significant differences in the size at harvest of ‘Topsi’ and ‘Rudi’ but ‘Rudi’ split significantly more than ‘Topsi’. These results suggest there could be a cultivar relationship between the rate of growth of a cultivar and how much that cultivar splits during growth suggesting that individual cultivars differ in their ability for cell expansion as opposed to cell rupture. If there was not a relationship between cultivar, growth rate and splitting it would be expected the cultivars which grew at the same rate would split at the same rate. These results are in contradiction to the conclusions drawn by Latimer (1991) who postulated the low splitting rate observed in radishes which were exposed to mechanical leaf damage was a result of slow growth and also in contradiction to the results of Dowker and Jackson (1977) who found a slower growth rate in carrot was correlated with higher levels of splitting. Results from this cultivar experiment demonstrate overall growth rate is not an explanation for the differences in splitting between cultivars.

Cultivars ‘Topsi’ and ‘Rudi’ were significantly smaller than ‘Celesta’ at harvest. Under commercial conditions ‘Topsi’ and ‘Rudi’ would have been grown for longer than ‘Celesta’,

as commercially grown radishes are harvested by size rather than a period of growing time. This would have given the 'Topsi' and 'Rudi' cultivar radishes a longer duration to develop splits. At harvest in this experiment there were no significant differences in growth rate for any of the factors measured for 'Topsi' and 'Rudi' but significant differences were observed in the number of growth splits. It is not known if the differences in the number of split radishes would have remained if the radishes had been grown to the same size as 'Celesta' before harvesting. It is not known if splitting occurs evenly throughout growth for all cultivars or if particular cultivars are more susceptible to splitting at particular points during growth. Had the radishes been harvested by size, the resultant differences in splitting may have been different. Further research is required to examine this effect.

Roundness: The third null hypothesis was supported as there was no significant (P=0.21) difference between cultivars in hypocotyl roundness, as calculated by ratio between hypocotyl length and width, suggesting there is no relationship between roundness measured as such and growth splitting. Emmons and Scott (1998) stated shape is an important factor in splitting susceptibility and the work by Considine and Brown (1981) suggests deviations from a globe shape result in increased differences in stress within tissue. Differences in the roundness of cultivars may have been missed if some radish cultivars were flatter or more irregularly shaped in general but had a similar maximum width and length ratio.

Mode of tissue failure: The mode of failure within the radish tissue which was sectioned was shown to be plasmoptysis which is similar to other vegetables such as kohlrabi (Lippert 1999) and carrot (McGarry 1993). It is likely this is due to the limited amount of intercellular space within the radish hypocotyl tissue, similar to other vegetable tissue. Therefore, the cell wall strength and composition and the pressure exerted within cells are likely to be important factors in determining splitting susceptibility.

Periderm thickness: 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. The fourth null hypothesis was supported as 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. If periderm thickness is under genetic control then splitting resistance in radishes has the potential to be improved through breeding.

Periderm thickness was not significantly different between cultivars (P=0.674) but the thickness of the radish periderm (P=0.045) was greater for the split radishes than the non-split radishes, it should be noted the only cultivar which had significant differences in periderm thickness was ‘Rudi’. There were not enough split radishes from the cultivar ‘Celesta’ to measure and although the mean periderm thickness of the ‘Topsi’ radishes was thicker for the split radishes there was no significant difference observed. These results reject the fifth null hypothesis. However, significant differences between the periderm of split and non-split radish were only observed for the cultivar ‘Rudi’. These results are in accordance with research into splitting in carrots where it has been shown that removing the periderm makes them more resistant to splitting. In contradiction to the results from this investigation, tomato cuticle and epidermal (epicarp) thickness has been shown to be negatively correlated with splitting susceptibility (Emmons 1998), as has cherry cuticle thickness (Demirsoy & Demirsoy 2004). In both cases a thicker skin was associated with a greater resistance to splitting. The differences in the results from this investigation, where a thinner skin was associated with a greater resistance to splitting, and the research on splitting in fruit may be due to the differences in cellular density within tissue between fruit and vegetables. Fruit tissue tends to have a greater proportion of intercellular space than vegetable tissue. Possibly the cells within fruit are able to squash together more when they are under stress and a rigid thick skin makes the fruit more resistant to splitting by causing the cells squash more before it ruptures. In vegetables there is less intercellular space for the cells to expand into when they are under pressure

so a thinner more elastic skin may absorb some of the pressure and allow cells to expand before rupturnig.

Hypocotyl water content: The results from this experiment showed ‘Rudi’, the cultivar which split significantly more than the other two cultivars, had a significantly lower hypocotyl water content thus rejecting the sixth null hypothesis. These results could suggest radishes with high hypocotyl water content are less likely to split, or another explanation for the lower mean water content of the cultivar ‘Rudi’ may be more rapid water loss from the radishes with split surfaces than through the periderm of the non-split radishes. If this was the case a difference in water content between split and non-split ‘Rudi’ would be expected which was not observed. However, it should be noted the residual degree of freedom for the analysis of hypocotyl water content was just 12; usually a value of 15 or greater is required for robust experimental work. This was due to a low number of replicates (n=17 non-split, n=18 split) and the trend was observed with mean water content for split radishes at 95.05% was slightly, but not significantly smaller than the non-split radishes at 95.13%.

If radishes which split less have a higher water content this may be as a result of greater tissue elasticity. Herppich *et al.* (2004) found radish tissue elasticity was positively correlated with water potential. Hypocotyls with increased tissue elasticity may be expected to split less. This theory requires further investigation to see if the moisture content crudely calculated by comparing the wet weight and the dry biomass of the hypocotyl is correlated to the water potential which would give a more accurate measure of the water status of the radish hypocotyl.

3.3 Experiments 3.2-3.3: Determining the growth stages of radishes

3.3.1 Experiments 3.2-3.3: Introduction

Two experiments (Experiment 3.2 and 3.3) were conducted to define the growth stages of radishes. The first experiment used the cultivar ‘Rudi’ to build on the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBHC) identification key for root and stem vegetables (Table 1-1) and gave timings for growth rate under glasshouse conditions to be used for future experiments. The second experiment investigated if these growth stages were common to different cultivars of radishes and correlated a range of physiological factors with splitting rates for the different cultivars.

3.3.2 Experiment 3.2: Determining the growth stages of *Raphanus sativus* 'Rudi'

3.3.2.1 Experiment 3.2: Introduction

Standardised codes which describe physiological growth stages enable accurate scientific descriptions and comparisons to be made between plants at specific ages. The general growth stages of radish are included in the BBHC-identification keys under root and stem vegetables (Table 1-1) (Meier 2001). However, the growth stages specific to radish have not been individually described. The scale lacks plant specific descriptions particularly during the development of the harvestable vegetative parts and gives no indication of the timings for each of the stages. Timings are essential when planning experiments, therefore the growth stages and growth rate of *Raphanus sativus* 'Rudi' grown under glasshouse conditions will be defined in this chapter.

Aim: To determine the specific growth stages for *Raphanus sativus* 'Rudi'.

3.3.2.2 Experiment 3.2: Materials and Methods

In this experiment two methods of growing radishes were used. Radishes were grown in rhizotrons to allow study of the root length, hypocotyl diameter and number of leave and radishes were grown in trays to allow study of hypocotyl diameter and number of leaves under conditions which were more similar to those used in the majority of other experiments in this thesis.

Twenty nine radishes were planted over three days: 17th April 2013 (11 radishes in rhizotron), 29th April 2013 (8 radishes in rhizotron) and 1st May 2013 (10 radishes in seed tray). Nineteen *Raphanus sativus* 'Rudi') plants were grown under glasshouse conditions in 7.5 L rhizotrons measuring 50 x 300 x 500 mm (Figure 3-12) and 10 radish were sown in 1.75 L plant trays (G18B half sized seed trays, Garland Products Ltd., Kingswinford, UK). Both 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 five minutes totalling 17 mm day^{-1}.

Figure 3-12 Radish plants (Day 9) growing in a rhizotron allowing measurement of roots

The glasshouse was set to 20/5°C day/night temperature and achieved a mean temperature of 18.2°C. The mean relative humidity was 57.9%.

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.

3.3.2.3 Experiment 3.2: Results

The proposed scale for the growth stages of radishes was derived from the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (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 physiological 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 32 mm in diameter giving a median size of 25 mm. This enables the median diameter for the hypocotyl during Growth Stage 4 to be calculated and has been included in the scale (Table 3-10).

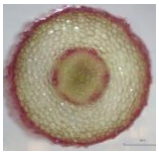
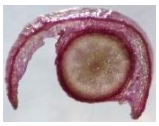
Table 3-10 Proposed growth stages for radishes during the commercial growing period including median hypocotyl diameters for principle Growth Stage 4

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 (~ 2.5 mm)
42	20 % of the final hypocotyl diameter reached (5 mm)

43	30 % of the final hypocotyl diameter reached (7.5 mm)
44	40 % of the final hypocotyl diameter reached (10 mm)
45	50 % of the final hypocotyl diameter reached (12.5 mm)
46	60 % of the final hypocotyl diameter reached (15 mm)
47	70 % of the final hypocotyl diameter reached (17.5 mm)
48	80 % of the final hypocotyl diameter reached (20 mm)
49	Expansion complete; typical form and size of hypocotyl reached (25 mm)

The BBCH growth stages adequately described growth of radish until Principal Growth Stage 4. At this stage in this investigation, the exodermis and outer cortex were observed to rupture and slough away exposing the periderm. The hypocotyl then began to expand (> 2.5 mm). The description of the BBCH Growth Stage 41 (Roots beginning to expand (diameter > 5 mm) was found to be inadequate therefore this description has been altered in the proposed growth standard 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 32 mm in diameter, less than 20% of this would be the range 3.6 mm to 6.4 mm. Therefore, they are not exclusively > 5 mm in diameter when they are less than 20% of the harvest size. Finally the BBCH description does not describe 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 3-11) therefore all splitting must happen after this point.

Table 3-11 Example pictures of whole radish and free-hand cross-sections of radishes at key growth stages. Principle Growth Stages 1 and 4 occur simultaneously

Day	Growth Stage	Whole plant	Hypocotyl cross section
2	01: Radicle emerged from seed		
5	10: Cotyledons completely unfolded; true leaf initial visible (diameter 1.2 mm)		
13	11: 1st true leaf or pair of true leaves unfolded (diameter 1.9 mm)		
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 3.5 mm)		

Growth Stage 41 can be identified non-destructively during growth as the radish hypocotyl is visible above the surface of the growing medium (Figure 3-13).

Figure 3-13 Growth Stage 41 can be identified non-destructively as in this photograph. Growth stage 41 is when the exodermis and outer cortex rupture and slough away exposing the periderm and the hypocotyl begins to expand more rapidly

3.3.2.4 Experiment 3.2: Discussion

In this investigation a clear pattern of growth for *Raphanus sativus* 'Rudi' was observed and described enabling a key to be developed for the growth stages of this cultivar. Timings for the growth stages of '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. It also needs to be established if all cultivars of radish follow the same pattern of growth and if they all pass through Growth Stage 41.

It should be noted the proposed key only represents the growth stages which have commercial relevance. Rates of growth had not plateaued at harvest and there had been no flowering showing radishes are not physiologically mature at commercial harvest.

It was concluded, radish phenology is well represented by the BBCH root and stem scale with the exception of Principle Growth Stage 4. 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. Further investigations will investigate if this growth stage is common to other cultivars of radish.

3.3.3 Experiment 3.3: Determining the growth stages and growth rate of five cultivars of radishes

3.3.3.1 Experiment 3.3: Introduction

In Experiment 3.1 all plants were grown for the same number of days which made it impossible to determine if growth rate was correlated with splitting as some radishes were harvested at an earlier growth stage than others. In this experiment plants were harvested when the radishes reached Growth Stage 49 i.e. commercial harvest size, rather than on a particular day to enable a comparison between growth rate and splitting.

Growth stages were established for the cultivar 'Rudi' in Experiment 3.2. This experiment aims to establish if these are valid for other radish cultivars of by growing a range of cultivars. Key growth stages were also recorded for each cultivar to allow comparison.

Water content was measured in Experiment 3.1 and found to be negatively correlated with splitting. However, due to the water content only being measured after 10 days of storage it was unclear if the differences were due to more rapid water loss from radishes with split surfaces. In this experiment the hypocotyl relative water content (RWC) was measured at harvest.

Aims: To determine if:

- The growth stages proposed in Experiment 3.2 are correct for other radish cultivars
- There is any difference in rates of splitting between cultivars
- The growth rate of different cultivars is correlated with growth splitting
- If hypocotyl roundness is correlated with splitting
- If radish hypocotyl water content at harvest is correlated with growth splitting

Null hypotheses:

1. There will be no difference in growth rate between cultivars
2. There will be no correlation between growth rate and splitting susceptibility of different radish cultivars
3. There will be no difference in susceptibility to splitting between cultivars
4. There will be no difference in roundness between cultivars

5. There will be no correlation between roundness and splitting susceptibility between cultivars
6. There will be no difference in total plant weight or hypocotyl weight between cultivars
7. There will be no difference in the number of leaves between cultivars
8. There will be no difference in hypocotyl RWC between cultivars
9. There will be no correlation between splitting and hypocotyl RWC

3.3.3.2 Experiment 3.3: Materials and Methods

Cultivar selection: This experiment investigated the splitting susceptibility of five different radish cultivars. The five cultivars which were chosen for this experiment were, 'Rudi', 'Celesta', 'Rougette', 'Kaspar' and 'Saxa 2'. The cultivars 'Celesta' and 'Rudi' were used in the previous cultivar experiment and split the least and most respectively, these varieties were also used in this experiment to ensure some differences in splitting were observed. The varieties 'Saxa 2' and 'Kaspar' were included as cultivars with different growth rates. 'Saxa 2' is described as cropping over a long period which may mean it has an inconsistent growth rate and 'Kaspar' is described as being ready to harvest after just three weeks which suggests a relatively rapid growth rate. The cultivar 'Rougette' was chosen because it is described as having a thick skin and results from the previous cultivar experiment suggest this may make it less resistant to splitting.

Replication: Each tray contained plants of all 5 cultivars. 10 plants were grown in each tray, two plants of each cultivar, one in each row, randomly arranged. Each pot was a block and the cultivars were randomised within the pot (block). Randomisation was generated by GenStat for Windows 15th Edition (VSN International 2011). Each plant was an experimental unit. There were 15 trays in total, containing 150 plants, 30 of each cultivar. Cultivars were identified with coloured stickers on the side of the pot (Figure 3-14).

Figure 3-14 Plant tray containing 10 plants of five different cultivars. There are two plants of each cultivar per tray, one in each row in the tray. The cultivars are identified by different coloured electrical tape on the rim of the tray.

Growth summary: Radish seeds were planted on 12.11.2013 (Day 1). Seedlings were thinned on Day 7 (18.11.2013). Each cultivar was harvested when mean diameter was greater than 25 mm. 'Rudi', 'Kaspar' and 'Saxa 2' were harvested on Day 28 (9.12.2013), 'Celesta' was harvested on Day 29 (10.12.2013) and 'Rougette' was harvested on Day 31 (12.12.2013).

Glasshouse conditions: The glasshouse was set to 20/5°C day/night temperature and achieved a mean temperature of 19.0°C with a range from 2.9°C to 33.0°C. The mean relative humidity was 63.7% with a range from 21.8% to 93.0%.

Growing conditions: Radishes were grown as described in general materials and methods.

Measuring substrate VWC: After the seedlings had been thinned on Day 7, the VWC of each pot was measured twice a week on Tuesday and Thursday.

Measurements during growth: Growth stage was recorded by measuring hypocotyl width and leaf number three times a week on Monday, Wednesday and Friday. The timing of Growth Stage 41 was also recorded.

Harvest: At harvest the radish hypocotyls were washed in tap water at ambient temperature and examined for splits. The mean temperature in the glasshouse on Day 28 during the harvest of 'Rudi', 'Kaspar' and 'Saxa 2' was 23.5°C, on Day 29 during the harvest of 'Celesta' it was 23.1°C and on Day 31 during the harvest of 'Rougette' the mean temperature was 22.4°C. To determine the rate of growth of the leaves and hypocotyl and their size at harvest, the radishes were weighed then defoliated, the white roots removed and the remaining hypocotyl was reweighed. The number of leaves on each plant was counted. The diameter and length of the hypocotyl to the first root hair was measured to enable a rough calculation of how spherical the radishes were to be made. This was done by dividing length by width, the closer the result was to 1 the more spherical the radish was considered to be.

RWC: Fifteen plants of each cultivar, one plant from each block, was weighed, saturated in deionised water and dried to calculate the RWC at harvest (see general materials and methods for more detail).

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Measurements of size during growth and at harvest, growth splits and hypocotyl RWC were all analysed using ANOVA. When a P value of less than 0.05 was observed a Tukey test was used to determine which results were different from each other.

Measurements of growth rate, number of growth splits, size and roundness at harvest were analysed using cultivar and block as factors (Table 3-12). Both radishes for each cultivar in each tray were measured and used for analysis.

Table 3-12 Skeleton ANOVA for measurements taken during growth and at harvest

Source of variation	df
Block	14
Cultivar	4
Residual	131
Total	149

RWC at harvest of one radish of each cultivar per tray was measured and analysed by ANOVA using block and cultivar as factors (Table 3-13).

Table 3-13 Skeleton ANOVA for hypocotyl RWC at harvest

Source of variation	df
Block	14
Cultivar	4
Residual	56
Total	76

The mean number of growth splits was correlated with RWC using general linear regression to perform the statistical analysis (Table 3-14).

Table 3-14 Summary of analysis for correlation between RWC and growth splits

Source	df
Regression	1
Residual	3
Total	4

3.3.3.3 Experiment 3.3: Results

VWC: The mean VWC was 64.1% ranging from a maximum of 67.5% to a minimum of 62.8 % (Figure 3-15).

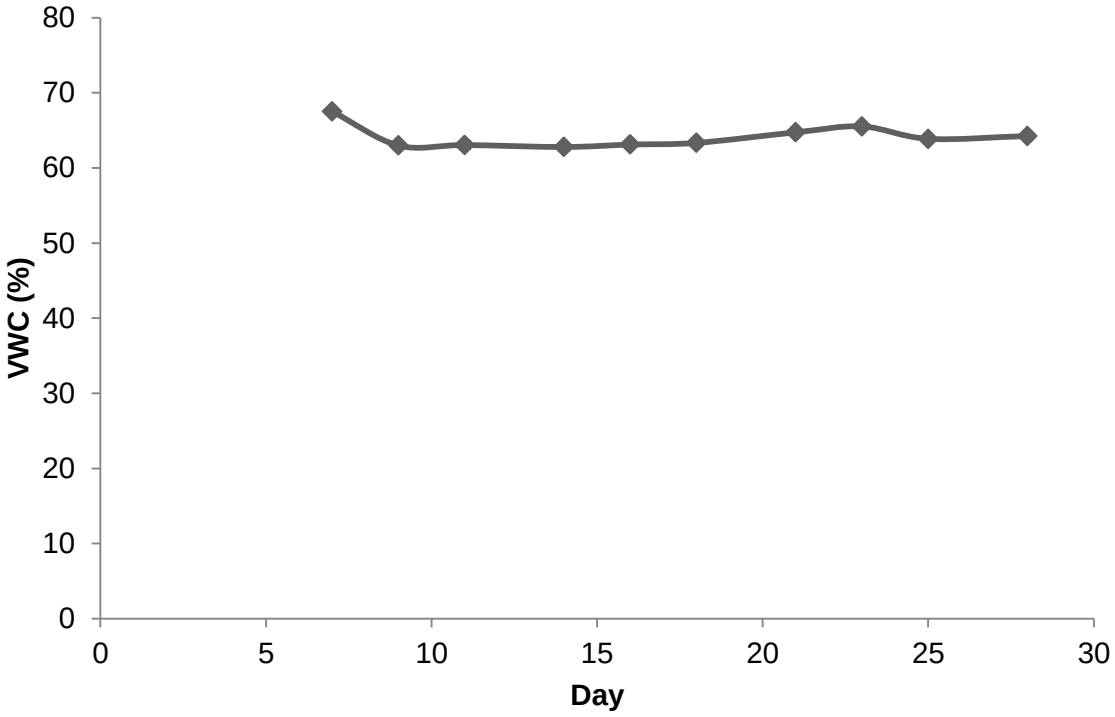


Figure 3-15 Mean VWC of compost in trays during growth (n=15)

Measurements during growth: There was no significant difference in the mean number of days it took for the different cultivars to form the first leaf bud (P=0.207) (Table 3-15), the mean was 7.9 days, or reach Growth Stage 41 (P=0.270) (Table 3-16) the mean was 14.6 days. There was no correlation between growth splits and days to first leaf bud (P=0.169), days to Growth Stage 41 (P=0.665) or days to harvest (P=0.615).

Table 3-15 Mean number of days for each cultivar to reach first leaf bud

Cultivar	Days to first leaf bud
Rudi	7.9
Celesta	8.1
Rougette	7.9
Kaspar	7.9
Saxa 2	7.6
P	0.207
LSD (5%)	0.395

Table 3-16 Mean number of days for each cultivar to reach Growth Stage 41

Cultivar	Days to Growth Stage 41
Rudi	14.4
Celesta	14.6
Rougette	14.9
Kaspar	14.5
Saxa 2	14.5
P	0.270
LSD (5%)	0.487

The pattern of hypocotyl expansion was very similar for all cultivars, expansion began slowly with an increase shortly after Day 15 (Figure 3-16). The rate of expansion up to Day 15, which was roughly the time of Growth Stage 41 was more variable between cultivars (Figure 3-17) than after Day 15 (roughly Growth Stage 41) when expansion rates were almost identical for all cultivars (Figure 3-18).

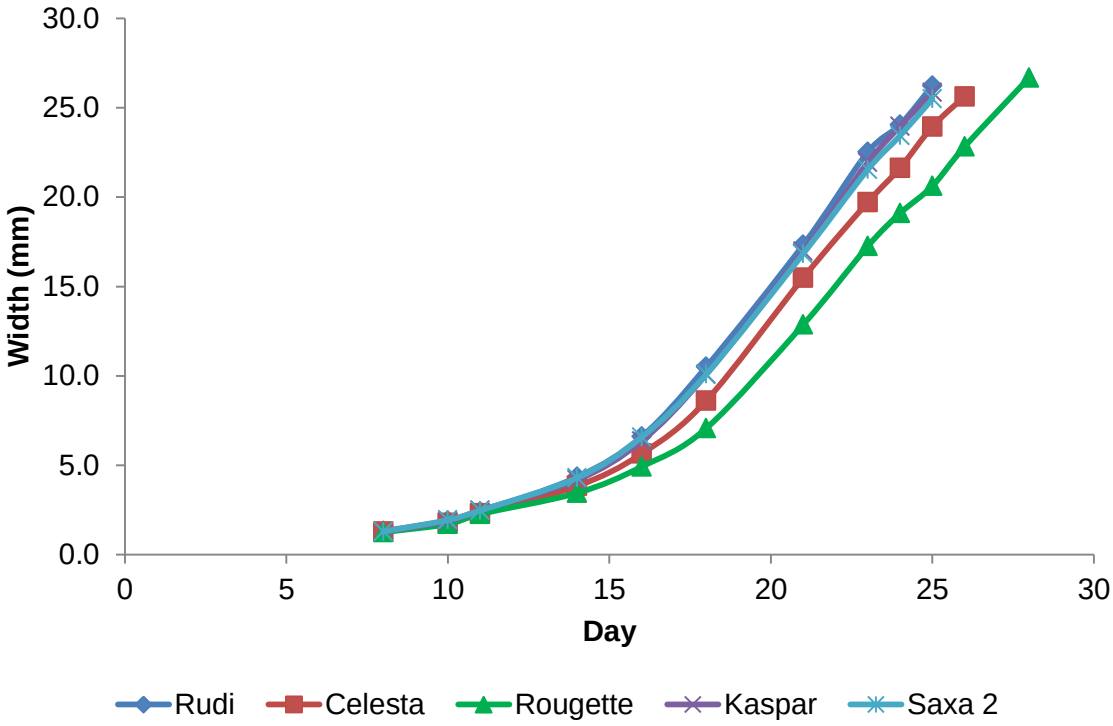


Figure 3-16 Hypocotyl expansion rate for different cultivars (n=30)

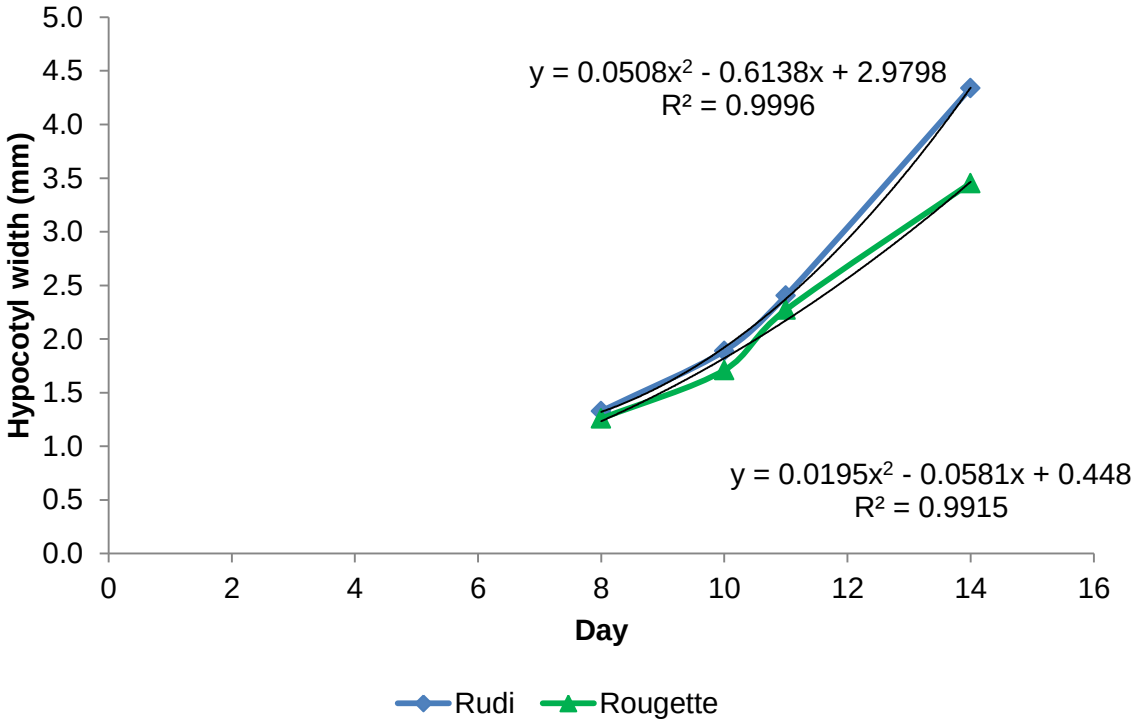


Figure 3-17 Hypocotyl expansion rate for 'Rudi' one of the fastest growing cultivars and 'Rougette' the slowest growing cultivar up to Day 15 (roughly Growth Stage 41). Only two cultivars are shown for clarity (n=30)

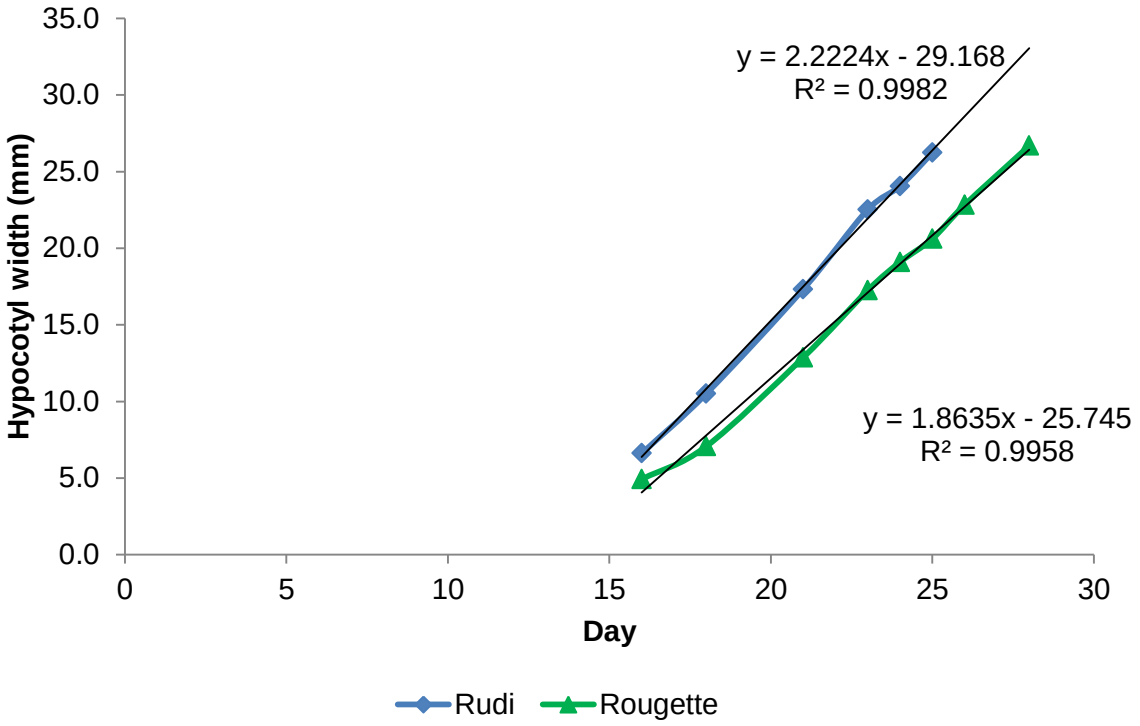


Figure 3-18 Hypocotyl expansion rate for 'Rudi' one of the fastest growing cultivars and 'Rougette' the slowest growing cultivar after Day 15 (roughly Growth Stage 41). Only two cultivars are shown for clarity (n=30)

The number of leaves increased at a similar rate and followed a similar pattern for all cultivars (Figure 3-19).

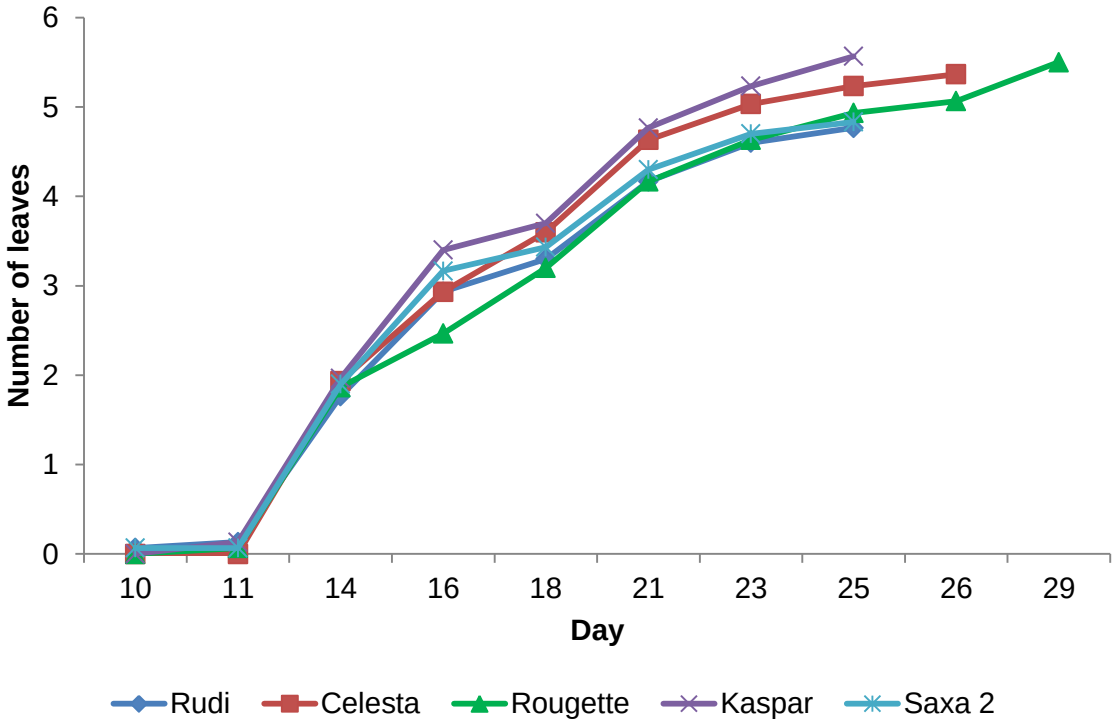


Figure 3-19 Mean number of leaves during growth for five different cultivars of radishes (n=30)

Growth splits: A significant difference in growth splitting was observed (P=0.031). The cultivar ‘Celesta’ split the least but not significantly less than ‘Rudi’, ‘Rougette’ or ‘Saxa 2’. The cultivar ‘Kaspar’ split the most but not significantly more than ‘Rudi’, ‘Rougette’ or ‘Saxa 2’.

Table 3-17 Differences in the number of growth splits for different cultivars of radishes (n=30)

Cultivar	Split (%)
Rudi	53.3ab[1]
Celesta	20.0a
Rougette	46.7ab
Kaspar	56.7b
Saxa 2	53.3ab
P	0.031
LSD (5%)	25.25

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Measurements at harvest: Plants in this experiment were harvested according to hypocotyl width rather than on a specific day, as a result there was no significant difference in hypocotyl width at harvest (P=0.951). There was a significant difference in hypocotyl length at harvest between the cultivars (P<0.001). 'Rougette' was significantly shorter than all the other cultivars. 'Celesta' was the longest cultivar but not significantly longer than 'Rudi', 'Kaspar' or 'Saxa 2' (Table 3-18). Significant differences (P<0.001) in roundness, as measured by dividing hypocotyl length by width, were observed. 'Rougette' had a roundness number significantly smaller than 'Rudi', 'Celesta' and 'Saxa 2' indicating the hypocotyl was significantly shorter. It also had the closest score to 1 indicating it had the most spherical hypocotyl. The cultivar 'Kaspar' was not significantly different in roundness to any of the other cultivars. There was no correlation between roundness and growth splits (P=0.53), length and growth splits (P=0.44) or width and growth splits (P=0.72).

Table 3-18 Mean hypocotyl length (L), width (W) and roundness for different cultivars of radish at harvest (radishes were harvested according to size when >50% of each cultivar was >25mm in diameter) (n=30)

Cultivar	Length (mm)	Width (mm)	Roundness (L/W)
Rudi	29.67bc[1]	26.23	1.210b
Celesta	34.34c	25.63	1.396b
Rougette	23.94a	26.72	0.908a
Kaspar	29.07b	25.86	1.161ab
Saxa 2	32.70bc	25.49	1.365b
P	<0.001	0.951	<0.001
LSD (5%)	3.488	3.312	0.1859

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

No significant differences were observed for total weight (P=0.954) or hypocotyl weight (P=0.857) at harvest. There were slight but significantly (P=0.002) different numbers of leaves on plants of different cultivars at harvest. ‘Rudi’ had the fewest leaves, although not significantly fewer than ‘Celesta’ or ‘Saxa 2’.’Kaspar’ had the greatest number of leaves but not significantly greater than ‘Rougette’ (Table 3-19).

Table 3-19 Mean number of leaves, total plant weight and hypocotyl weight at harvest (cultivars were harvested individually when >50% of each cultivar was >25mm in diameter) for different radish cultivars (n=30)

Cultivar	Number of leaves	Total weight (g)	Hypocotyl weight (g)
Rudi	4.767a[1]	19.30	12.39
Celesta	5.367ab	20.64	12.97
Rougette	5.500bc	19.47	12.40
Kaspar	5.567c	19.06	11.03
Saxa 2	4.833ab	19.61	12.25
P	0.002	0.954	0.857
LSD (5%)	0.5078	4.169	3.473

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

RWC: Significant differences were observed in the RWC for different cultivars (Table 3-20). 'Rougette' had the lowest RWC although it was not significantly different from the RWC of 'Celesta', 'Rudi' or 'Saxa 2'. 'Kaspar' had the greatest RWC but not significantly different from 'Saxa 2' or 'Rudi'.

Table 3-20 Hypocotyl RWC at harvest for different radish cultivars. Cultivars were harvested individually when >50% of each cultivar was >25mm in diameter (n=30)

Cultivar	RWC (%)
Rudi	87.50ab[1]
Celesta	84.79a
Rougette	85.57a
Kaspar	89.49b
Saxa 2	88.22ab
P	0.007
LSD (5%)	2.731

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

A non-significant linear trend was observed (P=0.082) positively correlating the mean RWC for each cultivar with the mean number of growth splits for each cultivar (Figure 3-20). The quadratic polynomial relationship between RWC and number of growth splits was not significant (P=0.105).

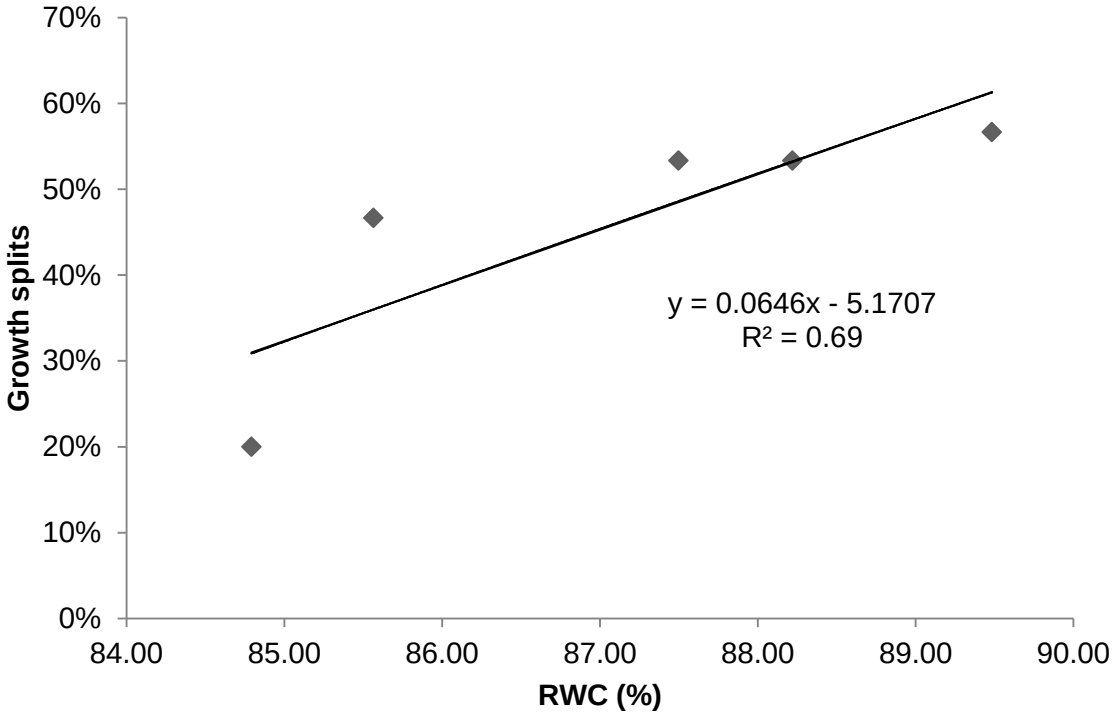


Figure 3-20 Non-significant correlation trend (P=0.082) between radish growth splits and hypocotyl RWC at harvest. Cultivars were harvested individually when >50% of each cultivar was >25mm in diameter. Standard error = 0.0965 (n = 15)

3.3.3.4 Experiment 3.3: Discussion

Rate of growth: The first null hypothesis was partially rejected because the rate of hypocotyl expansion varied between cultivars prior to Day 15, which is when Growth Stage 41 occurred in this experiment. After this however, the growth rates were very similar for the hypocotyls of all cultivars. Therefore, the rate of expansion prior to Day 15, or Growth Stage 41, appears to determine time to harvest and could be used by growers to predict harvest date for different cultivars.

The second null hypothesis was supported as no correlation was found between rate of growth and splitting. In this experiment all cultivars were harvested by hypocotyl width, as they would be commercially, rather than on a particular date as in Experiment 3.1. These results are in contradiction to the conclusions drawn by Latimer (1991) who postulated the low splitting rate observed in radishes which were exposed to mechanical leaf damage was a result of slow growth and also in contradiction to the results of Dowker and Jackson (1977) who found a slower growth rate in carrot was correlated with higher levels of splitting. Results from this cultivar experiment demonstrate overall growth rate is not an explanation for the differences in splitting between cultivars in radishes.

Effect of cultivars on splitting: A significant difference in splitting between cultivars was observed (P=0.031) and the third null hypothesis was rejected. The cultivar ‘Celesta’ split the least but not significantly less than ‘Rudi’, ‘Rougette’ or ‘Saxa 2’. The cultivar ‘Kaspar’ split the most but not significantly more than ‘Rudi’, ‘Rougette’ or ‘Saxa 2’.

As was found in Experiment 3.1, the cultivar ‘Celesta’ had the lowest number of splits and the greatest yield in terms of total and hypocotyl weight.

Roundness: The fourth null hypothesis was rejected as significant differences in hypocotyl roundness as calculated by dividing the length of the hypocotyl by the width were observed between cultivars. However, the fifth null hypothesis was supported as mean cultivar roundness was not found to be correlated with mean cultivar growth splits. This contradicts the work of Emmons and Scott (1998) who stated shape is an important factor in splitting susceptibility and the work by Considine and Brown (1981) which suggests deviations from a globe shape result in increased differences in stress within

tissue. However, in this experiment it appears relative roundness is not the main factor in determining growth splitting and other factors have a greater influence.

RWC: The eighth null hypothesis was rejected as significant differences were observed in the RWC for different cultivars. The ninth null hypothesis was also rejected as a non-significant trend (P=0.082) with a strong linear relationship (R^2=0.69) was observed positively correlating the mean RWC for each cultivar with the mean number of growth splits for each cultivar. These results suggest radishes with a higher RWC are more likely to split which contradicts the findings from the previous cultivar experiment where growth splitting was found to be negatively correlated with WC. In this experiment RWC was measured on the day of harvest whereas in Experiment 3.1 the WC was measured after 10 days of storage. Potentially, radish hypocotyls in Experiment 3.1 lost water more rapidly from the split surfaces than through the non-split periderm during storage and resulted in them having a lower WC. A high RWC being correlated with splitting susceptibility 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 (Konstankiewicz & Zdunek 2001; McGarry 1993; McGarry 1995). In these experiments it was concluded that an increase in tissue turgor pressure results in an increase in the tension of the cell walls, this may also be true for radishes although further work is required to investigate this.

3.3.4 Experiments 3.2-3.3: Conclusion

In conclusion, Experiment 3.2 successfully established the growth stages for *Raphanus sativus* 'Rudi' and Experiment 3.3 showed these were applicable to a range of other cultivars of radishes. This result suggests the proposed growth stages are likely to be applicable to radishes generally.

Both experiments gave an indication of the growth rates of radishes under glasshouse conditions. These results will be used in the planning of future experiments.

Experiment 3.3 investigated a number of physiological traits with splitting. A non-significant trend was observed linking a high RWC of the hypocotyl with splitting. This will be investigated further in future experiments.

3.4 Experiment 3.4: Analysis of commercial QA radish split data

3.4.1 Experiment 3.4: Introduction

Growth and harvest splitting is likely to be determined by both the physiological predisposition to splitting and on the environmental conditions which the radish hypocotyl is exposed to. These are not separate factors and environment will affect the way the radish grows and therefore its physiology. 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. Temperature at harvest and relative humidity at harvest may also affect the amount of harvest splitting which is observed. Radishes are harvested, washed, trimmed, placed into Dolavs (large storage bins) and quality assessed at ambient temperature and are not kept under managed humidity conditions prior to washing. Hence, by correlating commercial quality assessment data throughout the radish season with weather data during growth and at harvest it is possible to study the interaction between rainfall, temperature and relative humidity with splitting.

Weather conditions during growth may affect splitting susceptibility by changing turgor pressure within the radish hypocotyl. High turgor pressure has been shown to be related to increased splitting susceptibility in other crops (McGarry 1993; McGarry 1995). The theory is that less force is required to rupture cells which are already under stress. Turgor pressure could be increased through large amounts of rainfall during growth which may increase the hypocotyl water content or through high relative humidity during growth decreasing rates of evapotranspiration. Low temperature may also increase turgor pressure within the hypocotyl and increase splitting. Kokkoras (1995) suggested the cytoplasm and cell wall may contract to a greater extent than the vacuole at low temperatures causing an increase in turgor pressure. Low temperature and high RH at harvest and post-harvest could also affect splitting susceptibility again by affecting turgor pressure.

Experiment 3.4 analysed splitting data from G's Growers QA collected over three years for one growing site and one cultivar ('Celesta'). The main objective of the analysis was to

investigate commercial splitting trends and correlate these with the weather during growth and the weather conditions on the day of harvest to determine if environmental conditions during growth or at harvest appear to be related to splitting.

Aims: To determine:

- The magnitude of variation in splitting between years
- If rainfall, temperature and relative humidity during growth are correlated with splitting
- If temperature and relative humidity at harvest are correlated with splitting
- If there are differences in the extent or direction of effects between weather during growth or at harvest on splitting

Null hypotheses:

1. There will be no difference in the amount of splitting each year
2. There will be no correlation between the environmental parameters precipitation, temperature and relative humidity during growth and splitting
3. There will be no correlation between temperature and relative humidity on the day of harvest and splitting

3.4.2 Experiment 3.4: Materials and methods

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 analysis (QA) team, and weather data at harvest and during the growth of the radishes. The QA data which was used has been summarised in (Table 3-21). 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 3-21 Summary of commercial QA data provided by G's Growers which was used for analysis of correlations between weather conditions during growth and radish splitting

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	23.10.2014

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 3-22). Irrigation data was not included as this was not known for each batch of radishes, the lack of robust irrigation data is unfortunate and results should be remembered when interpreting results. It is imperative that any conclusions relating to soil moisture are tested using robust experimental design where VWC is measured accurately. To avoid duplication of data and to ensure the variables were as independent as possible, mean temperature and mean relative humidity during growth did not include the data for temperature and relative humidity on the day of harvest.

As the size of the batches and therefore the number of Dolavs harvested on any one day varied, 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 3-22 Summary of variables analysed for correlations between commercial splitting as recorded by G's Growers QA team and weather conditions during growth as recorded by BADC and the Met Office

Symbol	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 (%)

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

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.

Multiple regression was appropriate for the analysis because 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. Initially, a correlation matrix was performed in MS-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 for Windows 15th Edition (VSN International 2011) a full model containing main effects and interaction terms was fitted and the significance of terms tested by stepwise deletion. 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 RWC of the radishes. Using ANOVA the RWC 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.

Skeleton ANOVA:

Table 3-23 Skeleton ANOVA for the mean number of split radishes recorded by G's Growers QA team in 2012, 2013 and 2014

Source of variation	df
Year	2
Residual	643
Total	645

Table 3-24 Skeleton ANOVA for the multiple regressions comparing the number of split radishes recorded by G's Growers QA team with several weather parameters recorded by BADC and the Met Office over the years 2012, 2013 and 2014

Source of variation	df
Regression	5
Residual	640
Total	645

Table 3-25 Skeleton ANOVA for the multiple regressions comparing the number of split radishes recorded by G's Growers QA team with several weather parameters recorded by BADC and the Met Office in 2012

Source of variation	df
Regression	5
Residual	146
Total	151

Table 3-26 Skeleton ANOVA for the multiple regressions comparing the number of split radishes recorded by G's Growers QA team with several weather parameters recorded by BADC and the Met Office in 2013

Source of variation	df
Regression	4
Residual	208
Total	212

Table 3-27 Skeleton ANOVA for the multiple regressions comparing the number of split radishes recorded by G's Growers QA team with several weather parameters recorded by BADC and the Met Office in 2014

Source of variation	df
Regression	5
Residual	275
Total	280

3.4.3 Experiment 3.4: Results

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 3-28).

Table 3-28 Mean number of splits (%) observed in the years 2012, 2013 and 2014 by G's Growers QA team

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

[1]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 3-29).

Table 3-29 Accumulated precipitation, mean temperature and mean relative humidity during the commercial growing season at RAF Marham for the years 2013, 2013 and 2014

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 3-30).

Table 3-30 Correlation matrices between commercial splitting rates observed by G's Growers QA team and weather data measured by BADC and the Met Office at RAF Marham for 2012, 2013, 2014 and all three of these years together (numbers in bold are significantly correlated at the 5% level).

Year		S^1	R	T_g	RH_g	T_h	RH_h
All years	S	**1**					
n = 646	R	**0.251**	**1**				
ρ = 0.088	T_g	**-0.138**	**-0.178**	**1**			
	RH_g	**0.301**	**0.222**	**-0.301**	**1**		
	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	R	**0.226**	**1**				
ρ = 0.139	T_g	-0.008	0.082	**1**			
	RH_g	0.002	**-0.166**	**-0.862**	**1**		

	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	R	**0.389**	**1**				
ρ = 0.139	T_g	0.104	**-0.479**	**1**			
	RH_g	**0.577**	**0.442**	-0.020	**1**		
	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	R	**0.113**	**1**				
ρ = 0.113	T_g	**-0.431**	**0.189**	**1**			
	RH_g	**0.216**	0.004	-0.046	1		
	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 (%), R = 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 = critical value for Pearson product-moment correlation coefficient (5%)

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 variance in splitting explained by the weather variables for all three years combined was 16.6%. In 2014, 2012 and all years, all of the variables were included in the final model whereas in 2013, the mean relative humidity on the day of harvest, was not included (Table 3-31).

Table 3-31 Model relating weather conditions during growth, measured by BADC and the Met Office and splitting rates, recorded by G's Growers, as determined by multiple linear regression and stepwise deletion for 2012, 2013, 2014 individually and all years together

Year	Model fitted	P	Variance accounted for	SE
All years	$T_g+RH_h+R+T_h+RH_g$	<0.001	16.4%	1.75
2012	$T_h+T_g+RH_g+RH_h+R$	<0.001	12.9%	1.64
2013	$RH_g+P+T_g+T_h$	<0.001	39.6%	1.67
2014	$T_g+R+RH_g+RH_h+T_h$	<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 may have an effect on splitting (Table 3-32).

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 and was not included in the model for 2013 (Table 3-32).

Temperature both during growth and at harvest tended to have a negative parameter estimate when correlated with splitting. This suggests radishes are increasingly likely to split as the temperature decreases. 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 3-32).

Table 3-32 The significance of variation for splitting (measured by G's Growers QA team), parameter estimate, mean, standard error of the mean (SEM) and proportion of total sum of squares (TSS) accounted for by the accumulated sum of squares for each weather variable (measured by BADC and the Met Office) in 2012, 2013, 2014 and all years together

Variable	Year	P	Parameter estimate	Mean	SEM	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

3.4.4 Experiment 3.4: Discussion

The first null hypothesis was rejected as a difference in the amount of splitting each year was observed. There was significantly less splitting in 2014 (2.14%) compared to 2012 (2.76%) and 2013 (2.73%). The magnitude of differences in splitting between years was not great but the difference in the weather parameters which were measured was also limited in these years. The difference in accumulated precipitation between the year with the heaviest rainfall (2013) and the lightest rainfall (2014) was 85.4 mm, the difference in mean temperature between the warmest (2014) and coolest (2012 and 2013) years was 1.7°C and the difference in RH between the most (2012) and least (2013) years was 3.3%. To investigate the effects of weather on splitting more conclusively years with a greater variation in weather would be required.

In support of the theory 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 many 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. An additional limitation of the data is that splitting of batch samples was only recorded post washing and radishes split during growth versus those that split during the harvest process cannot be distinguished. Nevertheless, the results are encouraging and support further investigation into the effects of environment during growth and post-harvest during handling.

The second and third null hypotheses were rejected as results show that weather conditions during growth and at harvest affect splitting in commercial production. Relative humidity and rainfall both had exclusively positive parameter estimates when correlated with splitting indicating soil-plant water interactions have an important role in splitting. It was hypothesised increased rainfall during growth would lead to increased hypocotyl water content and turgor pressure. Similarly increased relative humidity may lower the

transpiration rates which could also increase turgor pressure. Higher turgor pressure has been shown in other crops (McGarry 1993, 1995) to result in increased splitting susceptibility.

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). 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. 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.

In conclusion rainfall, temperature and relative humidity during growth are correlated with splitting as are temperature and relative humidity at harvest. Temperature both during growth and at harvest tended to have a negative parameter estimate when correlated with splitting suggesting radishes are increasingly likely to split as the temperature decreases. 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.

3.5 Experiments 3.5-3.12: Investigating the effects of VWC during growth on the susceptibility to growth splits of radishes

3.5.1 Experiments 3.5-3.12: Introduction

At present, it is not known if VWC during growth, the magnitude of fluctuations in VWC during growth or the timing of water availability during growth have an effect on hypocotyl splitting in radishes. Therefore research is required to answer these gaps in knowledge.

In Experiment 3.4, the volume of rain which fell during growth was positively correlated with splitting in commercially grown radishes, linking greater available water content during growth with increased rates of splitting. These results are in keeping with those from other crops where one of the predominant environmental factors thought to affect growth splitting is water availability during growth. For instance, in sweet cherry fruit, rainfall has been linked to splitting. Rain induced growth splitting in cherries is thought to be caused by a combination of two mechanisms, firstly by rain water entering through the skin and degrading the dermal cell walls of the fruit and secondly by an increase in pressure on the skin from within the fruit as a result of water uptake by the vascular system (Sekse 1995). Similarly, water content during growth has been shown to affect splitting in winter radishes (Kang & Wan 2005) as has irrigation frequency (Wan & Kang 2005). Fluctuations in available water content have also been shown to effect splitting in carrot (Salter & Goode 1967) as has the timing of water availability (Sørensen *et al.* 1997). Eight experiments were conducted to investigate the relationships between VWC during growth and splitting. Initial investigations began by looking at the effect of VWC on splitting; three experiments (Experiments 3.5 to 3.7) were conducted into the effects of VWC on splitting. Experiment 3.8 investigated the effect of irrigation frequency on splitting. The section concludes with a series of four experiments (Experiments 3.9 to 3.12) which investigated the effects of timing of water availability on splitting. In the final experiment, water availability was linked to the growth stages which were developed in Experiment 3.2.

3.5.2 Experiments 3.5-3.7: The effects of VWC during growth on the susceptibility of radishes to growth splits

3.5.2.1 Experiments 3.5-3.7: Introduction

Three experiments were conducted to investigate the effects of VWC on splitting in radishes. The aim of these experiments was to determine firstly if water availability during growth has an effect on splitting and then to determine if these effects are similar for different cultivars. Kang and Wan (2005) investigated the effects of soil water potential on the large winter type of radish by attempting to maintain constant soil water content during growth. They grew radishes at five different soil water potentials, -0.015 MPa, -0.025 MPa, -0.035 MPa, -0.045 MPa and -0.055 MPa. In this investigation, the irrigation treatments had no effect on growth rate and yield of the radish crop but there was an effect on splitting with the highest levels being observed in the wettest treatment and the lowest level being observed at -0.035 MPa (Kang & Wan 2005), however, no explanation was proposed in the paper for potential causes of splitting as a result of soil water potential. In other crops the increase in splitting which is associated with greater water availability is thought to be due to an increase in pressure on the skin from within as a result of water uptake by the vascular system into the tissue (Sekse 1995). Higher pressure within the organ results in the tissue being more susceptible to splitting as less additional force is required to rupture tissue which is already under tension. To investigate this, the relationship between hypocotyl water pressure, irrigation treatment and splitting was explored.

3.5.2.1.1 Experiment 3.5: Preliminary experiment into the effects of VWC during growth on the susceptibility of *Raphanus sativus* 'Rudi' to growth splits

3.5.2.1.1.1 Experiment 3.5: Introduction

The aim of this experiment was to determine a method for manipulating and measuring the available water content and the conditions the radish plants are exposed to. Manipulating and measuring the VWC enabled trends in the relationship between VWC and splitting to be investigated. The results from this experiment were used to refine later experiments.

Aim: To investigate:

- The relationship between substrate available water content during growth and splitting at harvest and after storage;
- The relationship between substrate available water content during growth and rate of growth and physiology of radishes.

Null hypotheses:

1. There is no relationship between VWC and growth splitting or harvest splitting after 7 days of cold storage
2. There is no relationship between VWC during growth and the rate of growth and physiology of radishes

3.5.2.2.1.1 EXPERIMENT 3.5: MATERIALS AND METHODS

The cultivar 'Rudi' was chosen for this experiment as it had been shown in Experiments 3.1 and 3.3 to be highly susceptible to splitting.

Seedling establishment: Radish seeds were initially planted in 362 x 227 mm seed tray inserts (C24, LBS Horticulture Ltd., Lancashire) containing John Innes No. 2 growing medium, at a rate of 1 seed per cell. Like commercial seeds, the seeds were planted at a depth of 7 mm. They remained in seed trays for seven days to enable seedling establishment.

Start of treatments: After 1 week the most uniform seedlings were transferred to 4.2 L pots containing a 1:1 mix of horticultural sand and John Innes No. 2 growing medium. In each pot 6 uniform seedlings were transplanted and planted with equal spacing in a ring 25 mm from the rim of the plant pot.

The seedlings were transplanted to pots which had been uniformly filled two weeks previously. After the pots had been prepared they were left to dry in the glasshouse for two weeks, at this point the pots contained on average 20 % VWC, this was used as the driest treatment.

Treatments: The four treatments were: G1 24% VWC, G2 23% VWC, G3 21% VWC and G4 20% VWC. There were eight experimental and three destructive harvest pots for each treatment (Table 3-33), giving 11 pots per treatment, 44 pots in total containing a total of 264 radish plants, 192 of which were experimental plants.

Table 3-33 Summary of treatments used in Experiment 3.5

Treatment Number	VWC (%)	Replication
G1	24	8 pots containing 6 plants each
G2	23	8 pots containing 6 plants each
G3	21	8 pots containing 6 plants each
G4	20	8 pots containing 6 plants each

Randomisation: Experimental pots were arranged in a random block design (Figure 3-21) which was generated using GenStat for Windows 15th Edition (VSN International 2011).

Block				
1	G1	G4	G3	G2
2	G4	G1	G2	G3
3	G2	G4	G1	G3
4	G4	G2	G1	G3
5	G4	G2	G3	G1
6	G3	G4	G1	G2
7	G1	G4	G2	G3
8	G1	G4	G2	G3

Figure 3-21 Layout of pots on glasshouse bench. Blue lines represent the irrigation tape. G1=24 % VWC, G2=23 % VWC, G3=21 % VWC and G4=20 % VWC (n=8)

Irrigation: Pots were weighed and surface irrigated twice a week on Tuesday and Friday to maintain the water content of the treatments. During irrigation pots were surface watered to the weight at the VWC for their treatment. Pots were irrigated using a squeezable water bottle with a fine nozzle to ensure an even distribution of water over the surface without damaging the seedlings. Compensation was made for the increasing weight of the radish in the pots by performing destructive harvests twice a week on Tuesday and Friday. Additional plants were grown for the purpose of destructive harvests and these did not affect the number of replicates in the experiment.

Measurements during growth: The mean temperature was 18.2°C with a range of 42.4°C to 9.1°C. The mean relative humidity in the glasshouse was 69.2% ranging between 99.6% and 14.2%.

The width of the exposed hypocotyls was measured twice a week before irrigation. Radish plants were grown until more than 50% of radish in that treatment were 30 mm in diameter or greater, at which point they were harvested. This is in keeping with supermarket size requirements.

Harvest: As per Section 2.10.

Storage: The radishes were stored after harvest to replicate commercial practice. The radishes were placed into a labelled cryovac bag to simulate commercial packaging and moved to a Sanyo Versatile Environmental Test Chamber Model: MLR-351H for 14 days. Relative humidity and temperature were logged in the growth cabinet using TGP 4500 TinyTag logger. The growth cabinet achieved an average temperature of 2.1°C with a range between 8.4°C and -3.0°C. It achieved an average relative humidity of 77.6% with a range between 100% and 14.9%.

After storage the radish were again weighed and measured and the number of splits counted. They were then put in an oven at 105°C for at least 48 hours to calculate the dry biomass post-storage.

Growth summary: Seeds were planted on 21.05.2012 (Day 1) plants were transplanted and treatments commenced on 28.05.2012 (Day 8). G1 plants were harvested and moved to storage on 02.07.2012 (Day 47), G2 plants were harvested and moved to storage on 06.07.2012 (Day 51) and G3 and G4 plants were harvested and moved to storage on 09.07.2012 (Day 54). For G1 plants storage was terminated after 14 days on 16.07.2012, for G2 it was terminated after 14 days on 20.07.2012 and for G3 and G4 plants storage was terminated after 14 days on 23.07.2012.

G1 were harvested after 39 treatment days, G2 were harvested after 43 treatment days, G3 and G4 were both harvested after 46 treatment days. Growth time was longer than commercially grown radishes which usually takes four weeks. This was thought to be, in part due to the irrigation treatments which were imposed, but also possibly due to transplanting the radish at the start of the experiment.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

If data was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA (Table 3-34). Where data was not normally distributed with or without transformation the non-parametric Friedman's test was used. When a P value of less than 0.05 was observed a Tukey test was used for parametric data and Mann-U Whitney test was used for non-parametric data to determine which results were different from each other.

Table 3-34 Method of analysis for different factors measured in Experiment 3.5. Method of analysis (parametric or non-parametric) was decided according to normal distribution as determined by the Shapiro-Wilk test

Measurement	Shapiro-Wilk	Analysis used
Split data at harvest	P=0.184	ANOVA
Split data after storage	P=0.219	ANOVA
Number of leaves at harvest	P=0.618	ANOVA and Tukey Test
Hypocotyl width at harvest	P=0.971	ANOVA
Hypocotyl width after storage	P=0.153	ANOVA
Hypocotyl length at harvest	P=0.952	ANOVA
Hypocotyl length after storage	P=0.480	ANOVA

Skeleton AVOVA:

Table 3-35 Skeleton ANOVA for number of split radishes, number of leaves, hypocotyl width, hypocotyl length and hypocotyl weight at harvest (G1=Day 39, G2=Day 43, G3 and G4 = Day 46)

Source of variation	df
Treatment	3
Blocks	7
Residual	21
Total	31

Table 3-36 Skeleton ANOVA for number of split radishes, hypocotyl length, width and weight after 14 days of cold storage

Source of variation	df
Treatment	3
Blocks	7
Residual	21
Total	31

3.5.2.2.1.2 EXPERIMENT 3.5: RESULTS

VWC: Treatments successfully created a difference in VWC between groups (Table 3-37). However, the VWC overlapped between treatments (Table 3-37).

Table 3-37 Range of compost VWC, as calculated from the GWC, for each treatment group, up to Day 39 when G1 was harvested (n=8)

Treatment	Mean VWC (%)	Maximum VWC (%)	Minimum VWC (%)
G1	19.7	24	13.0
G2	18.7	23	13.3
G3	17.4	21	10.5
G4	16.1	20	10.3

Splitting: There was no significant difference between treatments in the number of split radishes at harvest (P=0.755) or after storage (P=0.940) (Table 3-38).

Table 3-38 The mean number (max = 6) of split radishes per pot for each treatment (G1=19.7% VWC, G2=18.7% VWC, G3 = 17.4% VWC and G4 = 16.1% VWC) at harvest (G1=Day 39, G2=Day 43, G3 and G4 = Day 46) and after 14 days of cold storage (n=8)

Treatment	Harvest	Storage
G1	2.13	2.50
G2	2.50	2.75
G3	3.00	3.00
G4	2.63	2.75
SEM (21 df)	0.808	0.794
CV (%)	63.1	57.8
P	0.755	0.940

Growth rate: Treatments had an effect on the growth rate of the radish plants; treatment group G1 grew the fastest and reached harvest size after 43 days, G2 was next at 47 days. G3 and G4 both took the longest to reach harvest size, 50 days. All treatments took longer than the usual growth time but this is thought to be due to disruptions from transplanting the radish seedlings at the beginning of the experiment.

There was a significant difference (P=0.004) in the number of leaves at harvest between treatment groups. G1 had the least leaves at harvest, G4 had the second least number of leaves, G3 had the second most number of leaves and G2 had the most number of leaves at harvest.

There was no difference in the size or weight of the radish at harvest or after storage between treatment groups (Table 3-39, Table 3-40).

Table 3-39 Measurements taken from the radish plants at harvest (G1=Day 39, G2=Day 43, G3 and G4 = Day 46) for each treatment group (G1=19.7% VWC, G2=18.7% VWC, G3 = 17.4% VWC and G4 = 16.1% VWC).

Treatment	Number of leaves	Hypocotyl width (mm)	Hypocotyl length (mm)	Hypocotyl weight (g)
G1	9.08a[1]	30.34	25.51	14.05
G2	10.38b	30.95	26.12	14.18
G3	10.21b	30.63	26.07	12.92
G4	9.98ab	29.37	25.84	13.12
SEM (21 df)	0.235	0.631	0.797	0.712
CV (%)	1.5	4.6	4.6	10.6
P	0.004	0.346	0.945	0.508

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Table 3-40 Measurements taken from the radishes after 14 days of cold storage for each treatment group (G1=19.7% VWC, G2=18.7% VWC, G3 = 17.4% VWC and G4 = 16.1% VWC).

Treatment	**Hypocotyl width (mm)**	**Hypocotyl length (mm)**	**Hypocotyl weight (g)**
G1	29.03	23.95	12.05
G2	29.00	24.22	12.61
G3	29.25	25.34	10.27
G4	28.85	25.74	11.45
SEM (21 df)	0.773	0.705	0.616
CV (%)	5.8	5.0	10.1
P	0.987	0.245	0.076

3.5.2.2.1.3 EXPERIMENT 3.5: DISCUSSION

The first null hypothesis was supported as here was no evidence for a relationship between VWC and splitting at harvest or after seven days of cold storage. Watering the radish plants at the same frequency but to different VWC did not result in any significant differences in growth splitting (P=0.755) or in any significant differences (P=0.940) in the amount of additional splitting which occurred post-harvest.

The second null hypothesis was rejected as a relationship between VWC during growth and the rate of growth of radishes was observed. As the radishes were harvested at the same hypocotyl growth stage there was no difference in hypocotyl width at harvest (P=0.346) or after storage (P=0.987) but there were differences in growth rate as it took the treatments different lengths of time to be of this harvest size. G1 was ready to harvest after 43 days, G2 after 47 days and G3 and G4 took 50 days. This finding is supported by previous research in which drought conditions were found to reduce or stop cellular division and cellular expansion in radishes which would reduce growth rate (Joyce *et al.* 1983). The shape of the hypocotyl appears to have remained similar for different treatments as there were no differences between treatments in hypocotyl length at harvest (P=0.945) or after storage (P=0.245) or hypocotyl weight at harvest (P=0.508) or after storage (P=0.076).

The effects of VWC on plant physiology and source to sink ratios were more difficult to interpret as the results did not follow a consistent pattern. Treatments appeared to affect leaf growth as differences in number of leaves at harvest were observed (P=0.004). G1 had significantly fewer leaves than G2 and G3 at harvest. The number of leaves on G4 plants was not significantly different to any other treatment group. However, no differences were observed in the size of the hypocotyls at harvest or after storage. These results suggest the treatments had different effects on leaves and hypocotyls and altered the source to sink ratios. However, the results are not easy to interpret as they do not follow a consistent pattern. The hypocotyls of the G1 plants, which received the most water grew the fastest as they were same size and weight as hypocotyls from the other treatments in less time, however the number of leaves was significantly fewer than the

plants grown in the mid-levels of water, G2 and G3. Suggesting the leaves from the G1 treatment grew more slowly than the hypocotyls. However, no differences were observed in the number of leaves of hypocotyl size of treatment G2 which received more water and grew faster than treatment G3 which received less water and grew more slowly. G2 and G3 were harvested after different periods of time but there was no difference in hypocotyl size or leaf number between the two treatments suggesting both their leaves and hypocotyls grew at a similar rate as the ratios were not altered. Treatment G4 was not different from any of the other treatments in terms of hypocotyl size or number of leaves at harvest.

The difference in the VWC between treatments was not large in this experiment and although differences in growth rate were observed showing the plants were responding to differences in VWC it would be interesting to investigate if larger differences have an effect on splitting. It was also noted during the experiment that the depth of the pots meant it was difficult to determine exactly the VWC the radishes were exposed to. Although weighing the pots gave an accurate indication of the water content of the whole pot, when the radishes were small they would not have had access to the water at the bottom of the pot.

In conclusion, VWC during growth did affect the rate of growth and physiology of radishes but the differences in VWC achieved in this experiment did not affect growth or harvest splitting.

3.5.2.2.2 Experiment 3.6: The effects of VWC during growth on the susceptibility of *Raphanus sativus* 'Rudi' to growth splits

3.5.2.2.2.1 Experiment 3.6: Introduction

In Experiment 3.5 no relationship between VWC and splitting was observed, however a very narrow and overlapping range of VWCs was used which made the results inconclusive. In this experiment a greater range in VWCs was used to determine more conclusively if water content during growth has an effect on growth splitting. To achieve this, shallower pots were used which allowed the compost to dry more rapidly. Shallower pots which were the same depth as the device used to measure water content were used to allow more accurate measurements of the conditions the radishes are exposed to, to be made.

For simplification compared to Experiment 3.5, only two treatments were used but these had a much greater difference. In Experiment 3.5, post-harvest splitting was measured by placing the radishes in storage this did not result in many additional splits. Under commercial conditions radishes are handled post-harvest therefore, in this experiment post-harvest splitting susceptibility was tested using a drop test which is quicker and more representative of commercial post-harvest handing than simply placing the radishes in storage and results in a greater number of radishes splitting.

The radishes were harvested at the same hypocotyl growth stage to enable differences in growth rate to be determined. However, in Experiment 3.5 it appeared that VWC during growth had an effect on leaf growth and the ratio of leaves to hypocotyl at harvest although the results were difficult to interpret as they did not follow a clear pattern. Therefore, in this experiment, more measurements of leaf size at harvest were taken to enable a greater understanding of the effects of VWC on leaf growth and how this compares to hypocotyl development.

Aim:

- To determine if VWC during growth has an effect on splitting of the radish hypocotyl at harvest and during post-harvest handling

- To determine if VWC during growth has an effect on growth rate and physiology of radish plants

Null hypotheses:

1. VWC during growth has no effect on splitting of the radish hypocotyl during growth or post-harvest handing
2. VWC during growth has no effect on the growth rate and physiology of radish plants

3.5.2.2.2.2 EXPERIMENT 3.6: MATERIALS AND METHODS

Seedling establishment: Radish seeds were initially planted and grown for seven days in seed trays measuring 350 mm in length by 210 mm in diameter and 55 mm in depth. These trays were watered by bench capillary matting for two minutes three times a day giving a total of 17 mm day $^{-1}$.

Transplanting and start of treatments: 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 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.

Treatments: Two treatments were used, Wet and Dry. For the Wet treatment the compost in trays was maintained at high water content close to pot capacity using capillary irrigation. The Dry treatment was maintained at low water content by hand watering three times a week to a low water content which was above permanent wilting point. During irrigation dry pots were watered to the weight at the VWC for their treatment. Compensation was made for the increasing weight of the radish in the pots by performing destructive harvests three times a week. Additional plants were grown for the purpose of destructive harvests and these did not affect the number of replicates in the experiment.

The VWC of each pot was measured three times a week.

Replication: 20 plus five extra dry pots for destructive harvests. Pots were arranged in a randomised block design (Figure 3-22) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block					Block
1	Dry	Wet	Dry	Wet	11
2	Dry	Wet	Wet	Dry	12
3	Wet	Dry	Dry	Wet	13
4	Dry	Wet	Wet	Dry	14
5	Dry	Wet	Wet	Dry	15
6	Wet	Dry	Wet	Dry	16
7	Wet	Dry	Dry	Wet	17
8	Dry	Wet	Dry	Wet	18
8	Dry	Wet	Wet	Dry	19
10	Wet	Dry	Wet	Dry	20

Figure 3-22 Randomised block design of pots on glasshouse bench. Blue lines represent irrigation tape

Planting date: The seeds were planted on 5th February 2014. The seedlings were transplanted and treatments started on Day 7, 11th February 2014.

Experiment duration: Plants were harvested from each treatment when more than 50% of the plants were a minimum of 25 mm 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 18.2°C with a range of 3.9°C to 35.0°C. The mean relative humidity was 63.9% ranging between a minimum of 31.3% and a maximum of 98.8%.

Drop test: During harvest, postharvest splitting susceptibility was tested using impact texture analysis. The number of radishes which split as a result of dropping was recorded for each tray.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

If data was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA. Where data was not normally distributed with or without transformation the non-parametric Friedman's test was used. When a P value of less than 0.05 was observed a Tukey test was used for parametric data and Mann-U Whitney test was used for non-parametric data to determine which results were different from each other.

Skeleton ANOVA:

Table 3-41 Skeleton ANOVA for growth splits, drop splits, hypocotyl width, hypocotyl length, fresh hypocotyl weight, hypocotyl water content, number of leaves, leaf area, fresh leaf weight and leaf water content

Source of variation	df
Block	18
Treatment	1
Residual	18
Total	37

3.5.2.2.2.3 EXPERIMENT 3.6: RESULTS

VWC: The different irrigation methods successfully created a difference in 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 3-42).

Table 3-42 Mean substrate VWC of the trays from the two treatments (Wet and Dry) during Experiment 3.6

Treatment	Mean VWC (%)	Maximum VWC (%)	Minimum VWC (%)
Wet	61.2	65.5	57.2
Dry	15.8	24.8	8.0

Splitting: VWC 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 the number of radishes which split during growth and post-harvest were significantly greater for radishes grown with the Wet Treatment (Table 3-43).

Table 3-43 Mean percentage of split radishes per tray (10 radishes) at harvest (growth split) and as a result of dropping from 1.4 m (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 differences in the hypocotyl width of radishes from the two treatments (Table 3-44), as radishes were harvested when the hypocotyls reached a commercial hypocotyl size rather than on a specific day this result was expected. The radishes grown with the Dry treatment were harvested five days later than the radishes which were grown with the Wet treatment. Having radishes of the same size for both treatments gives the advantage that any differences observed were not due to differences in the hypocotyl diameter of radishes.

The radishes grown with the Wet treatment were significantly (P<0.001) longer than the radishes grown with the Dry treatment and were less round as a result. The diameter to length ratio for radishes grown with the Wet treatment was 0.77 compared to 0.90 for the radishes grown with the Dry treatment. There was no significant difference (P=0.359) in hypocotyl fresh weight between the two treatments but the radishes grown with the Wet treatment had a greater hypocotyl water content at harvest (P<0.001) (Table 3-44).

Table 3-44 The mean hypocotyl diameter, length, fresh weight and water content for radishes grown under different irrigation treatments (Mean VWC Wet = 61.2%, Dry = 15.8%). Weight and water content are per tray containing 10 radishes (n=10).

Irrigation	**Hypocotyl diameter (W) (mm) (n=1)**	**Hypocotyl length (L) (mm) (n=1)**	**Hypocotyl fresh weight (g) (n=10)**	**Hypocotyl water content (%) (n=10)**
Wet	23.99	31.26	90.0	95.09
Dry	25.42	28.40	84.7	94.17
P	0.258	<0.001	0.359	<0.001
LSD (5%)	2.572	1.380	11.97	0.2727

Volumetric water content during growth had a significant effect on radish leaf growth. Despite being harvested five days earlier, the radishes which were grown with the Wet treatment 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 with the Dry treatment (Table 3-45).

Table 3-45 The mean leaf area, number, fresh weight and water content for radishes grown under different irrigation treatments. Weight and water content are per tray (n=10).

Irrigation	**Leaf area (n=1)**	**No. leaves (n=1)**	**Leaf fresh weight (g) (n=10)**	**Leaf water content (%) (n=10)**
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

3.5.2.2.2.4 EXPERIMENT 3.6: DISCUSSION

The first null hypothesis was rejected as growth rate was affected by VWC during growth. The radishes which were grown with the Dry treatment took five days longer to achieve commercial diameter size than radishes grown with the Wet treatment. 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).

In Experiment 3.5, leaf number was affected by VWC but the results were confusing. In this Experiment 3.6, leaf growth was reduced in the radishes grown with the Dry treatment. At harvest when the radishes grown with the Dry treatment 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 with the Wet treatment. This results means the source to sink ratio of leaves to hypocotyl was affected by VWC during growth. 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 second null hypothesis was rejected as VWC during growth had a significant effect on splitting both during growth and post-harvest. The amount of splitting observed at harvest and postharvest splitting susceptibility were both lower in radishes grown with the Dry treatment despite the radishes being grown for an additional five days allowing a greater amount of time for splitting to occur. The reduction in splitting may have been due to a reduction in pressure within the hypocotyl. The radishes grown under the Dry treatment 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, turgor pressure would need to be measured determine if this theory is correct. Differences in splitting susceptibility may have also been a result of difference in growth rate where 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 growth and in postharvest splitting susceptibility

could 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 also 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 with the Dry treatment as there was no significant difference in fresh weight between the two treatment groups but there was significantly less splitting observed in radishes grown with the Dry treatment. In addition, the radishes grown with the Dry treatment were rounder and potentially more attractive to the consumer. The mean width to length ratio for the radishes grown with the Dry treatment was 0.90 compared to 0.77 for the radishes grown with the Wet treatment.

In conclusion, VWC during growth has an effect growth rate and physiology of radish plants and also on growth and harvest spits.

3.5.2.2.3 Experiment 3.7: The effects of VWC on the susceptibility to growth splits of three cultivars of radish

3.5.2.2.3.1 Experiment 3.7: Introduction

In Experiment 3.6, VWC was shown to have a significant effect on growth splits and post-harvest splitting susceptibility in the cultivar 'Rudi'. In this experiment, the responses of three cultivars were studied to determine if the effects of VWC are dependent on cultivar. If there is a genotype x environment interaction for the effects of VWC on splitting it may not be possible to make recommendations for the agronomy of radishes as a whole. If the effects are similar for several different cultivars recommendations for radishes in general may be made.

In Experiment 3.6 the radishes grown with a higher VWC split more and had a greater hypocotyl water content. It was postulated the greater susceptibility to splitting may have been as a result of greater turgor pressure. In this experiment this was investigated further by measuring the hypocotyl water pressure at Day 21 and at harvest.

Radishes were harvested at the same hypocotyl width to enable comparisons to be made at the same growth stage. In Experiment 3.6 it was observed VWC affected the rate of leaf growth, altering the source to sink ratio at harvest. The effects of VWC on the growth rate of leaves in comparison to the hypocotyls for different cultivars were further investigated in this experiment.

Aim:

- To determine if radish cultivars differ in splitting response to VWC
- To determine if growth rate and physiology of radish cultivars differ in response to VWC

Null hypotheses:

1. The effect of VWC on radish hypocotyl splitting is not consistent between different radish cultivars
2. No conclusions can be made about the effects of VWC as changes in growth rate and physiology in response to VWC during growth differ significantly between cultivars

3.5.2.2.3.2 EXPERIMENT 3.7: MATERIALS AND METHODS

Seedling establishment: Radish seeds were initially planted and grown for seven days in seed trays measuring 350 mm in length by 210 mm in diameter and 55 mm in depth. These trays were watered by bench capillary matting for five minutes three times a day giving a total of 17 mm day $^{-1}$. The seeds were planted on Day 1 (15th July 2014). The seedlings were transplanted and treatments started on Day 7 (21st July 2014).

Transplanting and start of treatments: 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. The trays which the plants were transplanted into were at the correct 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.

Treatments: Six treatments were studied (Table 3-46). Cultivar and irrigation were factors. Irrigation had two levels, Wet and Dry. Cultivar had three levels 'Rudi', 'Celesta' and 'Saxa 2'.

Table 3-46 Six treatment groups used for Experiment 3.7

		Irrigation	
		Wet	**Dry**
Cultivar	**Rudi**	Rudi Wet	Rudi Dry
	Celesta	Celesta Wet	Celesta Dry
	Saxa 2	Saxa 2 Wet	Saxa 2 Dry

Replication: Ten plus three extra dry pots for each cultivar for destructive harvests. Pots were arranged in a randomised block design (Figure 3-22) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block						
1	CD	RD	SW	CW	SD	RW
2	SD	SW	RW	RD	CW	CD
3	CD	SD	RW	CW	RD	SW
4	RW	RD	SD	SW	CW	CD
5	SD	SW	RW	CW	CD	RD
6	RD	CD	RW	SW	CW	SD
7	RW	RD	CD	CW	SW	SD
8	SW	CW	CD	RD	RW	SD
8	RD	RW	CD	CW	SW	SD
10	RW	SD	CD	CW	RD	SW

Figure 3-23 Randomised block design of pots on glasshouse bench. Blue lines represent irrigation tape.

Experiment duration: Treatments were harvested when more than 50% of plants were 25 mm in diameter or greater as 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 with the Wet treatment were harvested first on Day 27, followed by the Wet treatment for ‘Rudi’ 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 3-47).

Table 3-47 Days to harvest, the number of days taken from drilling for 50% of the radish plants of each cultivar grown with Wet or Dry treatment 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: The mean temperature during the experiment 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%.

VWC: For the Wet treatment for all cultivars, the compost in trays was maintained at high water content close to pot capacity using capillary irrigation. The Dry treatment for all cultivars was maintained by hand watering three times a week. Compensation was made for the increasing weight of the radishes by performing destructive harvests of each cultivar three times a week. The weight of each cultivar was measured separately to account for any difference in growth rate of cultivars.

The VWC of each pot was measured three times a week.

Hypocotyl pressure: On Day 21 and at harvest, the water potential (bar) of 1 radish hypocotyl per tray was measured.

Harvest: Plants were harvested when >50% of the plants were 25 mm (median commercial size).

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

As the experimental design consisted of two factors each with more than one level a two-way ANOVA was used for statistical analysis.

Skeleton ANOVA:

Table 3-48 Skeleton ANOVA for number of splits, hypocotyl pressure, hypocotyl water content, hypocotyl width, hypocotyl fresh weight, hypocotyl dry weight, leaf fresh weight and leaf dry weight at harvest

Source of variation	df
Block	9
Cultivar	2
Irrigation	1
Cultivar x Irrigation	2
Residual	45
Total	59

3.5.2.2.3.3 EXPERIMENT 3.7: RESULTS

VWC: The different irrigation methods created a difference in VWC between the Wet and Dry treatments and there was not a large difference in VWC between the three cultivars in each treatment. 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 3-49).

Table 3-49 Mean 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: VWC during growth had a significant effect on the number of radishes which split during growth ($P<0.001$). The number of splits was greater for radishes grown under wet conditions. Cultivar had no effect on splitting ($P=0.746$) and there was no interaction between cultivar and irrigation treatment ($P=0.118$) (Table 3-50).

Table 3-50 Mean number of split radishes (%) per tray of 10 plants 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		P	LSD
Wet	37.94	35.56	33.33	35.61	Cultivar	0.746	5.59
Dry	1.00	6.56	10.78	6.11	Irrigation	<0.001	6.85
Mean	19.47	21.06	22.60	20.86	Cultivar x Irigation	0.118	9.68

Hypocotyl: There was no difference in hypocotyl pressure between cultivars, irrigation treatments or interaction between the two during growth on Day 21 (Table 3-51) or at harvest (Table 3-52).

Table 3-51 Mean hypocotyl pressure (bar) on Day 21 for the two irrigation treatments (Wet and Dry) and the three cultivars (‘Rudi’, ‘Saxa 2’ and ‘Celesta’)

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	1.78	2.42	2.58	2.26	Cultivar	0.958	0.987
Dry	2.46	1.99	1.94	2.13	Irrigation	0.742	0.806
Mean	2.12	2.21	2.26	2.19	Cultivar x Irigation	0.353	1.396

Table 3-52 Mean hypocotyl pressure (bar) 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		P	LSD
Wet	1.373	1.758	1.396	1.509	Cultivar	0.611	0.3543
Dry	1.667	1.623	1.741	1.677	Irrigation	0.248	0.2893
Mean	1.520	1.691	1.569	1.593	Cultivar x Irigation	0.334	0.5011

There was no significant difference in diameter between cultivars, treatments or interaction between the two (Table 3-53). As radishes were harvested when the treatment reached a commercial hypocotyl harvest size rather than on a specific day this result was expected.

Table 3-53 Mean hypocotyl width (mm) 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		P	LSD
Wet	27.19	30.18	23.93	27.10	Cultivar	0.487	18.75
Dry	28.08	24.36	24.71	25.72	Irrigation	0.163	15.31
Mean	27.63	27.27	24.32	26.50	Cultivar x Irigation	0.417	26.52

The Wet treatment radishes of the cultivar ‘Rudi’ were harvested five days before the Dry treatment radishes of the same cultivar. For the cultivars ‘Saxa 2’ and ‘Celesta’, the period between the harvest of the radishes grown with the Wet and Dry treatments was seven days for both cultivars. As in previous experiments, the radishes in the Wet treatment grew the most rapidly and were harvested first.

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 3-54).

Table 3-54 Mean hypocotyl fresh weight (g) per tray of 10 plants for the two irrigation treatments (Wet and Dry) and the three cultivars (‘Rudi’, ‘Saxa 2’ and ‘Celesta’).

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	100.9	119.7	86.6	12.4	Cultivar	0.189	17.9
Dry	66.8	72.1	75.3	71.4	Irrigation	<0.001	14.3
Mean	83.9	95.9	80.9	86.9	Cultivar x Irigation	0.111	24.3

Hypocotyl dry weight was significantly ($P=0.042$) affected by irrigation treatment with the radishes which received more water generally having a greater dry weight. This result was not consistent for all cultivars. There was a significant interaction between cultivar and irrigation treatment. Dry treatment ‘Celesta’ had a greater dry weight compared to Wet

treatment ‘Celesta’ unlike the other two cultivars. Overall, there was no effect of cultivar on hypocotyl fresh weight (P=0.095) (Table 3-55).

Table 3-55 Mean hypocotyl dry weight (g) per tray of 10 plants for the two irrigation treatments (Wet and Dry) and the three cultivars (‘Rudi’, ‘Saxa 2’ and ‘Celesta’)

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	9.44	10.30	8.62	9.45	Cultivar	0.095	0.681
Dry	8.63	8.88	9.12	8.88	Irrigation	0.042	0.556
Mean	9.04	9.59	8.87	9.17	Cultivar x Irigation	0.012	0.963

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 3-56).

Table 3-56 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		P	LSD
Wet	89.86	90.57	89.80	90.08	Cultivar	0.594	0.660
Dry	86.84	87.40	87.68	87.31	Irrigation	<0.001	1.02
Mean	88.35	88.98	88.74	88.69	Cultivar x Irigation	0.660	1.76

Leaves: Number of leaves was not affected by irrigation treatment or cultivar (Table 3-57).

Table 3-57 Mean number of leaves for the two irrigation treatments (Wet and Dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	4.70	5.40	5.40	5.17	Cultivar	0.150	0.456
Dry	5.20	5.00	5.40	5.20	Irrigation	0.858	0.373
Mean	4.95	5.20	5.40	5.18	Cultivar x Irigation	0.150	0.645

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 3-58).

Table 3-58 Leaf area (cm^2) per plant for the two irrigation treatments (Wet and Dry) and the three cultivars ('Rudi', 'Saxa 2' and 'Celesta')

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	199.2	211.1	204.4	204.9	Cultivar	0.982	34.37
Dry	147.0	136.6	148.0	143.9	Irrigation	<0.001	28.06
Mean	173.1	173.9	176.2	174.4	Cultivar x Irigation	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 3-59).

Table 3-59 Plant 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		P	LSD
Wet	88.7	93.2	97.0	93.0	Cultivar	0.396	5.26
Dry	65.5	66.3	64.0	65.2	Irrigation	<0.001	4.30
Mean	77.1	79.7	80.5	79.1	Cultivar x Irigation	0.181	7.44

Leaf dry weight was significantly (P<0.001) affected by irrigation treatment with the radishes which received more water having a greater leaf dry weight. This result was consistent for all cultivars. There was no effect of cultivar on leaf fresh weight at harvest (P=0.379) (Table 3-60).

Table 3-60 Leaf dry weight (g) per plant for the two irrigation treatments (Wet and Dry) and the three cultivars (‘Rudi’, ‘Saxa 2’ and ‘Celesta’)

Treatment	Rudi	Saxa 2	Celesta	Mean		P	LSD
Wet	9.88	10.07	9.92	9.96	Cultivar	0.918	0.357
Dry	9.39	9.16	9.45	9.33	Irrigation	<0.001	0.291
Mean	9.64	9.62	9.69	9.65	Cultivar x Irigation	0.379	0.504

3.5.2.2.3.4 EXPERIMENT 3.7: DISCUSSION

The first null hypothesis was rejected as VWC affected splitting with radishes which were grown under dryer conditions splitting less. As the size of the radish hypocotyls was the same at harvest there was a greater marketable yield for radishes grown under dry conditions for all cultivars.

Less rapid growth rates were observed for all three cultivars of radishes grown under dryer conditions and the second null hypothesis was rejected. This is in keeping with previous research where it has been shown the usual response of plants to drought is to limit growth (Wilson 1988). As in Experiment 3.6 the cultivar 'Rudi' had a five day difference in harvest time for the two irrigation treatments. 'Celesta' and 'Saxa 2' were affected to a greater extent by the VWC treatments and had a seven day difference in harvest time between Wet and Dry treatments. Growth rate did not correlate with the differences in splitting as there were no differences in splitting between cultivars but there were differences in growth rate.

No significant differences were observed for hypocotyl pressure during growth or at harvest. This may have been because the equipment used was not sensitive enough to detect any differences. Due to the small and delicate nature of the radish petioles it was difficult to maintain a seal and it was also difficult to observe when the xylem sap began to be extruded. Therefore, these results are inconclusive.

No significant effect of cultivar was found for any of the variables measured other than hypocotyl dry weight. 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.

As in Experiment 3.6, leaf growth rate was shown to be affected by irrigation treatment. Leaf area, fresh weight and dry weight were all consistently greater for cultivars grown with a greater VWC compared to lower VWC despite these radishes being harvested after less time. Again, this shows the source to sink ratios are affected by VWC during growth. This is in keeping with other research where source to sink ratios have been shown to decrease under drought conditions (Wilson 1988).

In contradiction to results from Experiments 3.1 and 3.3, splitting rates for ‘Celesta’ were not significantly lower than the other two cultivars. In addition, ‘Celesta’ did not have as great a reduction in splitting when grown under dry conditions as the other two cultivars. ‘Celesta’ also had a greater dry biomass when grown with lower VWC whereas ‘Rudi’ and ‘Saxa 2’ had a greater dry biomass when grown with a greater VWC. These results would suggest factors other than VWC are having an effect on the physiology and rates of splitting in this cultivar.

3.5.2.3 Experiments 3.5-3.7: Discussion

In the three experiments exploring the effects of VWC on radish hypocotyl splitting in this section, VWC was shown to have an effect on splitting in two out of three of the investigations. In Experiment 3.6 and 3.7 which showed an effect of VWC on splitting, greater VWC was associated with a greater amount of splitting and this was consistent for all cultivars tested. In Experiment 3.5 the failure to find a relationship between VWC and splitting is thought to have been due to the small and over lapping differences in VWC. In the two experiments where differences were observed, these results are supported by previous research into splitting in the larger winter radishes where VWC was also shown to have a significant effect on splitting (Kang and Wan 2005). However, in the experiment by Kang and Wan, the differences in VWC had no significant effect on growth rate or yield. In Experiment 3.6 and 3.7, VWC was shown to have both an effect on growth rate and marketable yield. Radishes grown under drier conditions had a slower growth rate but had a greater marketable yield due to decreased numbers of split radishes at the same hypocotyl weight and size. The decreased growth rate observed in radishes is in common with usual plant responses to drought which are to limit growth (Wilson 1988). In cherries, the reduction in growth rate in response to drought has been shown to be as a result of cessation in cell division and elongation (Sekse 1995).

Differences in growth rate of leaves (source) and hypocotyls (sink) were observed under the different irrigation regimes in both Experiment 3.6 and 3.7. The usual response to limited water availability is for assimilates to be directed more towards the root than the leaves thus reducing the shoot to root ratio (Wilson 1988). Results from Experiments 3.6 and 3.7 conducted in this section suggest in the case of radishes, which have a swollen hypocotyl and tap root, it appears assimilates are directed to this organ under conditions of drought in a similar way to which they would be towards the taproots and roots in other plants.

No evidence for a relationship between hypocotyl pressure and splitting was observed in Experiment 3.7, no differences were observed between any of the cultivar or treatments

and there was no interaction between the two. However, the equipment was difficult to use with radish hypocotyls therefore the results are not conclusive.

3.5.2.4 Experiment 3.8: Investigating the effects of irrigation frequency on the susceptibility of radishes to growth splits

3.5.2.4.1 Experiment 3.8: Introduction

Another aspect of water availability which has been linked to splitting is irrigation frequency.

In the large winter varieties of radishes, fluctuations in soil water potential during growth has been shown to affect splitting (Wan & Kang 2005). Radishes irrigated once every three days had the lowest cracking rate and well developed hypocotyls when compared to radishes irrigated daily, once every two days, once every four days, once every six days and once every eight days. Frequent irrigation during growth resulted in high levels of splitting as did large fluctuations in soil water potential. Wan and Kang (2005) suggested splitting in the infrequently irrigated treatments may have been due to cyclic water stresses on the hypocotyl due to swelling and shrinking. Similarly, splitting in carrots (Gracie & Brown 2004) tomatoes and pepper (Dorais *et al.* 2004) has been shown to have a diurnal pattern with higher incidences of splitting early in the morning and at the end of the afternoon. This is thought to be due to swelling and shrinking which occur as a result of changes in water status in the crop at these times.

The aim of this experiment into the effects of irrigation frequency on splitting in the cultivar 'Rudi' was to determine if there are any trends in the relationship between irrigation frequency and splitting. If any trends were observed this experiment would form the basis of further investigation.

Aim:

- To investigate if different irrigation frequencies have an effect on radish hypocotyl splitting during growth or after storage
- To investigate if different irrigation frequencies have an effect on the physiology of radishes during growth or after storage

Null hypothesis:

1. Irrigation frequency will have no effect on radish hypocotyl splitting during growth or after storage.

2. Irrigation frequency will have no effect on radish growth or physiology

3.5.2.4.2 Experiment 3.8: Materials and Methods

Environmental conditions during growth and storage: In the glasshouse the mean temperature during the experiment was 18°C with a range of 35°C to 4°C. The mean relative humidity was 54% ranging between 93% and 15%. Temperature loggers were inserted in the growing substrate to the same depth in the 1st, 3rd and 5th pots for each treatment; 12 loggers in total. The mean temperature for G1 pots was 17.14°C, for G2 was 17.03°C, for G3 was 17.17°C and for G4 was 17.66°C.

The controlled environment cabinet achieved an average temperature of 2.8°C with a range between 5.2°C and -0.8 °C. The average relative humidity was 70.3% with a range between 100% and 12.6%.

Treatments and Replication: The four treatments were: G1 irrigated daily, G2 irrigated every two days, G3 irrigated every four days and G4 irrigated every eight days (Table 3-61). At irrigation the pots were watered by hand to the weight at pot capacity as calculated in a preliminary experiment.

Table 3-61 Summary of treatments used in Experiment 3.8

Treatment Number	Irrigation Frequency	Replication
G1	1 day	6 pots containing 6 plants each
G2	2 day	6 pots containing 6 plants each
G3	4 day	6 pots containing 6 plants each
G4	8 day	6 pots containing 6 plants each

Figure 3-24 Pots were surface irrigated on the weighing scales to the correct GWC

All pots were irrigated on the day of harvest (Table 3-62).

Table 3-62 Irrigation schedule for the different irrigation treatments used in Experiment 3.8. G1 was irrigated daily, G2 was irrigated every 2 days, G3 was irrigated every 4 days and G4 was irrigated every 8 days (n=6).

Date	**13/03/12**	**14/03/12**	**15/03/12**	**16/03/12**	**17/03/12**	**18/03/12**	**19/03/12**
Day	**-**	**-**	**-**	**-**	**-**	**-**	**-**
G1: 1 day	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G2: 2 days	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G3: 4 days	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G4: 8 days	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Date	**20/03/12**	**21/03/12**	**22/03/12**	**23/03/12**	**24/03/12**	**25/03/12**	**26/03/12**
Day	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
G1: 1 day	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G2: 2 days	Yes	No	Yes	No	Yes	No	Yes
G3: 4 days	Yes	No	No	No	No	Yes	Yes
G4: 8 days	Yes	No	No	No	No	No	No
Date	**27/03/12**	**28/03/12**	**29/03/12**	**30/03/12**	**31/03/12**	**01/04/12**	**02/04/12**
Day	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
G1: 1 day	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G2: 2 days	No	Yes	No	Yes	No	Yes	No
G3: 4 days	No	No	No	No	Yes	Yes	No
G4: 8 days	No	Yes	No	No	No	No	No
Date	**03/04/12**	**04/04/12**	**05/04/12**	**06/04/12**	**07/04/12**	**08/04/12**	**09/04/12**
Day	**Day 15**	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
G1: 1 day	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G2: 2 days	Yes	No	Yes	No	Yes	No	Yes
G3: 4 days	No	No	No	Yes	Yes	No	No
G4: 8 days	No	No	Yes	No	No	No	No
Date	**10/04/12**	**11/04/12**	**12/04/12**	**13/04/12**			

Day	Day 22	Day 23	Day 24	Day 25
G1: 1 day	Yes	Yes	Yes	Yes
G2: 2 days	No	Yes	No	Yes
G3: 4 days	No	No	Yes	Yes
G4: 8 days	No	No	No	Yes

There were six experimental and three destructive harvest pots for each treatment, giving nine pots per treatment, 36 pots in total containing a total of 216 radish plants, 144 of which were experimental plants. Pots were arranged in a randomised block design (Figure 3-25) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block				
1	G3	G2	G1	G4
2	G1	G4	G2	G3
3	G2	G1	G3	G4
4	G4	G2	G1	G3
5	G4	G3	G2	G1
6	G3	G4	G2	G1
7	G2	G4	G3	G1
8	G4	G2	G3	G1
9	G2	G4	G3	G1
10	G3	G2	G1	G4

Figure 3-25 Randomised block design of pots on glasshouse bench. Blue lines represent irrigation tape. G1 was irrigated daily, G2 was irrigated every 2 days, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Harvest: Stomatal conductance was measured on the oldest leaf of each plant with an AP4 porometer (Delta-T Devices Ltd., Cambridge, UK) before harvest. Preliminary experiments showed taking porometer readings from the oldest leaf gave the most consistent results.

All plant pots were watered to field capacity at least two hours prior to harvest. The median (i.e. third lightest) radish from each pot was dried at 105°C for 48 hours to calculate the dry biomass at harvest and the remaining five radishes from each pot were put into a labelled cryovac bag to simulate commercial packaging and moved to a controlled environment cabinet for seven days. The median radish was chosen to be dried because it was considered this would be representative of the pot; choosing the largest or the smallest radish would have risked selecting non-representative outliers. After seven days of storage the radish were weighed again and the number of splits counted. The water content was calculated post-storage by drying all radishes at 105°C for 48 hours.

Growth summary: Seeds were planted on 13.03.2012, treatments commenced on 20.03.2012 (Day 1), plants were harvested and moved to storage on 13.04.12 (Day 25) as this was a day when all treatments were watered. After harvest, the radish hypocotyls were placed in storage. Storage was terminated on 20.04.2012. Plants were grown for 31 days.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Using a two-tailed, unpaired Student's T-test assuming equal variance, a comparison was made between the VWC of the split and non-split radishes. Pot temperature data was not normally distributed (Shapiro-Wilk $P<0.001$) and there was an unequal number of readings for all pots as three of the loggers (G1P2, G3P3 and G4P2) stopped logging shortly before the end of the experiment. Therefore, the non-parametric Kruskal-Wallis One Way ANOVA test was used. For all other data, if it was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA (Table 3-63). Where data was not normally distributed the non-parametric Friedman's test was used. When a P value of less than 0.05 was observed a Tukey test was used for parametric

data and Mann-U Whitney test was used for non-parametric data to determine which results were different from each other. If a significant difference was found for a measurement between treatments the relationship between irrigation frequency and the measurement was investigated to see if a linear or quadratic curve best described the association.

Table 3-63 Method of analysis for different factors measured in Experiment 3.8. Method of analysis (parametric or non-parametric) was decided according to normal distribution as determined by the Shapiro-Wilk test

Measurement	Shapiro-Wilk	Analysis used
Substrate temperature	<0.001	Kruskal-Wallis One Way ANOVA
Splits at harvest	<0.001	Friedman's test
Splits after storage	<0.001	Friedman's test
Stomatal conductance at harvest	0.005	Friedman's test and Mann-U Whitney test
Number of leaves at harvest	0.032	Friedman's test and Mann-U Whitney test
Hypocotyl width at harvest	0.052	ANOVA
Hypocotyl width after storage	0.285	ANOVA
Hypocotyl length at harvest	0.066	ANOVA
Hypocotyl length after storage	0.907	ANOVA
Plant weight at harvest	0.634	ANOVA and Tukey test
Hypocotyl weight at harvest	0.840	ANOVA and Tukey test
Hypocotyl weight after storage	0.773	ANOVA and Tukey test
Hypocotyl dry weight at harvest	0.525	ANOVA
Hypocotyl dry weight after storage	0.020	Friedman's test
Hypocotyl water content at harvest	0.964	ANOVA and Tukey test
Hypocotyl water content after storage	0.027	Friedman's test and Mann-U Whitney test

Skeleton AVOVA:

Table 3-64 Skeleton ANOVA for the number of split radishes at harvest and after storage, stomatal conductance, number of leaves at harvest, hypocotyl width, hypocotyl length, plant fresh weight, hypocotyl fresh weight, hypocotyl weight after storage, hypocotyl dry weight and hypocotyl water content

Source of variation	df
Treatment	3
Blocks	5
Residual	15
Total	23

3.5.2.4.3 Experiment 3.8: Results

Substrate temperature: Kruskal-Wallis One Way ANOVA gave a chi-squared value of 0.033. There were 3 degrees of freedom and the critical value at 5% level was 7.82 showing there was no significant difference in temperature at the 5% level between treatments. As differences in irrigation frequency in this experiment did not result in any statistically significant differences in substrate temperature any differences observed in the radish plants were not due to a difference in the temperature of the growing medium.

VWC: Fluctuations in substrate moisture of different magnitudes were created by different irrigation frequencies. Figure 3-26 shows the average daily VWC calculated from the GWC for each treatment group. It should be noted that VWC is impossible to determine exactly in a non-destructive way with current technology and therefore, this graph is only a representation of the soil water content for the whole pot. The exact values will be a range around each point and will not be homogenous throughout the pot. As a result of water being added to the surface during irrigation one would expect the fluctuations in VWC to be greatest at the surface of the compost mix around the radish hypocotyl. Despite this a clear pattern can be seen; G1 which was irrigated daily shows steady water content only fluctuating slightly around field capacity whereas G4 which was irrigated every eight days shows large changes in water content. The peaks and troughs for G2, G3 and G4 increase as the experiment progresses due to increasing plant size within the pots and increased transpiration and water consumption.

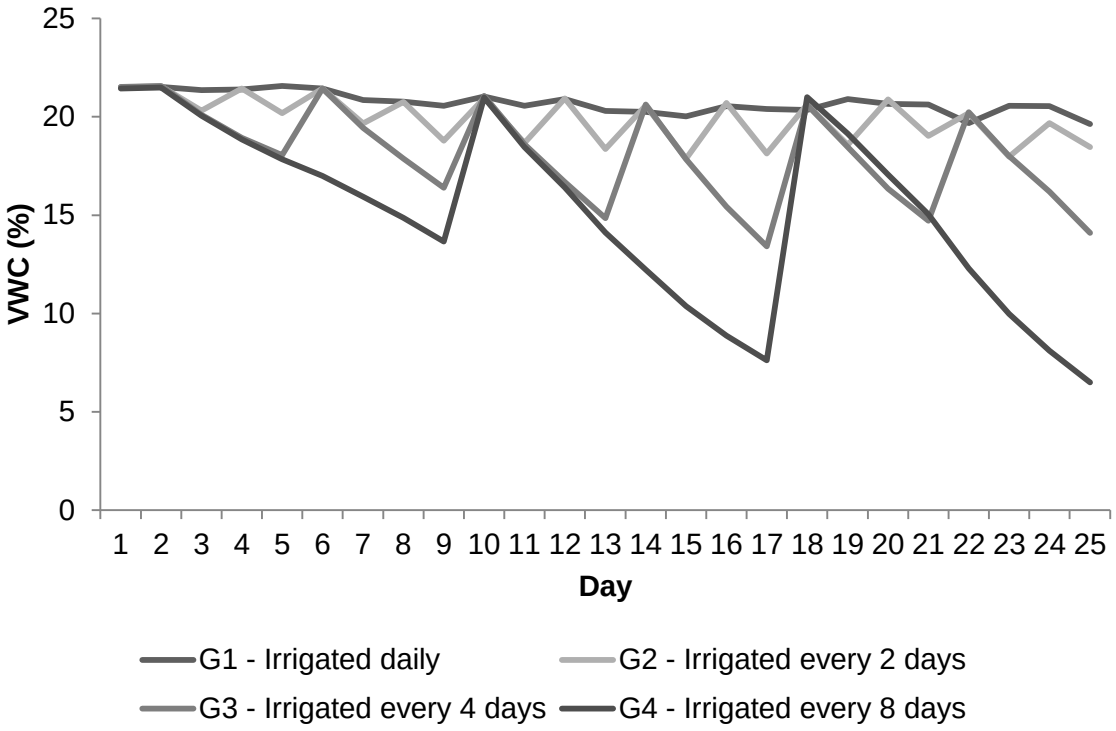


Figure 3-26 Mean substrate VWC for pots irrigated at different frequencies (n=6) calculated from the GWC using bulk density. Reading on Day 25 taken prior to irrigation to pot capacity.

Table 3-65 Substrate VWC calculated from the GWC for pots in each treatment group. Each treatment group was irrigated at a different frequency, G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Irrigation frequency	Max VWC (%)	Min VWC (%)	Mean VWC (%)
G1: 1 day	21.6	19.6	20.7
G2: 2 day	21.5	17.9	19.9
G3: 4 day	21.6	13.4	18.1
G4: 8 day	21.5	6.5	15.2

As would be predicted, a strong linear relationship between mean VWC and irrigation frequency was observed (R^2=0.96) (Figure 3-27).

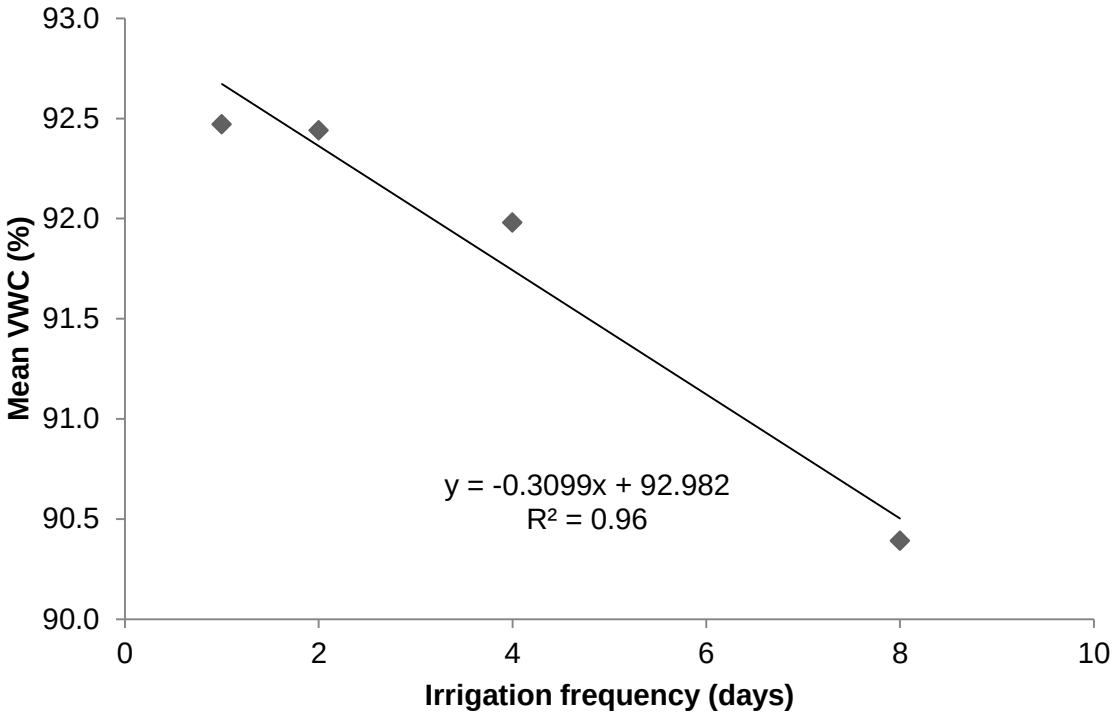


Figure 3-27 Negative correlation between mean substrate VWC, calculated from GWC and irrigation frequency. There were 4 treatment groups, G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Effect of treatments on splitting: There was no significant difference in the number of split radishes between treatment groups at harvest (P=0.912) (Figure 3-28).

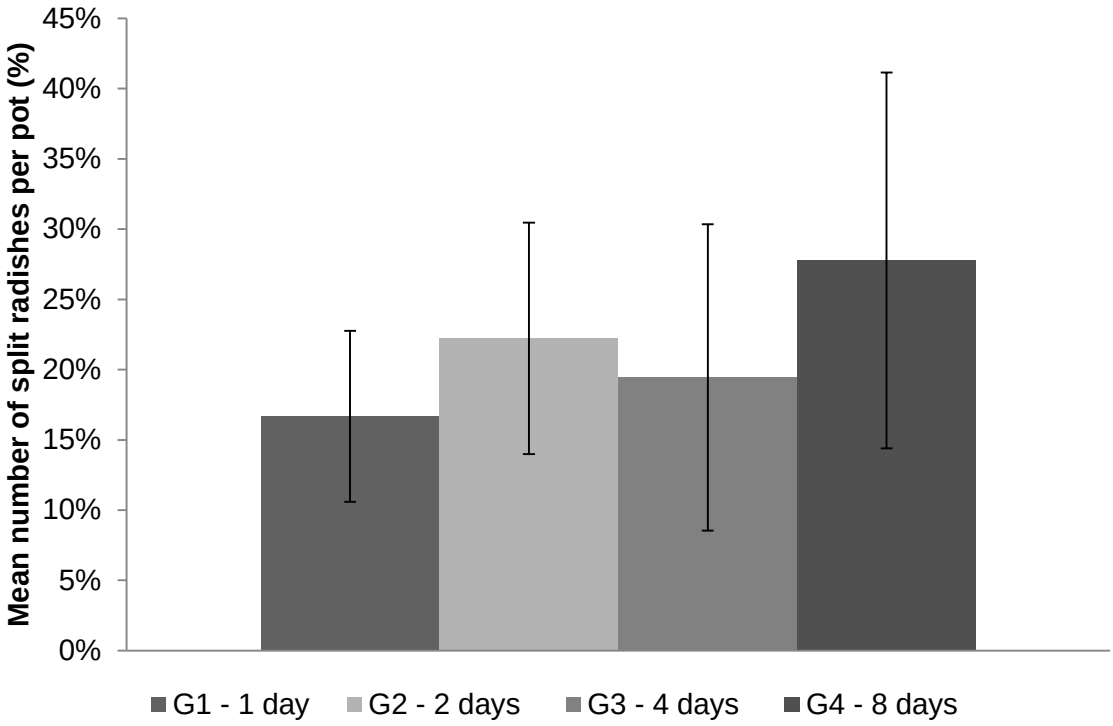


Figure 3-28 The mean percentage of split radishes per pot of 6 radishes at harvest (n=6) for different irrigation frequencies. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days. Bars represent standard error for each treatment.

There was only one additional split radish after storage which was in G2. There was no significant difference in the number of split radishes post-harvest (P=0.764) (Figure 3-29). The mean numbers of split radish post storage were slightly lower after storage than at harvest because one radish was removed from each pot at harvest to calculate the bulk density at harvest and not put into controlled environment storage.

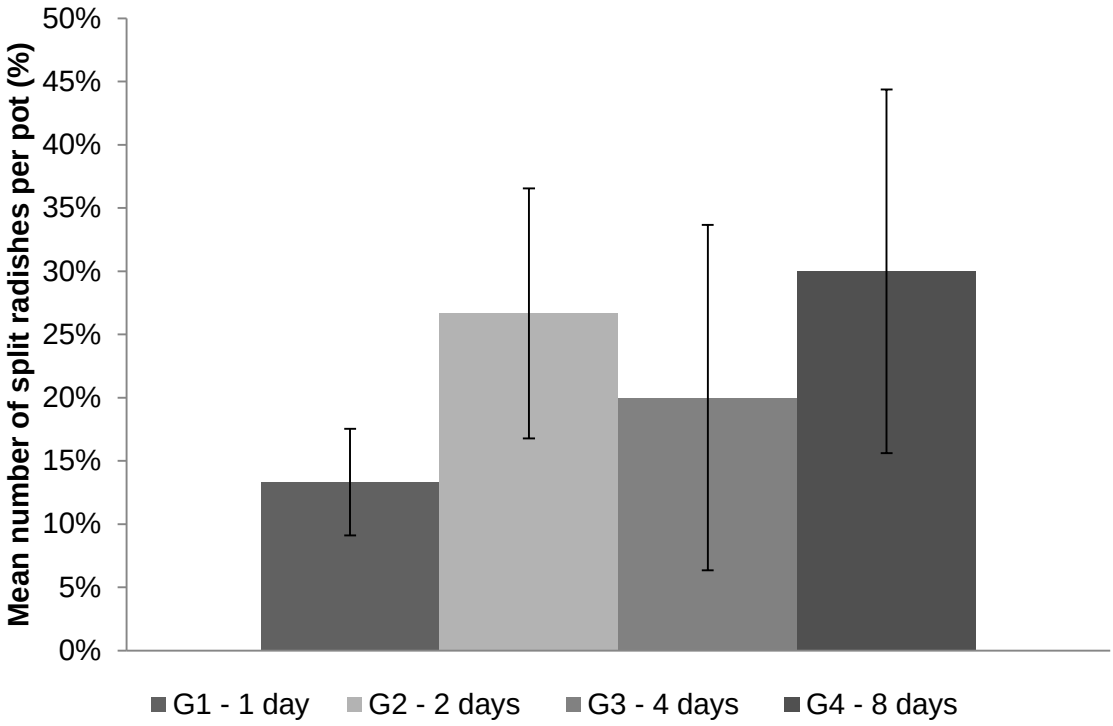


Figure 3-29 The mean percentage of split radishes per pot of 6 radishes for each irrigation treatment after seven days of cold storage (n=6). G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days. Bars represent standard error for each treatment

Although the results for splitting were not significantly different for the different treatment groups, there was a trend in the data both at harvest and after storage; greater fluctuations in soil moisture content were associated with a greater number of splits. G1, which was watered the most frequently and therefore experienced the smallest fluctuation in soil moisture, had the least number of splits whereas G4, which experienced the largest fluctuation in soil moisture, had the largest number of splits. In G2 and G3, which had smaller fluctuations in soil moisture than G4 but larger fluctuations than G1, the average number of split radish per pot was between the average G1 and G4 values. Not all treatments fit this pattern, G3 had larger fluctuations in soil moisture than G2 yet there were fewer split radishes in G3 than G2 both at harvest and after storage.

Stomatal conductance: Irrigating radish plants more frequently significantly (P=0.008) increased stomatal conductance by 450.89% when comparing plants irrigated daily with

plants irrigated every eight days (Table 3-66). Stomatal conductance for daily irrigation was significantly different to stomatal conductance in plants irrigated every eight days.

Table 3-66 The effect of irrigation frequency on stomatal conductance in radish leaves. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	**Mean stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)**
G1: 1 day	124.67a[1]
G2: 2 days	75.83ab
G3: 4 days	57.00ab
G4: 8 days	27.65b
P	0.008

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Number of leaves at harvest: Irrigating radish plants at different frequencies had a significant effect (P=0.032) on leaf number. Plants irrigated daily had 10.75% more leaves than plants irrigated every eight days.

Table 3-67 Effect of irrigation frequency on the number of leaves a radish plant has at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Mean number of leaves
G1: 1 day	6.58
G2: 2 days	5.99
G3: 4 days	5.98
G4: 8 days	5.94
SEM (15 df)	0.157
CV (%)	6.3
P	0.032

Hypocotyl width: Irrigation frequency did not significantly (P=0.509) effect radish hypocotyl width at harvest (Table 3-68).

Table 3-68 Effect of irrigation frequency on maximum hypocotyl width at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Hypocotyl width (mm)
G1: 1 day	28.89
G2: 2 days	28.80
G3: 4 days	26.14
G4: 8 days	27.78
SEM (15 df)	1.423
CV (%)	12.5
P	0.509

Irrigation frequency did not significantly (P=0.185) effect maximum hypocotyl width after seven days of cold storage (Table 3-69).

Table 3-69 Effect of irrigation frequency on maximum hypocotyl width after seven days of cold storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Hypocotyl width (mm)
G1: 1 day	28.08
G2: 2 days	28.09
G3: 4 days	25.67
G4: 8 days	26.37
SEM (15 df)	0.907
CV (%)	8.2
P	0.185

Hypocotyl length: Irrigation frequency did not significantly affect radish hypocotyl length at harvest (P=0.867) or after seven days of cold storage (P=0.579) (Table 3-70).

Table 3-70 Effect of irrigation frequency on hypocotyl length of radishes grown for 32 days at harvest and after seven days of cold storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Harvest hypocotyl length (mm)	Storage hypocotyl length (mm)
G1: 1 day	24.39	22.34
G2: 2 days	23.08	22.64
G3: 4 days	23.30	22.37
G4: 8 days	23.85	21.35
SEM (15 DF)	1.201	1.078
CV (%)	12.4	8.4
P	0.867	0.653

Hypocotyl fresh weight: Irrigating radish plants more frequently significantly (P=0.004) increased whole harvest weight by 13.05% when comparing plants irrigated daily with

plants irrigated every eight days. The linear trend between irrigation frequency and whole harvest weight was significant (P<0.001) but the quadratic trend was not (P=0.700) (Table 3-72) indicating a linear relationship between irrigation frequency and total harvest weight (Figure 3-30).

Table 3-71 Effect of irrigation frequency on trimmed (leaves and roots removed) harvest weight of radish plants. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Irrigation frequency	**Total weight (g)**	**Hypocotyl weight (g)**
G1: 1 day	21.96b[1]	14.81b
G2: 2 day	19.91ab	14.15ab
G3: 4 day	16.71a	11.74ab
G4: 8 day	15.54a	10.47a
SEM (15 df)	1.126	1.010
CV (%)	14.9	19.3
P	0.004	0.027

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Table 3-72 Effect of irrigation frequency on harvest whole weight of radish plants: trend data. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Source of variation	df	P
Treatment	3	0.004
Linear	1	<0.001
Quadratic	1	0.700
Deviations	1	0.538
Residual	15	
Total	23	

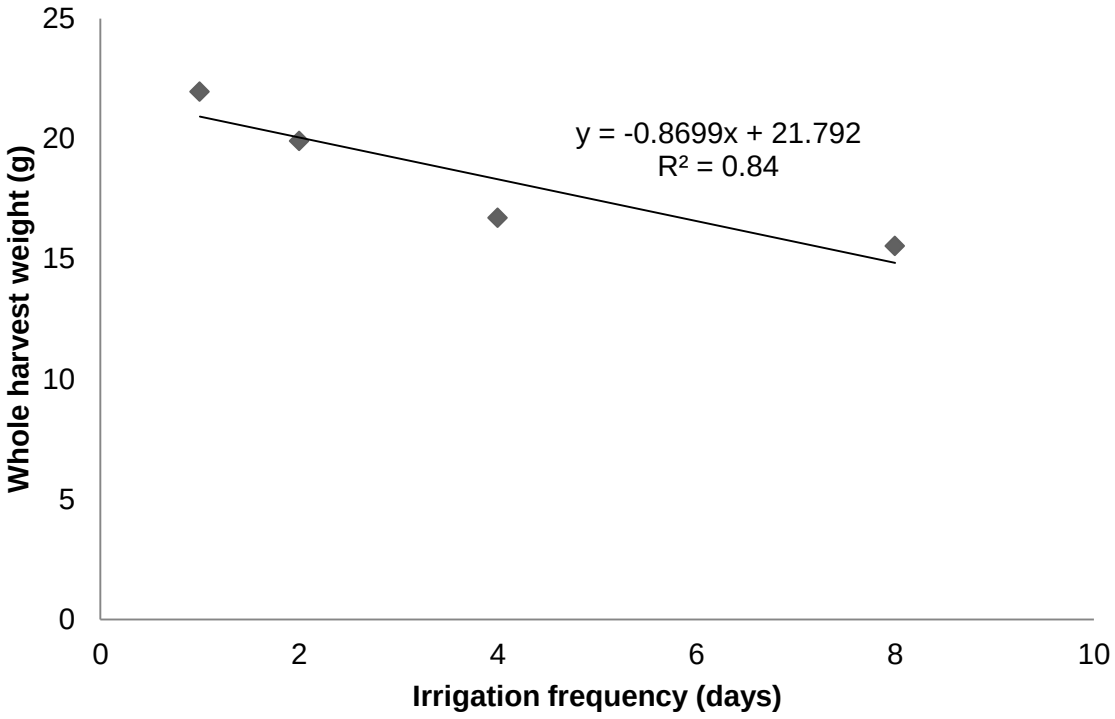


Figure 3-30 Linear relationship between irrigation frequency and whole weight of radishes at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Irrigating radish plants more frequently significantly (P=0.027) increased hypocotyl harvest weight by 41.5% when comparing plants irrigated daily with plants irrigated every eight

days. The linear trend was significant (P=0.004) but the quadratic trend was not (Table 3-73) indicating a linear relationship between irrigation frequency and hypocotyl weight at harvest (Figure 3-31).

Table 3-73 Effect of irrigation frequency on harvest hypocotyl weight of radish plants: trend data.

Source of variation	df	P
Irrigation frequency	3	0.027
Linear	1	0.004
Quadratic	1	0.765
Deviations	1	0.531
Residual	15	
Total	23	

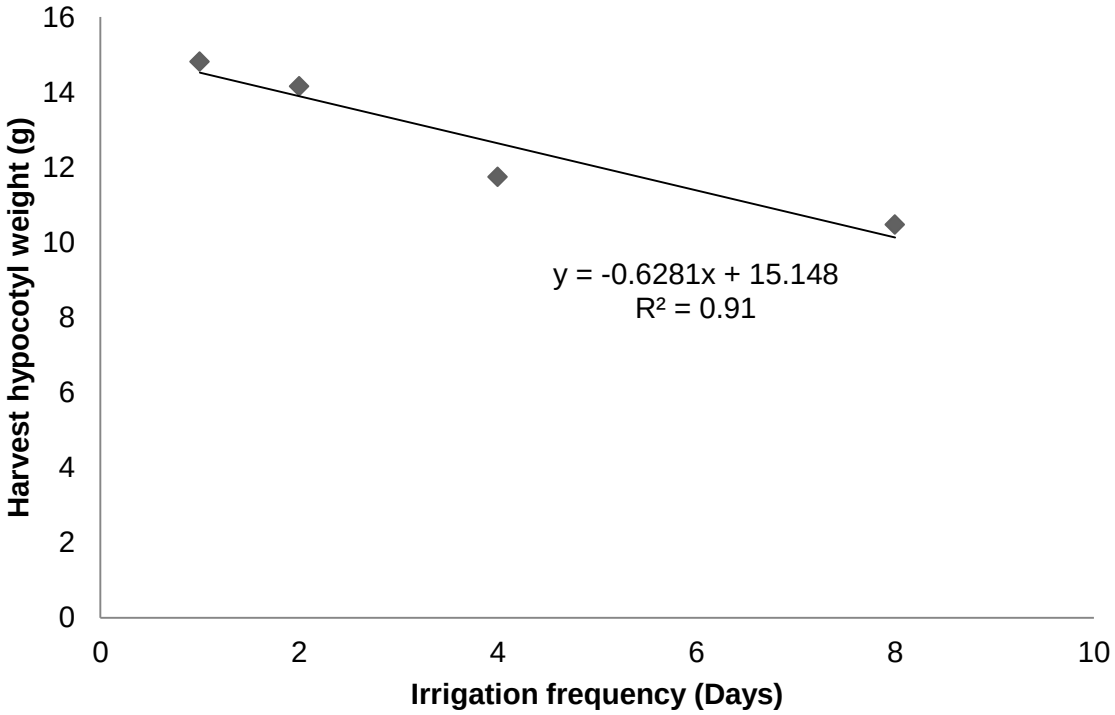


Figure 3-31 Linear relationship between irrigation frequency and hypocotyl weight of radishes at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Hypocotyl weight after storage: Irrigating radish plants more frequently significantly (P=0.026) affected radish weight after seven days of cold storage. Radish irrigated daily were 45.8% heavier than radish irrigated every eight days. The linear trend was significant (P=0.004) but the quadratic trend was not (P=0.988) (Table 3-75) suggesting a linear relationship between irrigation frequency and radish weight (Figure 3-32).

Table 3-74 Effect of irrigation frequency on radish hypocotyl weight after seven days of cold storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Weight (g)
G1: 1 day	12.82b[1]
G2: 2 days	12.12ab
G3: 4 days	10.05ab
G4: 8 days	9.32a
SEM (15 df)	0.821
CV (%)	18.2
P	0.026

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Table 3-75 Effect of irrigation frequency on radish weight after seven days of cold storage: trend data.

Source of variation	df	P
Irrigation frequency	3	0.026
Linear	1	0.004
Quadratic	1	0.988
Deviations	1	0.470
Residual	15	
Total	23	

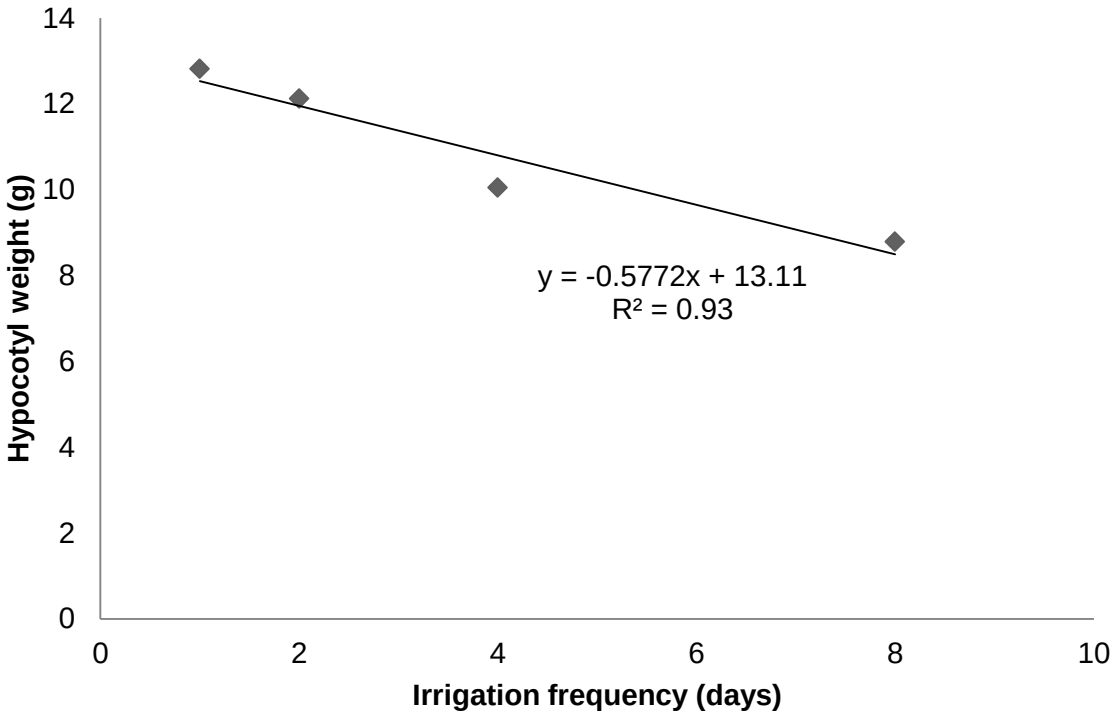


Figure 3-32 Linear relationship between irrigation frequency and radish weight after seven days of storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Hypocotyl dry weight: Irrigating radish plants more frequently did not significantly (P=0.974) affect radish dry weight at harvest (Table 3-76).

Table 3-76 Effect of irrigation frequency on radish dry weight at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Weight (g)
G1: 1 day	0.918
G2: 2 days	0.959
G3: 4 days	0.967
G4: 8 days	0.930
SEM (15 df)	0.0841
CV (%)	21.8
P	0.974

Irrigating radish plants more frequently did not significantly (P=0.615) effect radish dry weight after seven days of cold storage (Table 3-77).

Table 3-77 Effect of irrigation frequency on radish dry weight after seven days of cold storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Mean Weight (g)
G1: 1 day	2.00
G2: 2 days	1.94
G3: 4 days	1.88
G4: 8 days	1.88
P	0.615

Hypocotyl water content: Irrigating radish plants at different frequencies significantly affected (P=0.017) the water content of the radish hypocotyl at harvest of plants irrigated with different irrigation frequencies (Table 3-78). Plants irrigated daily had 2.19% more water than plants irrigated every eight days.

Table 3-78 Effect of irrigation frequency on radish hypocotyl water content at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Water content (%)
G1: 1 day	92.55a[1]
G2: 2 days	92.27ab
G3: 4 days	90.81ab
G4: 8 days	90.57b
SEM (15 DF)	0.467
CV (%)	1.3
P	0.017

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

The linear (P=0.003) trend between irrigation frequency and hypocotyl water content was significant but the quadratic trend was not significant (P=0.966) (Table 3-79) indicating a linear relationship between irrigation frequency and radish water content at harvest (Figure 3-33).

Table 3-79 Effect of irrigation frequency on radish hypocotyl water content at harvest: trend data

Source of variation	df	P
Irrigation frequency	3	0.017
Linear	1	0.003
Quadratic	1	0.966
Deviations	1	0.269
Residual	15	
Total	23	

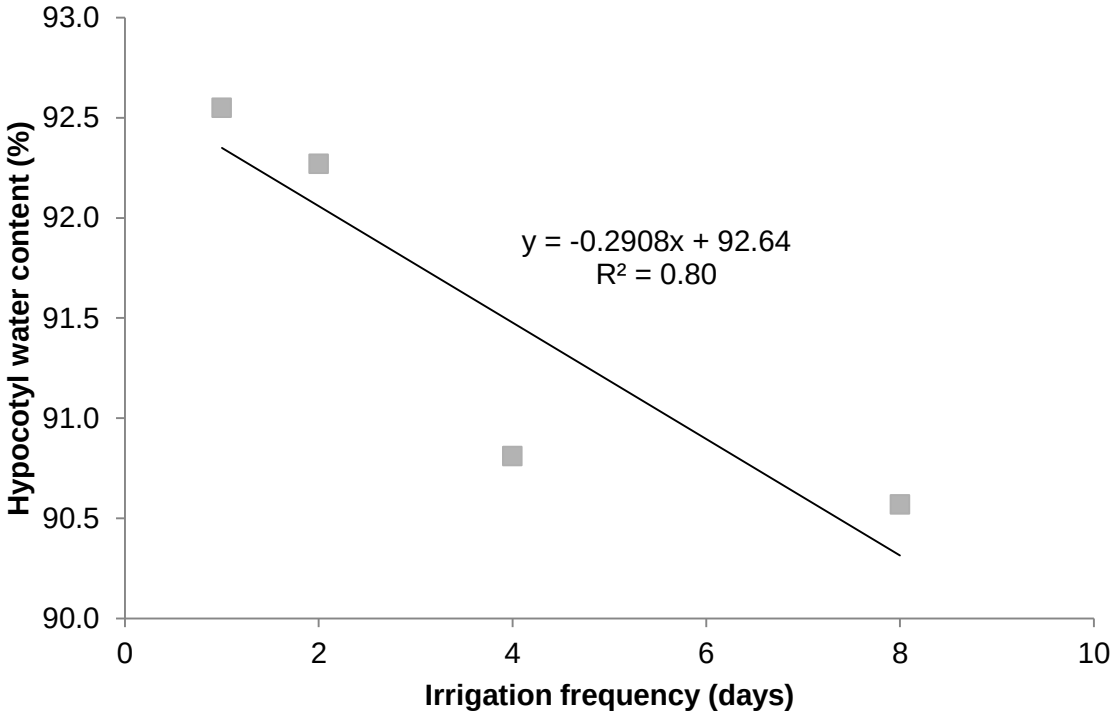


Figure 3-33 Effect of irrigation frequency on hypocotyl water content of radishes at harvest. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Irrigating radish plants at different frequencies significantly affected (P=0.02) the water content of the radish hypocotyl after seven days of cold storage (Table 3-80). Plants irrigated daily contained 2.25% more water than plants irrigated every eight days. Therefore, daily irrigation results in radish hypocotyls with higher water contents after seven days of storage than plants irrigated every eight days. Between groups there was no significant difference between plants irrigated every day with plants irrigated every two days and plants irrigated every four days. There was a significant difference between plants irrigated every eight days and plants irrigated daily.

Table 3-80 Effect of irrigation frequency on radish hypocotyl water content after seven days of cold storage. G1 was irrigated daily, G2 was irrigated every other day, G3 was irrigated every 4 days and G4 was irrigated every 8 days.

Treatment	Mean hypocotyl water content (%)
G1: 1 day	92.47b
G2: 2 days	92.44ab
G3: 4 days	91.98ab
G4: 8 days	90.39a
P	0.020

There was no correlation between the mean hypocotyl water content of each treatment and the mean number of growth splits for each treatment (P=0.478) or the mean number of total splits after storage for each treatment (P=0.613).

Mean substrate VWC: A significant difference (P=0.046) was observed in the average VWC of the pots of split and non-split radish (Figure 3-34). Pots containing radish which split had higher average VWC than pots containing radish which did not split.

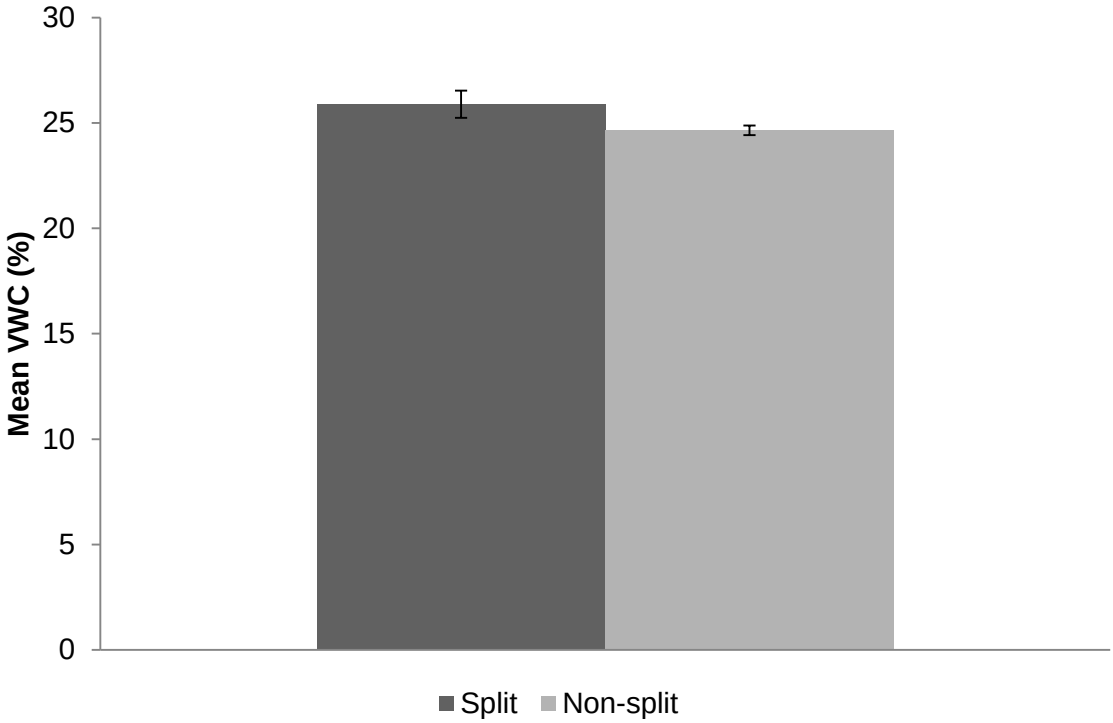


Figure 3-34 The Mean (± SE) VWC of the sand and compost mix in pots of split and non-split radish P=0.046. For non-split radish: n=113. For split radish: n=31.

3.5.2.4.4 Experiment 3.8: Discussion

Irrigation frequency did not affect rates of growth splitting (P=0.912) therefore, the first null hypothesis was supported.

A significant difference in the average pot VWC (P=0.046) of the plants which split and those which did not split was observed. Higher average water content was found in the pots with radish which split which is similar to results in previous experiments within this chapter where high VWC has also been correlated with high levels of splitting.

The second null hypothesis was rejected as the irrigation frequency treatments affected the way the radishes grew as the growth rate was affected. Radishes which were most frequently irrigated grew the greatest and the radishes which were irrigated least frequently grew least in terms of total weight, number of leaves, trimmed hypocotyl weight, hypocotyl length and hypocotyl width. These results correlate with the work of Bokhtiar *et al.* (2001) who found Radish 'Tasaki Mula'; a long white tropical radish variety grew the most under their most frequent irrigation regime, where plants were watered to field capacity every 10 days and they grew the least under no irrigation. The smaller size of radish irrigated less frequently suggests lower turgor pressure due to water deficit. In support of this theory, there was a significant difference (P=0.020) in the RWC of the radish hypocotyls after storage. Radishes which were irrigated more frequently had greater RWC and radishes which were irrigated less frequently had a lower RWC. The usual response for plants grown with limited water availability is to limit growth (Wilson 1988). Turgor pressure is known to regulate both cell division and enlargement in plants generally (Kirkham *et al.* 1972) and specifically in radish (Joyce *et al.* 1983). Having a water deficit for a period of time would reduce turgor and therefore reduce growth during this period. The plants which were irrigated the least frequently would have had the longest periods of deficit and therefore the longest periods with reduced cellular expansion and division.

In conclusion it would appear irrigation frequency does not have a significant effect on splitting but does have an effect on the growth and development rate of radishes. It is thought this is due to differences in the mean VWC of the compost affecting the RWC and

turgor pressure of the radish hypocotyls. Additionally VWC had a significant effect on splitting with radishes which were exposed to a greater mean VWC splitting more.

3.5.3 Experiment 3.9-3.12: Investigating the effects of timing of changes in VWC on the susceptibility of radishes to growth splits

3.5.3.1 Experiment 3.9-3.12: Introduction

Splitting in carrots has been shown to be affected by crop maturity with splitting mainly occurring later in crop development (Gracie & Brown 2004). It is also known growth stage affects when radishes are able to split as Experiment 3.2 showed the hypocotyl periderm does not become exposed until Growth Stage 41. As it is the periderm which splits, no splitting can occur until after Growth Stage 41. In apples growth stage is also known to have an effect on splitting. Throughout early growth, stress appears to be influenced by fruit size. However, during later growth stress is more affected by weather with large strains often associated with periods of heavy rain (Skene 1980). Similarly, the growth stage of carrots is thought to affect susceptibility to growth splitting as a result of water availability (Salter & Goode 1967; Sørensen *et al.* 1997). Salter (1967) found rates of splitting increased in carrots which experienced low water availability during mid-growth and then rain prior to harvest. Sørensen *et al* (1997) found splitting increased if there was a period of drought stress early in growth but decreased if there was a period of drought during mid-growth. It is difficult to compare the results from these two experiments as they do not refer to standardised growth stages so it is not known how the timing of their treatments compare. There is the potential radishes may also be more susceptible to growth splits as a result of VWC at certain growth stages. It may therefore be possible to reduce splitting by altering the environmental conditions and minimising stress at these key times.

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 effects 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, or retard the rate of growth to an extent which would not be commercially viable.

In this section, a series of four experiments were conducted to investigate the effects of timing of water availability on splitting. The initial three experiments, Experiments 3.9, 3.10 and 3.11, investigated how periods of drying during early or late growth affected the amount of splitting which was observed at harvest, the final experiment, Experiment 3.12, investigated how this was related to duration of drying and the growth stage at which drying occurs.

3.5.3.2 Experiment 3.9: Preliminary experiment investigating the effects of timing of changes in VWC on the susceptibility of radishes to growth splits

3.5.3.2.1 Experiment 3.9: Introduction

As mentioned previously, there is evidence that timing of water availability affects splitting in other crops (Salter & Goode 1967; Sørensen *et al.* 1997). This experiment studied the relationship between timing of water availability and splitting by altering the available water content at different times during radish growth. The aim of this first experiment into the effects of irrigation timing on splitting in the cultivar 'Rudi' was to begin to determine a method for manipulating the irrigation timing and measuring the resulting available water content and the conditions the radish plants are exposed to. Trends in the relationship between the timing of different available water contents and splitting were also investigated. The results from this experiment will enable greater refinement in future experiments. As radishes grow rapidly it was decided just to focus on two times in development, early to mid-growth and late growth. If differences were observed, this would allow more refinement of treatment times in future experiments. All radishes were irrigated for the initial period of development to ensure even germination. This also mirrors commercial production as radishes are irrigated after drilling to prevent scab.

Aim: To determine if:

- Timing of changes in VWC have an effect on splitting in radishes
- Timing of changes in VWC have an effect on the growth rate and physiology of radishes

Null hypothesis:

1. Timing of changes in VWC will have no significant effect on hypocotyl splitting in radishes
2. Timing of changes in VWC will have no significant effect on the growth rate and physiology of radishes

3.5.3.2.2 Experiment 3.9: Materials and Methods

Radish were grown in 4.2 L pots containing a 1:1 mix of horticultural sand and John Innes No. 2 growing medium. Before mixing the sand was air dried on trays in the glasshouse to make the mix as homogeneous as possible.

Treatments: For the purposes of this experiment the radish plants were grown for five weeks. In Week 1 all pots were watered to field capacity to allow germination and establishment. Treatments began after the first week and ran for two weeks. Treatments were applied twice a week on Tuesday and Friday. Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment (Table 3-81). Under deficit irrigation, plants were irrigated with 25% of the water lost due to evapotranspiration since the pervious irrigation. All plants were watered to field capacity at the end of Week 3 which was the end of the first treatment period and before the second treatment period.

Table 3-81 Summary of treatments used in Experiment 3.9

Treatment Number	Irrigation		Replication
	Weeks 2 -3	Weeks 4 -5	
G1	FC	FC	6 pots containing 6 plants each
G2	FC	Deficit	6 pots containing 6 plants each
G3	Deficit	FC	6 pots containing 6 plants each
G4	Deficit	Deficit	6 pots containing 6 plants each

Replication: There were six experimental and two destructive harvest pots for each treatment, giving eight pots per treatment, 32 pots in total containing a total of 192 radish plants, 144 of which were used for analysis.

Pots were arranged in a randomised block design (Figure 3-35) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block				
1	G4	G3	G1	G2
2	G1	G4	G2	G3
3	G1	G3	G4	G2
4	G4	G3	G1	G2
5	G4	G1	G2	G3
6	G2	G1	G4	G3

Figure 3-35 Randomised block design of pots on glasshouse bench for Experiment 3.9. Blue lines represent irrigation tape. Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment

Growth summary: Seeds were planted on Day 0 (30.07.2012) treatments commenced on Day 7 (06.08.2012) plants were harvested and moved to storage on Day 35 (03.09.2012) storage was terminated after 14 days on 17.09.2012.

Measurements during growth: Pots were weighed five times a week during the experiment and the VWC was calculated from the GWC. Compensation for the increasing weight of the radish plants was made by performing destructive harvests of plants grown under the same conditions but not used for analysis.

Measurements during and after storage: The radishes were placed into a labelled cryovac bag to simulate commercial packaging and moved to a Sanyo Versatile Environmental Test Chamber Model: MLR-351H for 14 days. Relative humidity and temperature were logged in the growth cabinet using TGP 4500 TinyTag logger.

After storage, the radish hypocotyls were examined for splits and weighed. The radish hypocotyls were then placed in an oven at 105°C until they were a constant weight to enable a calculation of hypocotyl water content to be made.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

If data was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA (Table 3-82). Where data was not normally distributed with or without transformation the non-parametric Friedman's test was used. When a P value of less than 0.05 was observed a Tukey test was used for parametric data and Mann-U Whitney test was used for non-parametric data to determine which results were different from each other.

Table 3-82 Method of analysis for different factors measured in Experiment 3.9. Method of analysis (parametric or non-parametric) was decided according to normal distribution as determined by the Shapiro-Wilk test

Measurement	**Shapiro-Wilk**	**Analysis used**
Number of splits at harvest	P=0.001	Friedman's test
Number of leaves at harvest	P=0.234	ANOVA and Tukey test
Plant weight at harvest	P=0.005	Friedman's test and Mann-U Whitney test
Hypocotyl weight at harvest	P=0.018 Log_e P=0.050	ANOVA and Tukey test (on log_e transformed data)
Hypocotyl length at harvest	P=0.707	ANOVA and Tukey test
Hypocotyl width at harvest	P=0.320	ANOVA and Tukey test
Number of splits after storage	P=0.008	Friedman's test
Hypocotyl weight after storage	P<0.001	Friedman's test and Mann-U Whitney test
Hypocotyl dry biomass after storage	P=0.769	ANOVA and Tukey test
Hypocotyl water content after storage	P=0.031	Friedman's test and Mann-U Whitney test

Skeleton ANOVA:

Table 3-83 Skeleton ANOVA for splitting at harvest and after storage, the number of leaves at harvest, the plant weight at harvest, the hypocotyl length, width and weight at harvest and the hypocotyl weight, dry weight and water content after storage

Source of variation	df
Treatment	3
Blocks	5
Residual	15
Total	23

3.5.3.2.3 Experiment 3.9: Results

Environmental growth and storage conditions: In the glasshouse the mean temperature was 20.4°C with a range of 43.5°C to 7.6°C. The mean relative humidity was 68.1% ranging between 99.0% and 17.1%.

The growth cabinet achieved a mean temperature of 4.2°C with a range between 6.5°C and 3.5°C. It had a mean relative humidity of 94.8% with a range between 100% and 90.6%.

Effect of treatments on soil water content: Treatments successfully created a difference in the VWC. G1 and G2 had higher water content in Weeks 2 and 3 then G1 and G3 had the highest water contents in Weeks 4 and 5. In the second half of the experiment the VWC was slightly lower. This is thought to be due to increased plant size and increased rates of transpiration (Table 3-84).

Table 3-84 Range of VWC for each treatment group in the first and second treatment period. Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment

	Week 2-3			**Week 4-5**		
	Average VWC (%)	**Maximum VWC (%)**	**Minimum VWC (%)**	**Average VWC (%)**	**Maximum VWC (%)**	**Minimum VWC (%)**
G1: FC/FC	20.6	23.2	16.5	17.9	22.0	14.7
G2: FC/D	20.4	23.2	15.6	10.4	16.5	2.9
G3: D/FC	16.1	20.5	11.6	17.3	21.8	13.7
G4: D/D	15.8	20.5	10.2	11.6	17.8	3.3

Effects of treatments on splitting: There was no significant effect of irrigation timing on splitting in radish at harvest (P=0.240) or after storage (P=0.161). However, there was a trend with G3, which received deficit irrigation for the first two weeks and then irrigation to field capacity for the final two weeks, appearing to have less splitting on average per pot than the other treatments which were all similar in their rates of splitting on average per pot (Figure 3-36). There were three additional split radishes after storage; one in G2 and two in G4.

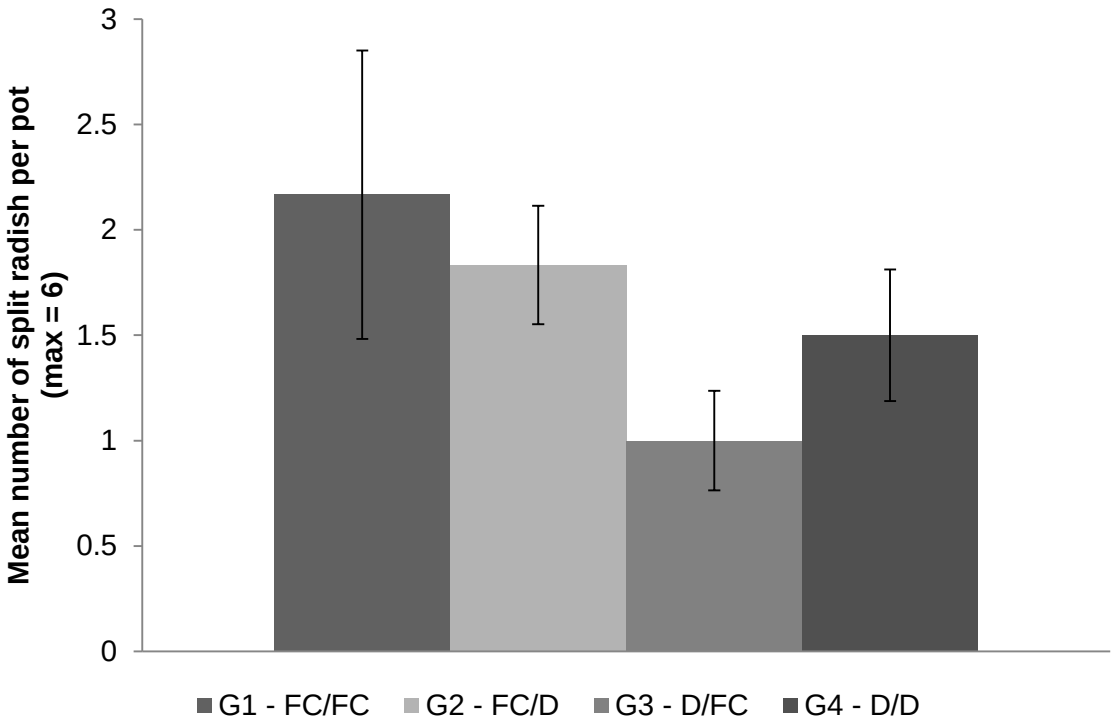


Figure 3-36 Mean (± SE) number of split radish per pot of 6 radishes at harvest (n=6). Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment

Effect of treatments on radish growth: Irrigation timing significantly affected radish growth in terms of number of leaves (P<0.001), hypocotyl width (P<0.001), hypocotyl

length ($P<0.001$), total plant weight ($P=0.002$) and trimmed hypocotyl weight ($P<0.001$) at harvest. Irrigation timing also significantly affected the yield in terms of hypocotyl weight after storage. The weight of the radish hypocotyl after storage was significantly different between treatments due to both significantly different hypocotyl water contents ($P=0.001$) between treatments and a significant difference in dry biomass ($P<0.001$) between treatments. The mean hypocotyl water content for split radishes (93.71%) was greater than the mean hypocotyl water content for non-split radishes (93.19%) although this difference was not statistically different ($P=0.897$). Plants which were irrigated to field capacity in the last two weeks had the highest yields in terms of weight and size compared to plants which had deficit irrigation in the last two weeks. Marketable yield was greatest in plants which were given deficit irrigation in the first two weeks and irrigation in the final two weeks as they had the large hypocotyls with few splits.

Table 3-85 Measurements (mean) taken at harvest for each treatment group (n=6). Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment

Treatment	**No. leaves**	**Hypocotyl width (mm)**	**Hypocotyl length (mm)**	**Plant weight (g)**	**Hypocotyl weight (g)**	**Split radishes (max = 6)**
G1: FC/FC[1]	6.00	30.98b[2]	30.93c	25.12b	15.87 (2.76)b	2.17
G2: FC/D	5.44	23.73a	26.74ab	13.54a	8.29 (2.11)a	1.83
G3: D/FC	6.44	30.08b	30.23bc	24.69b	14.72 (2.67)b	1.00
G4: D/D	5.64	21.90a	25.37a	13.23a	7.34 (1.99)a	1.50
P	<0.001	<0.001	<0.001	0.002	<0.001	0.240
LSD (5 %)	0.3956	2.591	2.633		2.383 (0.1742)	

[1]FC = Field Capacity, D= Deficit irrigation replacing 25% of water lost since previous irrigation

[2]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

Table 3-86 Measurements (mean) taken after storage for each treatment group (n=6). Treatment G1 was watered to field capacity throughout the experiment. Treatment G2 was watered to field capacity for the first half of the experiment then deficit irrigation was applied for the second half of the experiment. Deficit irrigation was applied to Treatment G3 for the first half of the experiment then watered to field capacity for the second half of the experiment. Treatment G4 received deficit irrigation throughout the experiment

Treatment	**Hypocotyl weight (g)**	**Dry biomass (g)**	**Water content (%)**	**Split radishes (max = 6)**
G1: FC/FC[1]	19.31b	0.763c	94.90b	2.17
G2: FC/D	7.45a	0.633ab	91.25a	2.00
G3: D/FC	13.66b	0.735bc	93.85b	1.00
G4: D/D	6.65a	0.529a	91.94a	1.83
P	0.002	<0.001	0.001	0.161
LSD		0.0865		

[1]FC = Field Capacity, D= Deficit irrigation replacing 25% of water lost since previous irrigation

[2]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

3.5.3.2.4 Experiment 3.9: Discussion

Experiment 3.9 supported the first null hypothesis that timing of changes in VWC will have no significant effect on splitting in radishes. However, there was an indication of a trend, with radish plants which were irrigated throughout the experiment having the largest number of split radishes and radish plants which were given an early period of deficit irrigation having the fewest split radishes. This is supported in part by the research of Sørensen (1997) who found timing of irrigation had a significant effect on splitting in carrots. Similar to the results from this experiment, Sørensen (1997) found high levels of splitting in carrots which were irrigated to high water contents throughout growth. Sørensen (1997) also found early periods of drought led to high levels of splitting; this was not tested in this experiment as all radishes were given irrigation in the first week to ensure there was even germination between treatments. The shorter growth period of radishes compared to carrots makes it more difficult to have distinctly different VWCs during early, mid and late growth. Again, similar to the results from this experiment, Sørensen (1997) also found a period of drought during mid-growth resulted in a low number of split carrots. Sørensen (1997) attributed the differences in the effects of water content at different growth stages to the different modes of enlargement which occur during different growth stages. During mid-growth when a period of drought stress reduces splitting, carrot growth is created by rapid radial root expansion caused by cell enlargement whereas during early growth, when drought leads to high levels of splitting, carrot growth is characterised by cell division. Similar differences may also be true for radishes. Further investigation with greater numbers of replicates and more refined methodology are required to determine if the trends observed in this experiment are statistically significant or not.

The second null hypothesis that timing of irrigation and water availability will have no significant effect on the growth rate and physiology of radish plants was rejected by the results from this experiment. Irrigation later in growth and in the case of this experiment, in the fourth and fifth week, appears to determine growth rate and consequently as the radishes were harvested at the same time, the width at harvest and after storage, weight

at harvest and after storage and the water content after storage. There was a significant difference between G1 and G3 when compared to G2 and G4 but no significant difference between G1 and G3 which were watered to field capacity in the last two weeks or G2 and G4 which had deficit irrigation in the last two weeks. In contradiction, total irrigation over the lifetime of the radish and timing of water availability appears to affect the hypocotyl length and the dry biomass. For these two factors, the hypocotyl length and dry biomass was greatest for G1 and least for G4 suggesting the total amount of irrigation was important. However, the second largest was G2 then followed by G3. As G2 received less water in total than G3 these results suggest it is timing of water availability in combination with total water availability which is important and radishes are more sensitive to drought later in growth. High water content throughout growth results in the most growth and high water content later in growth result in the second largest amount of growth in terms of hypocotyl length and dry biomass.

The effects of water content on leaf number are less easy to interpret and do not appear to be solely dependent on VWC as they do not follow a clear pattern in relation to mean water content. The plants which received a period of deficit irrigation followed by watering to field capacity had the largest number of leaves followed by the treatment which was irrigated to field capacity for the duration of the experiment. After this, the treatment which received deficit irrigation for the duration of the experiment had the second lowest number of leaves and finally the treatment which received irrigation to field capacity followed by deficit irrigation had the fewest leaves. Work on carrots (Hutmacher *et al.* 1990) has been shown both prolonged and sudden decreases in water availability result in reductions in leaf area, therefore it would be expected the treatment which was irrigated to field capacity for the duration of the experiment would have the greatest number of leaves and the treatment which received deficit irrigation for the duration of the experiment would have the fewest leaves. However, it would appear the radish plants which received a period of deficit irrigation followed by a period of irrigation were conditioned to grow under dry conditions and then were able to exceed the growth rate late deficit of the radish plants which received irrigation for the duration of the experiment. Similar responses to an

increase in water availability after a period of stress have been observed in *Nitella* cells, corn plants, sugar beets, tomato, alfalfa, barley, corn and the stems of pine seedlings (Kramer 1983), where growth is very rapid after the stress is alleviated. This is often termed stored or compensatory growth. It is thought metabolites are accumulated during the period of stress while cell enlargement is inhibited by lack of turgor. Once turgor is restored these metabolites are thought to then be available for rapid cell wall synthesis and other processes associated with growth.

Similarly, the radishes which were watered to field capacity and then received deficit irrigation had fewer leaves than the radishes which received deficit irrigation for the duration of the experiment despite receiving more water in total. Again it would appear the plants had been conditioned to grown under environments experienced early in growth and reacted more severely to the decrease in water compared to plants which had experienced it from early in growth. It is thought if water stress develops slowly, osmotic adjustment may occur (Kramer 1983). This enables growth to continue at lower water potentials than would otherwise be possible. Osmotic adjustment has been shown to occur in leaves of wheat plants (Kramer 1983) and may explain the results observed in this experiment.

There were several problems observed with the methodology used for this experiment which will need to be improved in future experiments. Firstly the addition of sand made it 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. Secondly, the depth of the pots was 183 mm. In Experiment 3.9, VWC was calculated from GWC for the whole pot. It became apparent 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. It is simpler and quicker to measure the VWC using a Theta Probe (Delta T Devices, Cambridge, UK). However, the length of the prongs is only 60 mm which would only give you the VWC for the surface of the pot. Decreasing the pot

depth would allow more accurate measurements of the conditions the radish plant was experiencing.

3.5.3.3 Experiment 3.10: Experiment investigating the effects of timing of changes in VWC on the susceptibility of radishes to growth splits

3.5.3.3.1 Experiment 3.10: Introduction

The design for Experiment 3.10 is a refinement of Experiment 3.9 in which a non-significant trend was observed suggesting high levels of splitting were associated with high VWC in the second and third weeks and that yield was determined by high VWC in the final two weeks prior to harvest. In Experiment 3.10 several improvements to the method which had been used in Experiment 3.9 were made. The number of replications was increased from six to 32 and the number of radishes in a pot was increased from six to ten plants. The substrate which the plants were grown in was changed from a 50:50 mix of sand and John Innes No. 2 to 100% John Innes No. 2. The depth of the pots was decreased from 183 to 60 mm which is the same length as the Theta Probe prongs (Delta T Devices, Cambridge, UK) which were used to measure the volumetric water content during the experiment.

Aim: To determine if:

- Timing of changes in VWC have an effect on splitting in radishes
- Timing of changes in VWC have an effect on the growth rate and physiology of radishes

Null hypothesis:

1. Timing of changes in VWC will have no significant effect on hypocotyl splitting in radishes
2. Timing of changes in VWC will have no significant effect on the growth rate and physiology of radishes

3.5.3.3.2 Experiment 3.10: Materials and Methods

Planting date: Seeds were planted on 19.02.2013, this was Day 1. Treatments commenced on Day 8 (26.02.2013).

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 29 (19.03.2013) and 30 (20.03.2013).

Treatments: The experimental period, which commenced after an initial seven day establishment period, was divided into two treatment periods. The first treatment period lasted for 11 days and the second treatment period lasted for 11 or 12 days depending on block. There were three treatment groups. The first group (W/W) received irrigation for the duration of the experiment, the second group (D/W) received no irrigation for the first treatment period and irrigation for the final treatment period, the third group (W/D) received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment (Table 3-87).

Table 3-87 Irrigation regimes for the three treatment groups used in Experiment 3.10

Treatment	Day 8 to 18	Day 19 to harvest
W/W	Irrigation	Irrigation
D/W	No Irrigation	Irrigation
W/D	Irrigation	No Irrigation

Replication: n = 34. Pots were arranged in a randomised block design (Figure 3-37) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block							Block
1	W/D	D/W	W/W	W/D	W/W	D/W	18
2	D/W	W/W	W/D	W/W	W/D	D/W	19
3	W/W	D/W	W/D	D/W	W/W	W/D	20
4	D/W	W/W	W/D	D/W	W/W	W/D	21
5	W/W	W/D	D/W	D/W	W/W	W/D	22
6	W/D	W/W	D/W	W/D	W/W	D/W	23
7	D/W	W/D	W/W	W/W	D/W	W/D	24
8	W/W	D/W	W/D	W/D	W/W	D/W	25
9	D/W	W/D	W/W	W/W	D/W	W/D	26
10	W/D	W/W	D/W	W/D	W/W	D/W	27
11	W/D	W/W	D/W	W/D	D/W	W/W	28
12	D/W	W/W	W/D	W/D	W/W	D/W	29
13	D/W	W/D	W/W	D/W	W/W	W/D	30
14	W/W	W/D	D/W	D/W	W/W	W/D	31
15	W/D	D/W	W/W	W/W	D/W	W/D	32
16	D/W	W/D	W/W	D/W	W/D	W/W	33
17	D/W	W/D	W/W	W/W	D/W	W/D	34

Figure 3-37 Randomised block design of pots on glasshouse bench for Experiment 3.10. Blue lines represent irrigation tape. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

VWC: VWC of all pots was measured three times a week. The maximum hypocotyl diameter was measured for each radish. Differences in VWC were analysed when the seeds were planted, at the start of treatments, at the end of the first treatment and mid-

way through the second treatment to ensure all treatments received the same conditions during establishment and to coincide with the other measurements taken during growth.

Measurements at the end of the first treatment period (Day 18): After the first treatment period and before treatments were changed the number of leaves in each pot was counted and the leaf temperature from one leaf per pot was measured. The leaves were too small to measure stomatal conductance. The hypocotyl width was measured of one plant in the same position from each pot; only the part of the hypocotyl which was visible above the soil surface was measured, the compost was not disturbed around the radish hypocotyl.

Measurements on Day 22: On Day 22 stomatal conductance and hypocotyl width was measured as described above.

Measurements on Day 24: On Day 24 the number of leaves, the leaf temperature, stomatal conductance and hypocotyl width were measured.

Measurements before harvest (Day 28): On Day 28 hypocotyl width, the number of leaves, leaf temperature and stomatal conductance were measured.

Measurements at harvest: Mean temperature in the glasshouse at harvest was 24.2°C on 19.03.2013 and 22.9°C on 20.03.2013). At harvest, the maximum hypocotyl width was measured not just the hypocotyl which was exposed above the surface of the compost as had been measured previously.

Marketable yield: At harvest, marketable yield was calculated. This is the weight of radishes which are of a commercial size and have no splits. Uniformity in radish diameter is desirable as supermarkets typically require radishes which are have widths between 18 mm and 32 mm, anything outside of this range is too small or too large for commercial sale.

Measurements after storage: The controlled environment cabinet achieved an average temperature of 4.3°C with a range between 3.9°C and 5.1°C. The average relative humidity was 98.6% with a range between 94.3% and 100%.

Plants were removed from storage after two days of storage on 21 and 22.03.2013 depending on which day they had been harvested.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Using a two-tailed, unpaired Student's T-test assuming equal variance, a comparison was made between the VWC at the end of the first treatment period for treatments W/W and W/D. there were 34 observations for each treatment and 66 degrees of freedom.

Using general linear regression the number of split radishes per tray for treatments W/W and D/W were correlated with the mean hypocotyl water content of the radishes in that tray.

For all other analysis there were three treatment groups therefore a T-test was not appropriate for analysis. If data was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA. Where data was not normally distributed with or without transformation the non-parametric Friedman's test was used (Table 3-88). When a P value of less than 0.05 was observed a Tukey test was used for parametric data and Mann-U Whitney test was used for non-parametric data to determine which results were different from each other.

Table 3-88 Method of analysis for different factors measured in Experiment 3.10. Method of analysis (parametric or non-parametric) was decided according to normal distribution as determined by the Shapiro-Wilk test

Measurement	Shapiro-Wilk	Analysis used
Seeds planted VWC	<0.001	Friedman's test
Day 9 VWC	<0.001	Friedman's test
Day 18 width	<0.001	Friedman's test
Day 18 leaf temperature	<0.001	Friedman's and Mann-U Whitney test
Day 18 number of leaves	<0.001	Friedman's and Mann-U Whitney test
Day 22 width	0.228	ANOVA and Tukey test
Day 22 leaf temperature	<0.001	Friedman's and Mann-U Whitney test
Day 22 stomatal conductance	0.153	ANOVA and Tukey test
Day 24 width	0.379	ANOVA and Tukey test
Day 24 leaf temperature	<0.001	Friedman's and Mann-U Whitney test
Day 24 stomatal conductance	<0.001	Friedman's and Mann-U Whitney test
Day 24 number of leaves	0.178	ANOVA and Tukey test
Day 25 VWC	<0.001	Friedman's and Mann-U Whitney test
Harvest splits	<0.001	Friedman's and Mann-U Whitney test
Harvest width	<0.001	Friedman's and Mann-U Whitney test
Harvest plant weight	<0.001	Friedman's and Mann-U Whitney test
Harvest hypocotyl weight	<0.001	Friedman's and Mann-U Whitney test
Harvest leaf weight	<0.001	Friedman's and Mann-U Whitney test
Harvest leaf area	<0.001	Friedman's and Mann-U Whitney test
Storage hypocotyl weight	<0.001	Friedman's and Mann-U Whitney test
Storage hypocotyl water content	<0.001	Friedman's and Mann-U Whitney test
Storage hypocotyl dry biomass	<0.001	Friedman's and Mann-U Whitney test

Skeleton ANOVA:

Table 3-89 Skeleton ANOVA for Day 22 stomatal conductance, Day 24 stomatal conductance, Day 24 width, Day 24 number of leaves

Source of variation	df
Block	16
Treatment	2
Residual	32
Total	50

Table 3-90 Skeleton ANOVA for number of split radishes, VWC, Day 18 width, Day 18 leaf temperature, Day 18 number of leaves, Day 22 Width, Day 22 leaf temperature, Day 24 leaf temperature, leaf area at harvest

Source of variation	df
Block	33
Treatment	2
Residual	66
Total	101

Table 3-91 Skeleton ANOVA for linear regression of number of split radishes per tray and mean hypocotyl water content per tray for treatments W/W and D/W. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period

Source of variation	df
Regression	1
Residual	66
Total	67

3.5.3.3.3 Experiment 3.10: Results

Measurements during growth

VWC:

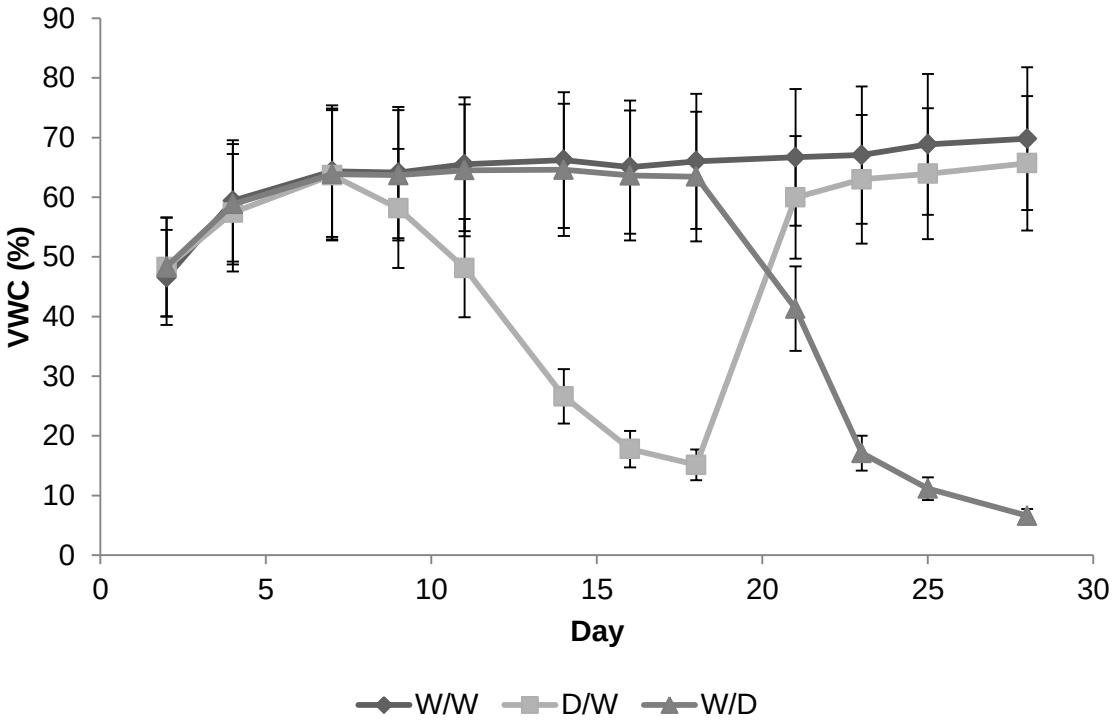


Figure 3-38 The VWC of pots undergoing different irrigation treatments. Bars represent the standard error. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

There was no significant difference in VWC between treatments when the seeds were planted ($P=0.385$), at the start of the first treatment period ($P=0.092$) or between the W/W and W/D treatments ($P=0.223$) 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. On Day 25, mid-way

through the second treatment period, there was a significant difference in the VWC for all treatments (P<0.001) W/W had a VWC of 68.86%, D/W was 63.94% and W/D was 11.14%. The VWC for W/D ultimately fell below the minimum VWC which D/W achieved 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 a mean VWC of 63.2% compared to a mean VWC of 68.1% for W/W (Figure 3-38). The mean VWC for W/W was 64.1%, for D/W was 49.0% and for W/D was 47.3%.

Hypocotyl width:

Table 3-92 Width of exposed hypocotyl above the compost surface of radish grown under different irrigation treatments. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experimentDay 18 was before the second treatment started, Day 22 and Day 24 were mid-way through the second treatment and Day 28 was before harvest (n=34)

Treatment	Day 18	Day 22	Day 24	Day 28
W/W	5.85	14.13b[1]	18.65b	24.06b
D/W	4.72	12.25a	15.41a	22.46b
W/D	5.35	13.04ab	13.86a	11.39a
P	0.085	0.039	<0.001	<0.001
LSD		1.444	2.231	

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only.

The width of the radish was affected by VWC but there was a delay (Table 3-92). On Day 18, at the end of the first treatment period, there was no significant difference in the widths of the radish from each group regardless of the different treatments. On Day 22, midway

through the second treatment, the W/W treatment group had significantly larger (P=0.039) mean widths than D/W even though they were both receiving irrigation at this point. Treatment D/W was not significantly different from either of the other treatment groups despite not receiving any irrigation at this point. On Day 24 the mean width of radishes grown with treatment W/W was significantly larger than either W/D or D/W which had both received periods without irrigation. However, by the end of the experiment the D/W treatment group had rapidly increased in size and there was no longer a significant difference in width between W/W and D/W. Treatment W/D decreased in width between Day 24 and Day 28 (Figure 3-39) resulting in hypocotyls from this treatment being significantly smaller than hypocotyls in the other two treatment groups.

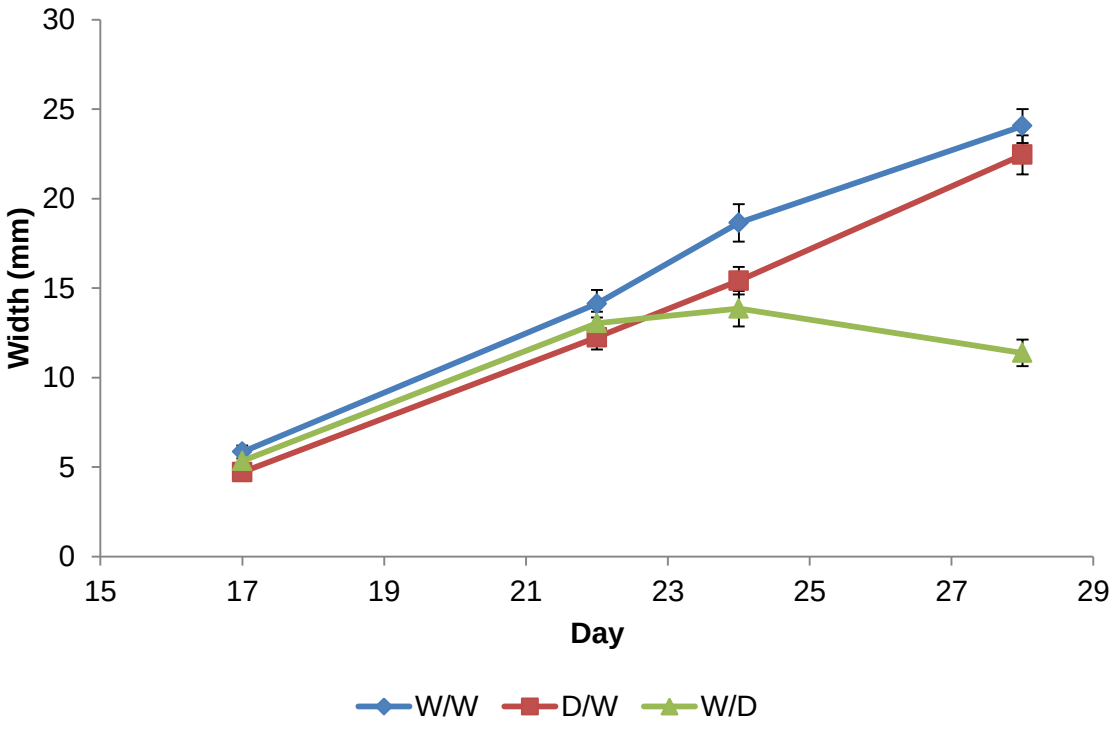


Figure 3-39 The mean hypocotyl widths (mm) of radishes grown under different irrigation treatments. Bars represent standard error for each treatment. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

Number of leaves:

Table 3-93 Mean number of leaves per pot (n=10) for radishes grown under different irrigation regimes. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment. Measurements before irrigation Treatment 2 (Day 18), mid-way through Treatment 2 (Day 24) and before harvest (Day 28) (n=34)

Treatment	Day 18	Day 24	Day 28
W/W	23.64b[1]	35.46b	47.47b
D/W	20.32a	32.56a	46.03a
W/D	23.35b	35.91b	44.56a
P	<0.001	<0.001	<0.001
LSD		1.902	

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only

The number of leaves was affected by irrigation regime. At the end of the first treatment the plants which had been exposed to the wet treatment had significantly ($P<0.001$) more leaves than the plants which had been given a dry treatment. Midway through the second treatment the pattern remained the same but by harvest the W/W treatment group plants had significantly more leaves than either of the other groups (Table 3-93).

Unlike hypocotyl width the number of leaves continued to increase for all treatments for the duration of the experiment (Figure 3-40).

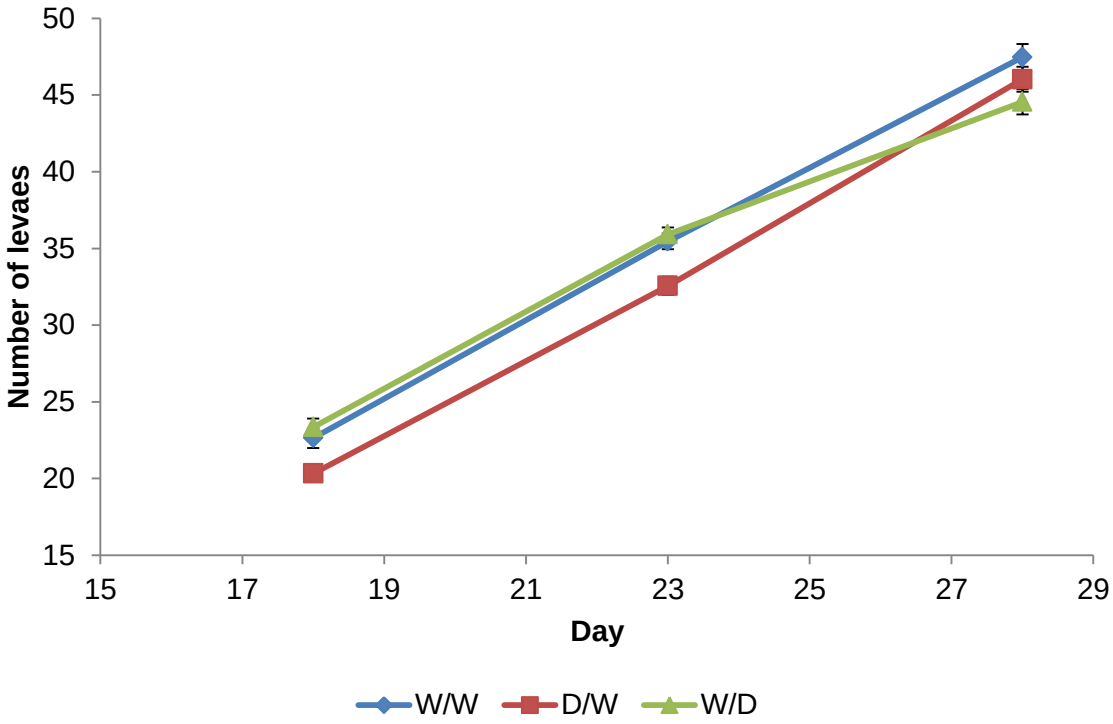


Figure 3-40 The mean number of leaves on 10 radish plants in a tray grown under different irrigation treatments. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

Leaf temperature:

Table 3-94 The mean leaf temperature (°C) for radish plants grown under different irrigation regimes. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment. Day 18 was before Treatment 2, Day 24 was mid-way through the second treatment, Day 28 was before harvest (n=34)

Treatment	Day 18	Day 24	Day 28
W/W	17.79a[1]	17.86a	16.28a
D/W	20.60b	17.20a	16.28a
W/D	17.55a	21.32b	21.28b
P	<0.001	<0.001	<0.001

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only.

Leaf temperature (°C) was affected by irrigation regime. At the end of the first treatment plants which had been exposed to a wet treatment had a significantly ($P<0.001$) (Table 3-94) lower temperature on average than plants which had been exposed to a dry treatment. Within five days after treatments changed the plants which changed from a dry to wet treatment decreased in temperature and plants which had been exposed to a wet environment and were now exposed to a dry treatment had increased in temperature. Plants which were given a wet treatment for the start and end of the experiment had a more constant leaf temperature throughout the experiment. By the end of the second treatment the W/W and D/W plants were significantly ($P<0.001$) cooler than the W/D.

Stomatal conductance:

Table 3-95 Effects of irrigation treatment on stomatal conductance (mmol m^{-2} s^{-1}). W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment (n=34)

Treatment	Day 22	Day 24	Day 28
W/W	383.2b	544.9b	274.6b[1]
D/W	337.0ab	501.4b	389.2c
W/D	280.6a	68.5a	17.2a
P	0.003	<0.001	<0.001
LSD	56.2		

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only.

Irrigation had an effect on stomatal conductance (mmol m^{-2} s^{-1}). On Day 22 the W/W treatment had the greatest stomatal conductance suggesting these plants were the least stressed and W/D had the lowest stomatal conductance suggesting these plants were the most stressed which is to be expected as they were in a drying down phase at this point. D/W plants had a stomatal conductance mid-way between the other two treatment groups suggesting they had not entirely recovered from the period of drying they had experienced. On Day 24 results were as expected, the two treatments which were receiving irrigation, W/W and D/W had a stomatal conductance which was not significantly different but is significantly greater than the plants which were in a drying phase (W/D). By Day 28 all three treatment groups had a different stomatal conductance. Treatments D/W had the greatest stomatal conductance, followed by W/W and finally by W/D which had a much smaller stomatal conductance than the other two groups suggesting these plants were experiencing drought stress.

Measurements at harvest

Table 3-96 Effects of irrigation treatment on splitting and plant size at harvest. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment (n=34)

Treatment	Split radish per tray (max=10)	Hypocotyl width (mm)	Plant weight (g)	Hypocotyl weight (g)	Leaf weight (g)	Leaf area (cm^2)
W/W	4.2b[1]	27.3b	185.6c	116.0c	65.99c	102.42c
D/W	1.0a	25.9b	139.8b	95.7b	44.67b	67.25b
W/D	3.6b	11.2a	25.6a	13.5a	10.20a	35.74a
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.770					

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only.

Growth splits: The mean number of split radish per tray at harvest was significantly (P<0.001) lower for treatment D/W (9.7%) than either W/W or W/D (42 and 36% respectively) (Table 3-96).

Hypocotyl width: Both W/W (mean width of 27.3 mm) and D/W (mean width of 25.9 mm) radishes were significantly (P<0.001) larger in width than the W/D (mean width of 11.2 mm) radishes at harvest. There was no significant difference in width between W/W and D/W radishes. However, D/W radishes were more uniform in shape and diameter than the other two treatments (Figure 3-41).

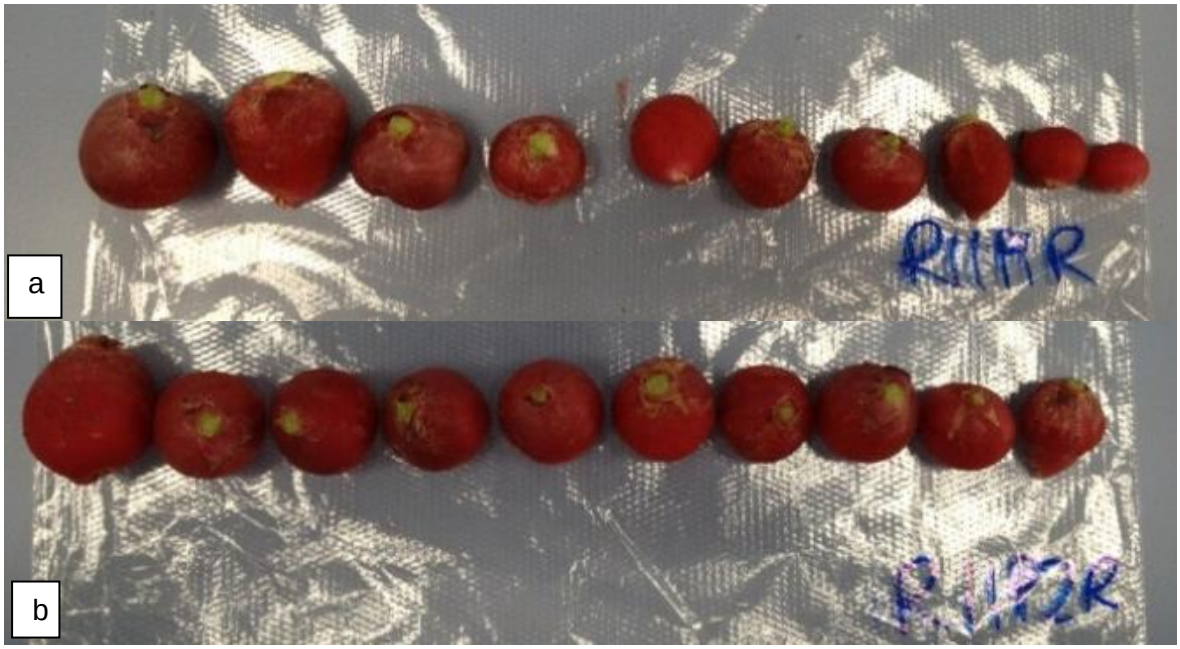


Figure 3-41 Ten radishes harvested from one experimental tray from treatment W/W (a) and D/W (b). The radishes from W/W (a) appeared to be less uniform than the radish from D/W (b). W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period

Radishes below 18 mm in width and above 32 mm in width are outside of the typical commercial range in the UK. Commercial radishes are usually graded into two groups, small radishes are 18 to 25 mm and large radishes are 25 to 32 mm. In this experiment, 91% of radishes from D/W were of commercial width compared to 67% of W/W and 0% of W/D (Figure 3-42).

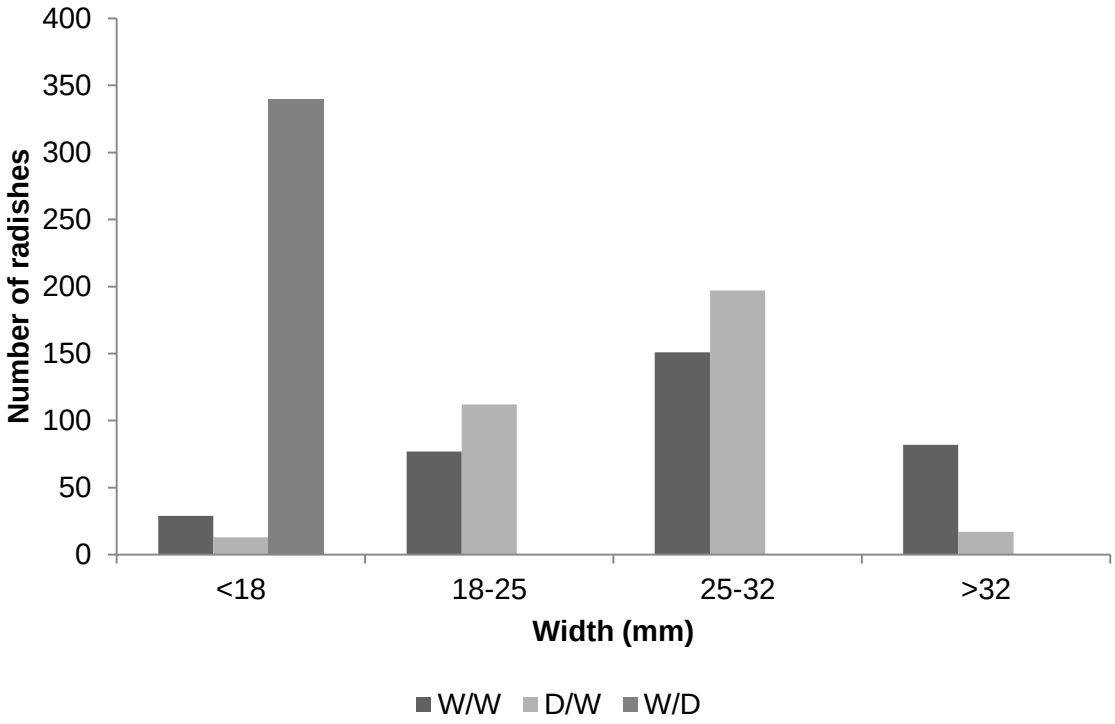


Figure 3-42 Distribution of sizes of radishes from different irrigation treatments. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

Plant weight: The radish plant weight at harvest was significantly different ($P<0.001$) for all treatments. Plant weight was heaviest for treatment W/W with a mean weight of 185.6 g per tray, D/W radishes were second heaviest with a mean weight of 139.8 g per tray and W/D radishes were the lightest with a mean weight of 25.6 g per tray (Table 3-96).

Hypocotyl weight: Following the same pattern as plant 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 116.0 g per tray, D/W radishes were second heaviest with a mean weight of 95.7 g per tray and W/D radishes were the lightest with a mean weight of 13.4 g per tray (Table 3-96).

Leaf weight: Again following the same pattern, the leaf weight at harvest was significantly different ($P<0.001$) for all treatments. Leaf weight was heaviest for treatment W/W with a

mean weight of 65.99 g per tray, D/W radishes were second heaviest with a mean weight of 44.67 g per tray and W/D radishes were the lightest with a mean weight of 10.20 g per tray (Table 3-96).

Leaf area: The leaf area at harvest also followed a similar pattern and was significantly different ($P<0.001$) for all treatments. Leaf area was greatest for treatment W/W with a mean leaf area of 102.42 cm^2, D/W radishes were second largest with a mean leaf area of 67.25 cm^2 and W/D radishes were the smallest with a mean leaf area of 35.74 cm^2 (Table 3-96).

Marketable yield: Marketable yield is the weight of saleable radish and can be roughly calculated from the data gathered in this experiment (Table 3-97). The calculated marketable yield for W/D was greatest at 78.6 g per tray, the marketable yield for W/W was 45.1 g per tray and there was no marketable yield for treatment W/D as the radishes were all less than 18 mm in width. Commercially, the minimum acceptable hypocotyl width is 18 mm.

Table 3-97 Marketable yield calculations for radishes from different irrigation treatment groups. Marketable yield calculations presume radish splitting is evenly distributed throughout the different sizes and the hypocotyl weight is evenly spread across the different widths. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment

Treatment	Hypocotyl weight (g) = W	Commercial size (%) = C	Not split (%) = S	Marketable yield (g) = W*(C/100)*(S/100)
W/W	116.0	67.25	57.94	45.08
D/W	95.7	91.15	90.29	78.64
W/D	13.4	0	64.12	0

Measurements after storage

Table 3-98 Hypocotyl weight, water content and dry biomass after two days of storage in a controlled environment. W/W received irrigation for the duration of the experiment, D/W received no irrigation for the first treatment period and irrigation for the final treatment period, W/D received no irrigation for the initial treatment period and irrigation for the final treatment period of the experiment (n=34)

Treatment	Split radish per tray (max = 10)	Hypocotyl weight (g)	Hypocotyl dry biomass (g)	Hypocotyl water content (%)
W/W	4.2b[1]	112.9c	6.043c	94.66b
D/W	1.0a	92.99b	4.134b	95.54b
W/D	3.6b	13.06a	2.710a	78.70a
P	<0.001	<0.001	<0.001	<0.001
LSD	0.770			

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. Least significant difference (LSD) available for results analysed using ANOVA only.

Harvest splits: There were no additional splits expressed during storage.

Hypocotyl weight: The weight of the trimmed hypocotyls was significantly (P<0.001) different for all treatment groups after storage. The W/W radishes which received the most water were the heaviest, followed by the D/W radishes which were irrigated prior to harvest and finally the radishes which were in a drying period prior to harvest were the lightest. After drying the hypocotyls at 105°C to a constant weight the dry biomass (g) was also found to be significantly (P<0.001) different for all treatment groups (Table 3-98). Like fresh weight, the dry biomass was greatest for the W/W radishes and least for the W/D radishes.

Hypocotyl water content: As a percentage of the total weight, 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 3-98).

For the W/W and D/W treatments a significant (P<0.001) correlation expressed by the equation: y = -21.24x + 2046 (R^2=0.39) was found linking mean hypocotyl water content with the percentage of split radishes per tray (Figure 3-43).

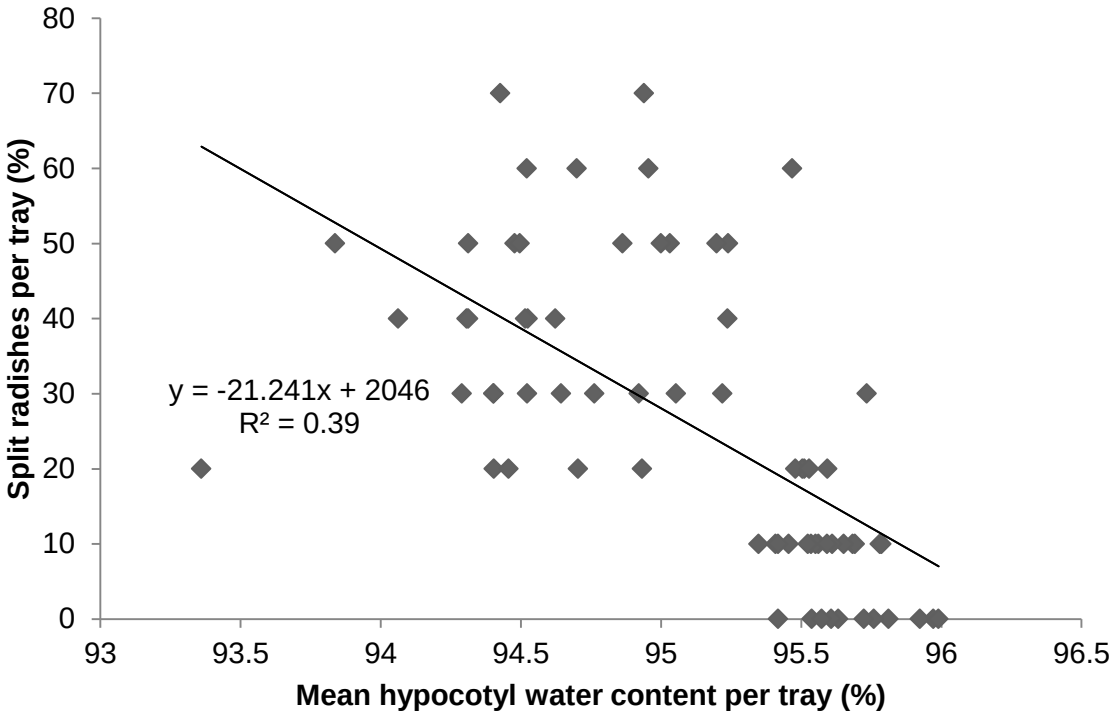


Figure 3-43 Percentage of split radishes per tray for trays with different mean hypocotyl water contents (%)

3.5.3.3.4 Experiment 3.10: Discussion

VWC: 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 on average than the D/W plants did at the end of the 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.

End of treatment 1 (Day 18): At the end of the first treatment period before treatments were changed the leaf temperature, maximum exposed hypocotyl width and the number of leaves per pot were recorded. There was no significant ($P=0.085$) difference in the width of the radishes but there was a significant difference in the leaf temperature ($P<0.001$) and the number of leaves ($P<0.001$). Radishes which had been given a wet treatment were significantly ($P<0.001$) cooler than radish which had been given a dry treatment suggesting the radishes which were being grown under dry treatments were closing their stomata to reduce water loss through transpiration and as a result increasing their temperature. The number of leaves on the radishes in the dry treatment was significantly fewer than the number of leaves for the wet treatments. This would suggest

growth rate was being limited by available water content in the dry treatment. At this point in the experiment, there was no significant difference in any of the factors measured between the two groups of plants which had received the wet treatment. This is as would be expected as there had been no differences in the irrigations treatments between the W/W and W/D treatment groups up to this point.

Early treatment 2 (Day 22): By Day 22, the second null hypothesis had been rejected as timing of irrigation was having a significant effect on hypocotyl width and therefore growth rate. Despite there being no difference in hypocotyl width on Day 18 when the treatments changed, by Day 22 the earlier period of drought appeared to be having an effect on the hypocotyl width of the plants from the D/W treatment as these were significantly smaller than the W/W treatment. The width of the plants from the W/D treatment was midway between the widths of the other two treatments but not significantly different from either of them suggesting the second drought period was beginning to have an effect on limiting hypocotyl expansion rate. The stomatal conductance was affected more rapidly than hypocotyl expansion by changes in available water content and mirrored the VWC of the compost. W/W had the greatest VWC (Day 21 VWC = 66.7%, Day 23 VWC = 67.1%) and the greatest stomatal conductance, D/W had a stomatal conductance which was between the other two groups and not significantly different from either and a VWC between the other two treatments (Day 21 VWC = 60.0%, Day 23 VWC = 63.0%) and W/D had the lowest stomatal conductance and the driest compost (Day 21 VWC = 41.3%, Day 23 VWC = 17.1%). These results are unexpected as it is usually considered that cell growth is more sensitive to water stress than stomatal opening (Kramer 1983). However, the responsiveness of stomata to water stress varies greatly between species, environmental conditions, leaf age and past treatment making it difficult to generalise (Kramer 1983).

Late treatment 2 (Day 24): On Day 24 the stomatal conductance again mirrored the VWC. The highest stomatal conductance was observed for W/W and D/W which were receiving irrigation. W/D had the lowest VWC as it was in a period of drying and the plants had the lowest stomatal conductance. Leaf temperature followed the same pattern as

stomatal conductance. This is as would be expected if transpiration rates were being reduced by the reduction in stomatal conductance and thus increasing leaf temperature.

The hypocotyl width was significantly greater for the W/W plants than either of the treatments which had received a period of drying. This suggests the plants from the W/D treatment were responding to water stress as the growth rate of the hypocotyls had slowed. The plants from the D/W treatment were increasing at a steady rate and do not appear to have been limited by the earlier period of water stress. This is in contradiction to the number of leaves which remains to be significantly fewer for the D/W treatment compared to the W/W and W/D treatments which suggests radish leaves are slower to respond to changes in available water content than radish hypocotyls.

End of treatment 2 (Day 28): Before plants were harvested the stomatal conductance, leaf temperature, maximum exposed hypocotyl width, number of leaves and leaf area were measured.

Between Day 24 and Day 28, the rate of hypocotyl expansion was greatest for D/W as there was no significant difference in the width of the W/W and D/W treatments at harvest when previously the W/W plants had been larger than the D/W plants. This finding is in keeping with the previous experiment where plants which had received a period of drought grew rapidly when the stress was alleviated. It was thought this may have been due to compensatory growth and results from this experiment are in accordance with this theory. At harvest W/W and D/W hypocotyls were both significantly ($P<0.001$) larger than the W/D treatment radish hypocotyls which had decreased in size since Day 24. The decrease in size suggests these plants are experiencing severe water stress and the plants are wilting.

There was no significant difference in the number of leaves in the W/D and D/W radish at harvest but these both had significantly ($P<0.001$) fewer leaves than the W/W radish. This is in contrast to the previous experiment where the plants which received a period of drying followed by irrigation had the largest number of leaves at harvest. However, the number of leaves for D/W increased at a faster rate in the final few days compared to W/W and it may be that if the plants had been harvested later then a similar pattern to the

previous experiment would have been observed. In contrast to leaf number, the leaf area at harvest was significantly ($P<0.001$) different between all treatment groups. The radishes grown with W/W treatment had on average the greatest leaf area (102.4 cm^2), the radish grown with D/W treatment were second largest on average (67.3 cm^2) and the W/D radish had the smallest leaf area on average (35.7 cm^2). This shows that although there was no significant difference in the number of leaves between W/W and D/W there was a difference in the size of the leaves between treatments and this pattern followed the amount of irrigation the plants received.

There was no difference in leaf temperature between the W/W and D/W treatments but these were both significantly ($P<0.001$) lower than the leaf temperature for the W/D treatment. These results suggest the leaf temperature is being determined by available water content as the two treatments which were being irrigated had a lower temperature than the treatment which was drying down. Unexpectedly, the stomatal conductance did not follow the same pattern as leaf temperature and it was significantly ($P<0.001$) different between treatments; it was greatest for the D/W treatment and lowest for the W/D treatment. However, if the amount of gas which is being transpired is calculated by multiplying the stomatal conductance by leaf area, the rates for the two treatments which are receiving irrigation are similar (W/W = 281.25 mmol s^{-1}, D/W = 261.74 mmol s^{-1}) and the rate for the treatment which is not receiving irrigation was far lower (W/D = 6.15 mmol s^{-1}). Water loss as a result of transpiration follows the pattern of leaf temperature. The higher stomatal conductance for D/W compared to W/W may have been due to a priming effect caused by the earlier period of drought stress which the D/W plants experienced. Some plants which have experienced drought stress in the past have been shown to have reduced sensitivity to abscisic acid (ABA) and increased water loss due to transpiration (Bruce *et al.* 2007).

Growth splits: The first null hypothesis was rejected as the amount of splitting in radishes at harvest was affected by the VWC of the substrate between Day 8 and Day 18, 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. Although it was not recorded in this experiment, Day 18 has been shown in previous experiments to be approximately when Growth Stage 41 occurs. The majority of the hypocotyl expansion in radishes occurred after Day 18, the radish hypocotyl diameter increased 10 fold after Day 18.

Marketable yield: The radish which received the D/W treatment had the greatest marketable yield of all three treatments despite not having the greatest mean hypocotyl weight, it is therefore recommended that where possible, radishes are grown with a period of drying up to Day 18 and then irrigation after this point. This is because the D/W treatment group had a greater proportion of radish of a commercial size and far fewer split radishes than the other two treatment groups. It should be noted the numbers are an indication of yield only as calculations presume the weight is even for all widths which is unlikely. The calculations also presume the likelihood of splitting is independent of width which has not been investigated in this experiment.

Measurements after storage: The hypocotyl water content was affected by VWC 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 and therefore less water is taken up by the plant.

In conclusion, the results from this experiment showed timing of available water content was crucial in predicting splitting. The substrate VWC on Day 18, approximately Growth Stage 41, was important and explained far more of the splitting than the mean substrate VWC. Timing of water availability also had significant effects on the growth rate, marketable yield and physiology of the radish plants.

3.5.3.4 Experiment 3.11: A further experiment investigating the effects of timing of changes in VWC on the susceptibility of radishes to growth splits

3.5.3.4.1 Experiment 3.11: Introduction

Experiment 3.10 showed a significant difference in hypocotyl splitting of radishes when they are exposed to different VWC at different periods in growth. Specifically, a reduction in splitting was observed the when the radishes were given a period of drying between Day 8 and Day 18. This experiment aims to repeat the previous work to reinforce the results.

Aim:

- The aim of this experiment was to confirm if irrigation and more specifically timing of irrigation application has a significant effect on the rate of splitting in radishes

Null hypothesis:

1. Different irrigation regimes will have no significant effect on the amount of splitting at harvest

3.5.3.4.2 Experiment 3.11: Materials and Methods

Planting date: Seeds were sown on 14.05.2013, this was Day 1, and treatments began on Day 7.

Glasshouse conditions: In the glasshouse the mean temperature was 19.0°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 seven days of irrigation for establishment. There were two treatment periods each lasting 10 days. Treatment W/W received irrigation for the duration of the experiment; Treatment D/W received no irrigation for the first 10 days and irrigation for the final 10 days (Table 3-99). 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 17 mm day $^{-1}$.

Table 3-99 Irrigation regimes for the two treatment groups used in Experiment 3.11

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

Replication: For the main harvest n = 34 (Figure 3-44). 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. Pots were arranged in a randomised block design (Figure 3-22) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block					Block
1	D/W	W/W	D/W	W/W	18
2	W/W	D/W	W/W	D/W	19
3	W/W	D/W	W/W	D/W	20
4	D/W	W/W	W/W	D/W	21
5	D/W	W/W	D/W	W/W	22
6	D/W	W/W	W/W	D/W	23
7	D/W	W/W	D/W	W/W	24
8	D/W	W/W	W/W	D/W	25
9	D/W	W/W	W/W	D/W	26
10	W/W	D/W	W/W	D/W	27
11	W/W	D/W	W/W	D/W	28
12	D/W	W/W	W/W	D/W	29
13	D/W	W/W	D/W	W/W	30
14	W/W	D/W	W/W	D/W	31
15	W/W	D/W	W/W	D/W	32
16	D/W	W/W	W/W	D/W	33
17	D/W	W/W	D/W	W/W	34

Figure 3-44 Layout of experimental pots on glasshouse bench in Experiment 3.11. Blue lines represent the capillary tubing. Treatment W/W received irrigation for the duration of the experiment; Treatment D/W received no irrigation for the first 10 days and irrigation for the final 10 days

Measurements taken during growth: On Day 18 leaf area and temperature were measured. On Day 28 leaf area, temperature and stomatal resistance were measured.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

All data was analysed using a two-tailed, unpaired Student's T-test assuming equal variance.

Skeleton ANOVA:

Table 3-100 Skeleton ANOVA for all analysis

Source of variation	df
Treatment	1
Residual	61
Total	63

3.5.3.4.3 Experiment 3.11: Results

VWC: There was no significant difference in 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 3-45).

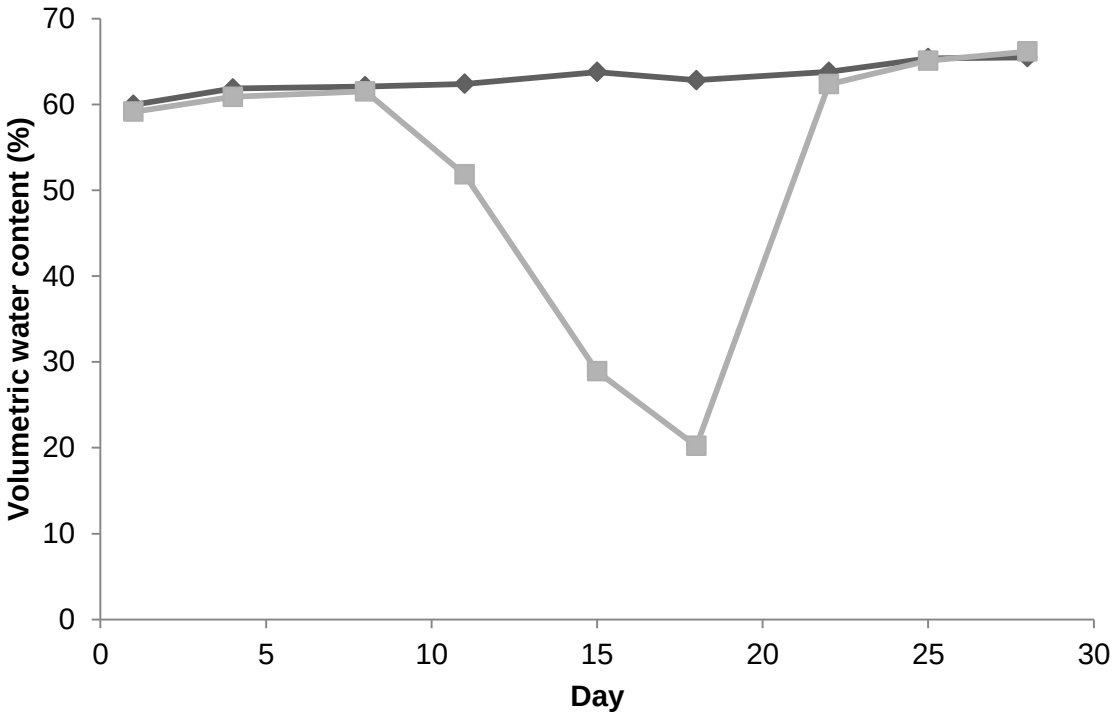


Figure 3-45 The VWC of pots undergoing different irrigation treatments. W/W pots (dark grey line) were irrigated for the duration of the experiment. D/W pots (light grey line) received no irrigation for 10 days between Day 8 and 18 (n=3)

Stomatal resistance, leaf area and leaf temperature: By Day 18 there were no significant differences between treatments in 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 3-101).

Table 3-101 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	Leaf temperature (°C)	Leaf area (cm^2)
W/W	21.6a[1]	12.8
D/W	22.5b	12.6
P	0.034	0.923

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

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 3-102).

Table 3-102 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 (°C)	Leaf area (cm^2)
W/W	1.0	15.9	128.5
D/W	1.0	15.6	130.7
P	0.208	0.091	0.369

Harvest: At harvest there were significant differences in the number of split radishes 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 3-99).

Table 3-103 Effects of irrigation treatment on splitting and yield at harvest. Treatment W/W received irrigation for the duration of the experiment, Treatment D/W received no irrigation for the first 10 days and irrigation for the final 10 days (n=34)

Treatment	**Split radish (max=10)**	**Plant weight (g)**	**Hypocotyl water content (%)**	**Hypocotyl weight (g)**	**Hypocotyl width (mm)**	**Hypocotyl length (mm)**
W/W	7.7a[1]	93.7a	94.8a	41.3a	16.9a	27.6
D/W	6.5b	113.5b	95.7b	53.8b	18.6b	28.1
P	<0.001	<0.001	<0.001	0.011	0.024	0.491

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

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

A greater proportion of radishes from D/W were of a commercial size at harvest (Figure 3-46).

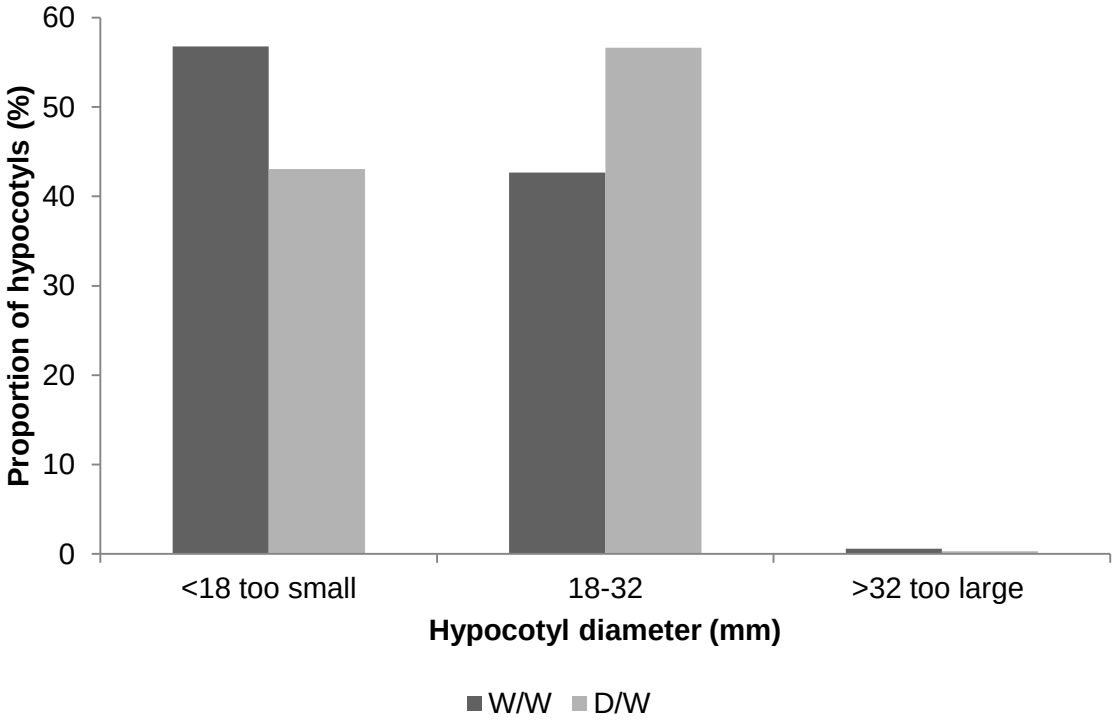


Figure 3-46 The size distribution of radishes grown with different irrigation regimes. W/W (dark grey bars) were irrigated for the duration of the experiment but D/W (light grey bars) received no irrigation for 10 days from Day 8 to Day 17 (n=340)

3.5.3.4.4 Experiment 3.11: Discussion

As in Experiment 3.10, on Day 18 a significant difference was observed in leaf temperature between treatment groups with D/W plants having a greater leaf temperature than W/W (Table 3-104). This again suggests the plants which had received a period of drying had reduced the rate of transpiration to reduce water loss and as a result leaf temperature had increased.

Plants in Experiment 3.11 appear to have retarded growth compared to plants in Experiment 3.10. 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 the previous experiment. 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 Experiment 3.10.

The null hypothesis was rejected, as differences were again observed in the number of split hypocotyls at harvest. As with Experiment 3.10 the treatment which received a period of drying down from Day 7 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 the previous experiment. 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.

Table 3-104 A comparison between the results from the previous experiment (Experiment 3.10) with the results from this experiment (Experiment 3.11)

	Previous experiment	**This experiment**
Stomatal conductance Day 28	D/W > W/W	No difference
Leaf temperature Day 18	D/W > W/W	D/W > W/W
Leaf temperature Day 28	No difference	No difference
Split hypocotyls at harvest	W/W > D/W	W/W > D/W
Plant weight at harvest	W/W > D/W	D/W > W/W
Hypocotyl width at harvest	No difference	D/W > W/W
Hypocotyl weight at harvest	W/W > D/W	D/W > W/W
Hypocotyl water content	No difference	D/W > W/W
Leaf area at harvest	W/W > D/W	No difference

In Experiment 3.11, no significant difference in leaf area was observed at harvest whereas in Experiment 3.10 W/W plants had a greater leaf area. This suggests the higher VWC in Experiment 3.11 compared to Experiment 3.10 did not cause enough water stress to have a significant effect on leaf area.

At harvest, in Experiment 3.11 as in the Experiment 3.10, there was no significant difference in leaf temperature between the two treatment groups. However, unlike Experiment 3.10 there were also no differences in leaf area or stomatal resistance at harvest. However, this results in a similar amount of gas being lost from the plant as the AP4 porometer measures the rate of diffusion conductance over an area and there were no differences in rate or area. When the amount of gas being lost from the plant was calculated for the previous experiment, this was also found to be the same for the two treatments.

At harvest, 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.

In conclusion, the results from this experiment again showed a period of drying mid-growth up to Day 17 around the time of Growth Stage 41 was crucial in predicting splitting. Further experiments are now required to determine if it is the VWC at Growth Stage 41 which is important or if any period of drying mid-growth will reduce splitting.

3.5.3.5 Experiment 3.12: Experiment investigating the effects of changes in VWC at Growth Stage 41 on the susceptibility of radishes to growth splits

3.5.3.5.1 Experiment 3.12: Introduction

Building on work from Experiments 3.9, 3.10 and 3.11 where a reduction in splitting has been observed in plants which have been grown with a period of drying mid-growth compared to plants which have been irrigated for the duration of the experiment, the objective of Experiment 3.12 was to determine the factors which result in this reduction in splitting.

In Experiments 3.9, 3.10 and 3.11 the driest point in the treatments which received a period of drying and a reduction in splitting coincided with Growth Stage 41. Growth Stage 41 is when rapid expansion of the hypocotyl begins following the rupture of the exodermis and outer cortex which exposes the periderm. There is support for timing of water stress having an effect in splitting from literature. Sørensen (1997) 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 or shortly prior to harvest when rapid radial expansion is occurring. This investigation aimed to investigate if Growth Stage 41 was associated with the reduction in rates of splitting observed at harvest in previous experiments.

In Experiments 3.6 and 3.7 a lower VWC for the duration of the experiment was shown to result in a reduction in splitting. This experiment also investigated if the length of the period of drying down affects the amount of splitting which is observed at harvest.

Aim:

- The aim of this experiment was to identify the features of a period of drying mid-growth which result in a reduction in splitting.

Null hypothesis:

1. Different irrigation treatments will have no significant effect on the amount of splitting observed at harvest.

3.5.3.5.2 Experiment 3.12: Materials and methods

Planting date: Seeds were planted on 22.05.2013, treatments commenced on Day 7.

Experiment duration: 27 to 29 days depending on block.

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 seven 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 and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days (Table 3-105). It was expected that T2 and T4 would be driest at the point when the hypocotyl begins to swell (Growth Stage 41), Treatments T1 and T3 would have the greatest VWC at the point when the hypocotyl swells (Growth Stage 41) and Treatment T5 would have a VWC between the other groups. If splitting was related to the VWC at Growth Stage 41 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. T4 had the longest drought period of 15 days, T2 and T5 had the second longest drought period of 10 days, T3 had the shortest drought period of five days and treatment group T1 had no drought period.

Table 3-105 Irrigation regimes for the five treatment groups used in Experiment 3.12

Treatment	Day 1-7	Day 8-12	Day 13-17	Day 18-22	Day 23 -27
T 1	Irrigation				
T 2	Irrigation	No Irrigation		Irrigation	
T 3	Irrigation	No Irrigation		Irrigation	
T 4	Irrigation	No Irrigation			Irrigation
T 5	Irrigation		No Irrigation		Irrigation

Replication: n = 24. Pots were arranged in a randomised block design (Figure 3-47) which was generated by GenStat for Windows 15th Edition (VSN International 2011).

Block 1	Block 5	Block 9	Block 13	Block 17	Block 21
T4	T3	T3	T4	T2	2
T3	T2	T5	T5	T3	5
T1	T4	T2	T2	T1	3
T2	T1	T4	T3	T4	1
T5	T5	T1	T1	T5	4
Block 2	**Block 6**	**Block 10**	**Block 14**	**Block 18**	**Block 22**
T5	T5	T5	T5	T3	5
T2	T1	T1	T2	T5	2
T1	T2	T3	T1	T4	4
T3	T3	T4	T4	T1	3
T4	T4	T2	T3	T2	1
Block 3	**Block 7**	**Block 11**	**Block 15**	**Block 19**	**Block 23**
T1	T2	T3	T2	T4	1
T5	T4	T5	T1	T3	3
T3	T1	T1	T3	T5	4

T4	T3	T2	T4	T2	2
T2	T5	T4	T5	T1	5
Block 4	**Block 8**	**Block 12**	**Block 16**	**Block 20**	**Block 24**
T4	T3	T1	T1	T4	1
T5	T5	T4	T2	T2	2
T2	T4	T2	T3	T1	4
T3	T1	T3	T5	T3	5
T1	T2	T5	T4	T5	3

Figure 3-47 Layout of experimental pots on glasshouse bench for Experiment 3.12. Blue lines represent the capillary tubing. 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 and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days

Measurements taken during growth: The VWC of the compost was measured at the start of the experiment on Day 1 and again on before treatments were changed on Day 8, before treatments were changed on Day 13, before treatments were changed on Day 18, before treatments were changed on Day 22 and before plants were harvested on Day 27.

At harvest, stomatal resistance, leaf temperature and leaf area were taken from plants in even numbered blocks (n=12).

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

If data was parametric as confirmed by Shapiro-Wilk test for normal distribution it was analysed using ANOVA. Where data was not normally distributed with or without transformation the non-parametric Friedman's test was used. When a P value of less than 0.05 was observed a Tukey test was used for parametric data and Mann-U Whitney test

was used for non-parametric data to determine which results were different from each other (Table 3-106).

Table 3-106 Method of analysis for different factors measured in Experiment 3.12. Method of analysis (parametric or non-parametric) was decided according to normal distribution as determined by the Shapiro-Wilk test

Measurement	Shapiro-Wilk	Analysis used
VWC Day 18	<0.001	Friedman's test and Mann-U Whitney
Number of splits at harvest	<0.001	Friedman's test and Mann-U Whitney
Leaf area	0.059	ANOVA and Tukey test
Leaf temperature	0.088	ANOVA
Stomatal resistance	<0.001	Friedman's test
Hypocotyl length	<0.001	Friedman's test and Mann-U Whitney
Hypocotyl width	<0.001	Friedman's test and Mann-U Whitney
Roundness	<0.001	Friedman's test and Mann-U Whitney
Plant weight	<0.001	Friedman's test and Mann-U Whitney
Hypocotyl weight	<0.001	Friedman's test and Mann-U Whitney
Hypocotyl water content	<0.001	Friedman's test and Mann-U Whitney

Skeleton ANOVA:

Table 3-107 Skeleton ANOVA for VWC Day 18, harvest splits, roundness, hypocotyl length, hypocotyl width, roundness, plant weight, hypocotyl weight, hypocotyl water content

Source of variation	df
Block	23
Treatment	4
Residual	92
Total	119

Table 3-108 Skeleton ANOVA for stomatal resistance, leaf area and leaf temperature

Source of variation	df
Block	11
Treatment	4
Residual	44
Total	59

3.5.3.5.3 Experiment 3.12: Results

VWC: There was no significant difference (P=0.204) in 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 3-48).

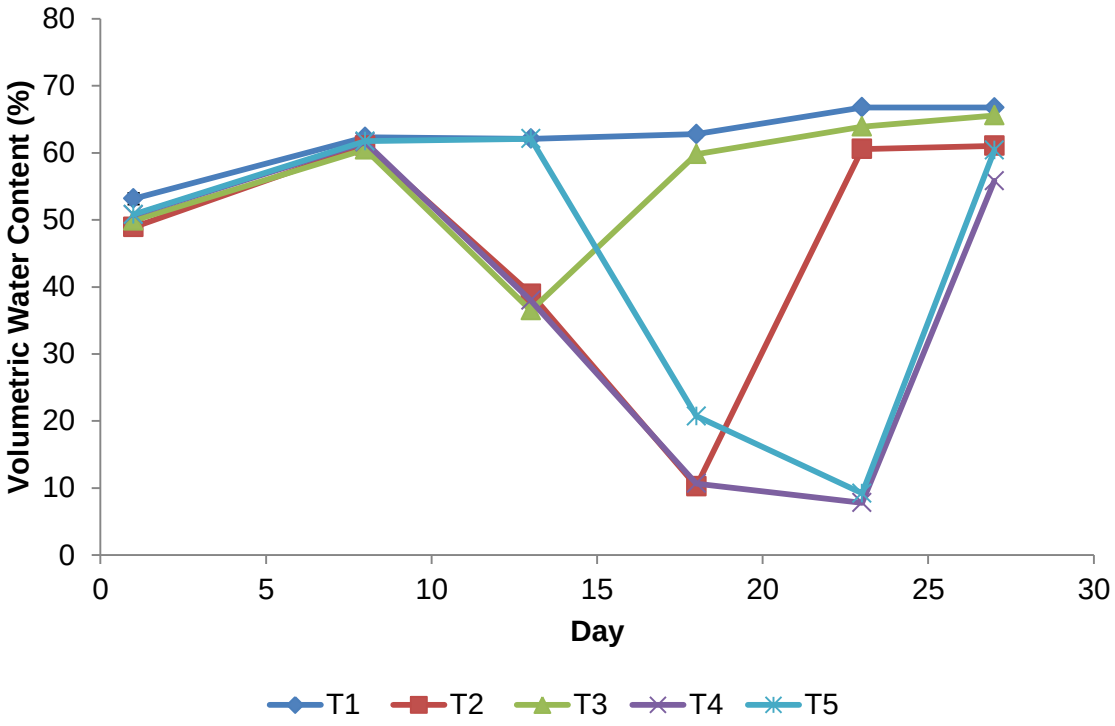


Figure 3-48 The VWC of pots undergoing different irrigation treatments. T1 pots were irrigated for the duration of the experiment. T2 pots received no irrigation for 10 days between Day 8 and Day 17, T3 pots received no irrigation for five days between Day 8 and Day 12, T4 pots received no irrigation 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: There was no significant difference in stomatal conductance (P=0.231) or leaf temperature (P=0.636) between treatment groups but there was a difference (P<0.001) in leaf area. T1 and T3 had the largest leaf area and T4 had the smallest leaf area (Table 3-109).

Table 3-109 Stomatal resistance, leaf temperature and leaf area taken from plants in even numbered blocks. 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 and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days (n=12)

Treatment	Stomatal resistance (m^2 s mol^{-1})	Leaf temperature (°C)	Leaf area (cm^2)
T1	1.11	17.95	157.90c[1]
T2	1.32	17.88	128.76bc
T3	1.07	18.01	156.56c
T4	1.77	18.09	66.19a
T5	1.75	17.95	100.41ab
P	0.231	0.636	<0.001
LSD	n/a	0.278	25.96

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. LSD only available for plants analysed using ANOVA.

Significant differences were observed between treatments for the number of split radishes per tray (Table 3-111), the total weight of the radishes, the trimmed hypocotyl weight, the length and width of the hypocotyls and the water content of the hypocotyls (Table 3-110). The average number of split radish per pot was significantly lower (P<0.001) for plants in T2 and T4 (mean of 1.38 and 1.67 split radish per pot respectively) than T5 (mean of 3.79 split radish per pot) and T5 radishes had significantly fewer (P<0.001) splits than radishes from T1 and T3 (mean of 6.5 and 7.5 split radishes per pot 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 3-110).

Table 3-110 Effects of irrigation treatment on splitting and yield at harvest. 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 and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days (n=24).

Treatment	**Plant weight (g)**	**Hypocotyl weight (g)**	**Hypocotyl width (D) (mm)**	**Hypocotyl length (L) (mm)**	**Roundness (L/D)**	**Hypocotyl water content (%)**
T1	197.2d[1]	98.8c	23.3c	30.3c	1.2a	96.0a
T2	138.1c	66.1b	21.5c	26.4b	1.2a	96.6b
T3	196.9d	104.8c	25.7d	31.4c	1.2a	96.2ab
T4	51.7a	10.4a	9.7a	19.2a	2.1b	96.6b
T5	104.9b	59.9b	18.7b	24.2b	1.3a	96.6b
P	<0.001	<0.001	<0.001	<0.001	<0.001	0.004
LSD	n/a	n/a	n/a	n/a	n/a	n/a

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. LSD only available for plants analysed using ANOVA.

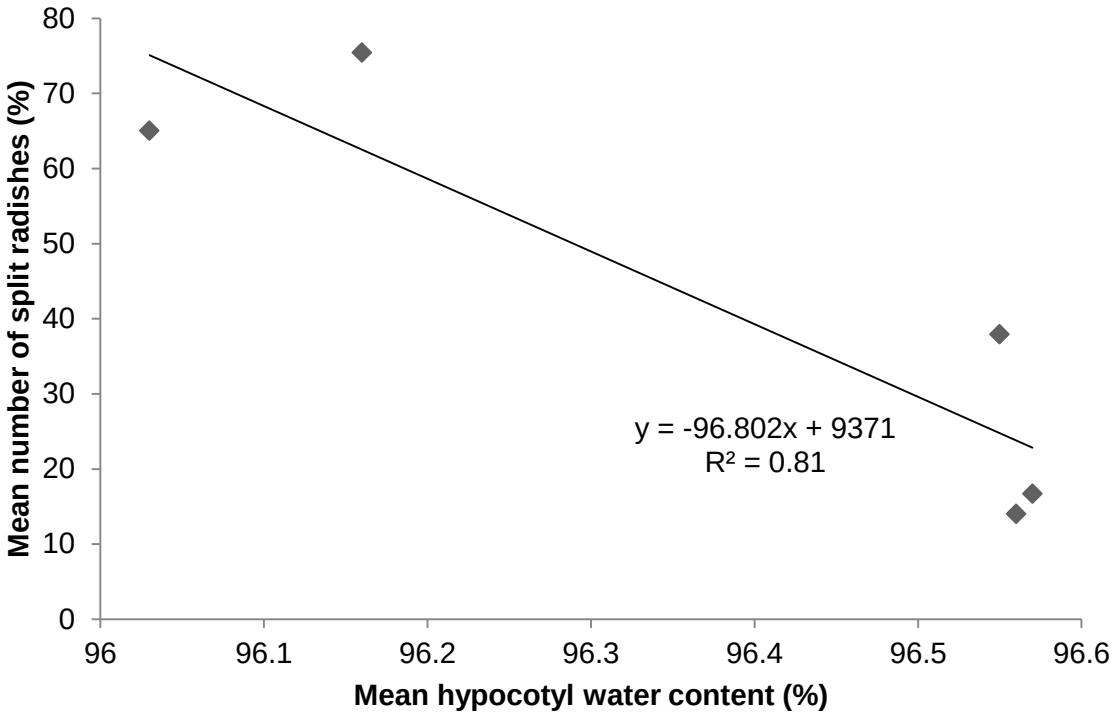


Figure 3-49 Mean splits (%) per treatment correlated with mean hypocotyl water content per treatment (%)

The mean number of splits per treatment was found to be negatively correlated (P=0.036) with the mean hypocotyl water content per treatment (Figure 3-49). The strong linear relationship between splitting and hypocotyl water content was expressed by the equation:

$$y = -96.802x + 9371 \ (R^2=0.81)$$

This finding would suggest radishes which split in this experiment had lower hypocotyl water contents.

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 3-111). The duration of the dry period and the trimmed hypocotyl weight at harvest were found to be negatively correlated.

Table 3-111 Number of split radishes compared to duration of dry period and VWC on Day 18 when the hypocotyl has begun to expand. 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 and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days

Treatment	**VWC (%) on Day 18**	**Duration of dry period (days)**	**Split radishes per tray**
T1	62.8c[1]	0	6.50c
T2	10.0a	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
LSD	n/a		n/a

[1]Denotes difference at the 5% level, where letters are shared no significant difference is present between values. LSD only available for plants analysed using ANOVA.

Size distribution: Commercially only radishes which are between 18 and 32 mm in diameter are sold. Radishes which fall outside of these restrictions are graded out and discarded. It was found the greatest proportion of commercial size radishes at harvest were from T1 and T3 (Table 3-51).

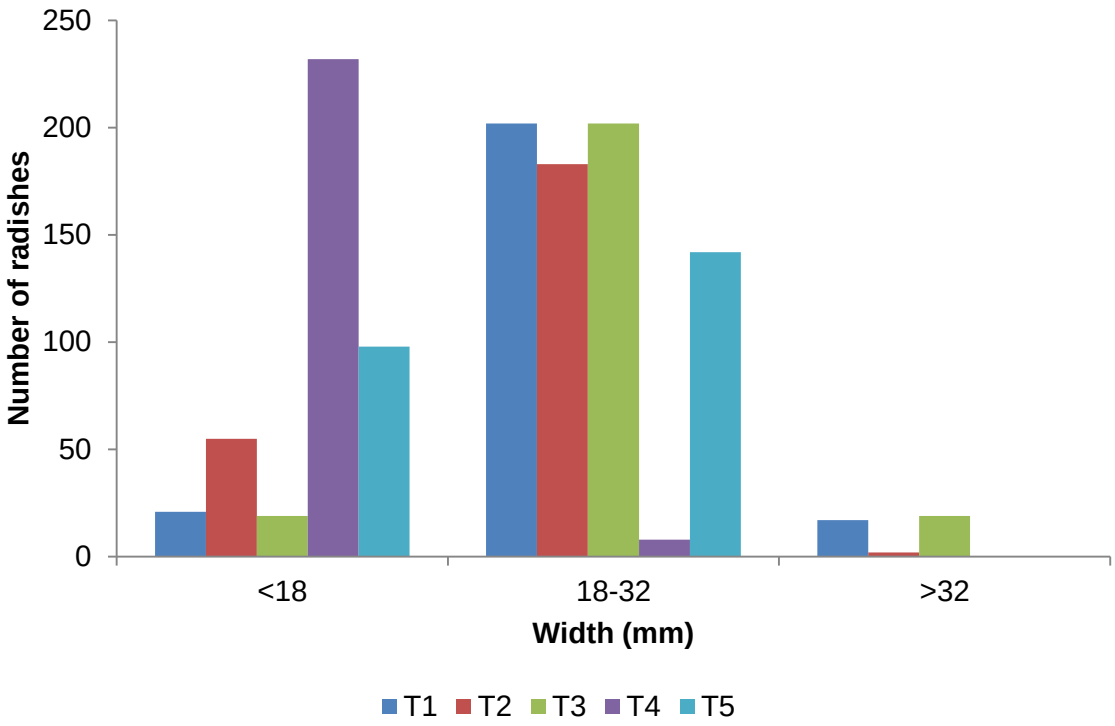


Figure 3-50 The size distribution of radishes grown with different irrigation regimes. T1 received irrigation for the duration of the experiment; T2 received no irrigation for the first 10 days and irrigation for the final 10 days, T3 received no irrigation for the first five day and irrigation for the final 15 days, T4 received no irrigation for the first 15 days and irrigation for the final five days and T5 received irrigation for the first five days, no irrigation for the following ten days and then irrigation for the final five days

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 29.1 g per tray, then for T5 was 22.0 g per tray, for T3 was 21.7 g per tray and the marketable yield for T4 was least at 0.3 g per tray.

3.5.3.5.4 Experiment 3.12: Discussion

In Experiment 3.12, the null hypothesis was rejected as different irrigation treatments had a significant effect on the amount of splitting observed at harvest. This experiment showed splitting does not appear to be related to the duration of the drying period or the minimum VWC reached during growth but it is affected by the VWC around Day 18 as the number of split radishes was found to be positively correlated with the VWC on Day 18. Day 17 is when Growth Stage 41 occurs and the hypocotyl begins to expand rapidly. All splitting occurs after Growth Stage 41 because it is the periderm which splits and the periderm is not exposed until this point. Sørensen (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 or shortly prior to harvest when rapid radial expansion is occurring. Sørensen (1997) attributed the differences in timing of water stress to differences in the type of growth occurring at each development stage (Sørensen *et al.* 1997). During the early period of drought stress which failed to reduce splitting, carrot growth is characterised by cell division whereas during mid-growth when a period of drought stress reduced splitting, carrot growth is created by rapid radial root expansion caused by cell enlargement. As splitting is thought be affected by cell wall strength and composition, factors which affect this may affect splitting susceptibility. Sørensen (1997) suggested the decrease in splitting in carrots may have been due to a decrease in the rate of expansion during this period. In contradiction to this theory, previous experiments on radishes have shown the rate of hypocotyl expansion is more rapid in plants which have received a period of drought stress which has been relieved compared to ones which have received a constant supply of water. This difference may be explained by compensatory growth where it is thought metabolites are accumulated during the period of stress while cell enlargement is inhibited by lack of turgor. Once turgor is restored these metabolites are available for rapid cell wall synthesis and other processes associated with growth. A reduction in growth rate is not likely to explain the differences in splitting. There was also no clear pattern relating hypocotyl dry matter content with splitting in radishes suggesting there were not any

obvious relationships between cell wall structure as measured crudely by dry matter content and splitting susceptibility. However, dry matter content gives no measurement of cell wall components and these may have been affected by the irrigation treatments.

Both timing and duration of the period of drying 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. In keeping with results from previous experiments, T2 (equivalent to D/W) 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. However, in contradiction to previous experiments T2 resulted in the largest proportion of radishes of a commercial size yet in this experiment T1 (equivalent to W/W) and T3 resulted in slightly more radishes in the commercial size range than T2. In this experiment T4 which had the lowest average VWC due to the longest drought period had the lowest yield in terms of weight. T1 and T3 had the greatest yield in terms of weight and they also had the greatest mean VWC due to the shortest drought periods. The results from this experiment would suggest yield is linked to mean VWC.

At harvest, there was no significant difference in stomatal resistance ($P=0.231$) or leaf temperature ($P=0.636$) between treatment groups but there was a difference ($P<0.001$) in leaf area. This is in keeping with the results from previous experiments where leaf temperature and stomatal resistance have been shown to mirror VWC. Also in keeping with the results from previous experiments the water loss from T1 (equivalent to W/W) and T2 (equivalent to D/W) would have been similar. Overall T1, T2 and T3 which had the largest leaf areas must have had a greatest rate of water loss due to transpiration as they had the greatest leaf area.

In this experiment, T4 produced radishes which were less spherical in shape than the radishes from the other treatments. This was as the radishes from T4 were proportionally longer in length than width 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.

The VWC of the compost at harvest was significantly different between groups. T1 and T3 which had received either no dry period or the shortest duration of dry period and had the greatest minimum VWC had the greatest VWC at harvest, T2 and T5 which received the medium length of dry period had the median minimum VWCs and the median VWC at harvest. Finally T4 which received the longest duration of dry period had the driest minimum VWC over all and at harvest. Groups T4 and T5 both received irrigation for the same amount of time before harvest these results would suggest it is the duration of the dry period which determines how dry the compost becomes and how much and how quickly the compost absorbs water following irrigation.

In conclusion, VWC at Growth Stage 41 affects the amount of splitting observed at harvest and VWC later in radish growth affects the yield. Marketable yield is determined by both of these factors.

3.5.3.6 Experiment 3.9-3.12: Discussion

Timing of water availability and water stress appears to have a significant effect on hypocotyl splitting during growth in radishes. The four experiments in this section consistently showed a period of drying down from Day 8 to Day 18 or Growth Stage 41 reduced splitting compared to plants which were irrigated for the duration of growth or were given periods of drying down at different times or for different lengths of time. The final experiment in this section gave strong evidence in support of the reduction in splitting being associated with the VWC at Growth Stage 41.

Results from this series of experiments have shown that unlike apples splitting does not appear to be associated with size. Skene (1980) showed in apples stress developed at particular fruit sizes whereas results from these experiments have shown radish hypocotyls of different sizes split at similar rates and radishes of similar sizes can split at different rates depending on irrigation treatment. Stress was not measured in this series of experiments therefore it is not known if the stress in different sizes of fruit is different and the treatments have caused the radishes to be more resistant to these differences or if there is no difference in stress. However, the results consistently show fruit size cannot be used to indicate splitting susceptibility.

In tomato, splitting rates have been shown to be at a peak when growth rates are at a maximum (Dorais *et al.* 2004) this is also broadly true of radishes. Hypocotyl expansion is at a maximum after Growth Stage 41 and splitting also occurs after Growth Stage 41. However, this association is due to physical reasons rather than growth rate causing splitting as the periderm does not become exposed until Growth Stage 41 and consequently cannot split. In fact, experiments in this section have provided evidence contrary to the theory that rapid growth rates are associated with splitting as hypocotyls from the D/W treatments which received a period of drying from Day 8 to Day 18 and then irrigation to harvest had hypocotyls which expanded more rapidly and split less than the hypocotyls from W/W plants which received irrigation for the duration of the experiment.

In this series of experiments, timing of water stress was consistently shown to have an effect on growth splitting. With drying down mid-growth from Day 8 to 18 causing the

greatest reduction in splitting whilst maintaining yield at harvest. Sørensen (1997) also found the timing of water stress had an effect on splitting in carrot. Similar to results from these experiments, Sørensen (1997) observed more splitting in carrots which were grown under fully irrigated conditions compared with carrots grown with a period of drought stress mid-growth or shortly prior to harvest. In carrots, similar to radishes, this period is when rapid expansion is occurring. Sørensen (1997) thought the differences in timing of water stress could be explained by differences in the type of growth occurring during early and late growth (Sørensen *et al.* 1997). Early carrot growth is characterised by cell division whereas during mid-growth the rapid radial root expansion is caused by cell enlargement. Sørensen (1997) suggested the decrease in splitting in carrots may have been due to a decrease in the rate of expansion during the cell enlargement period. In contradiction to this theory experiments on radishes have shown the rate of hypocotyl expansion is more rapid in plants which have received a period of drought stress compared to ones which have not. Since the radishes which have received a period of drying split less yet grow more rapidly, differences in growth rate are not likely to explain the differences in splitting.

The reductions in radish hypocotyl splitting may result from a change in physiology which has made them more resistant to splitting. For example, it is known lignin in cell walls provides structural rigidity and may therefore be associated with splitting susceptibility. Experiments 3.1 in this thesis showed radish splits propagate through the cell wall in a process called plasmoptysis. Lignin biosynthesis is affected by water stress (Lee *et al.* 2007) so it follows if stress occurs at a key time when cell walls are being produced then the lignin content and therefore cell wall strength may be affected. Early plant growth is typically characterised by cell division and this may coincide with the time when the plants are given a period of drying. Growth Stage 41 and the start of rapid hypocotyl expansion may indicate the time when growth changes from predominantly cell division to cell enlargement although further research is required to establish if this is the case.

Another explanation in the reduction in splitting caused by a period of drying and water stress may be priming. Priming is known to have long lasting effects in some species and

the resulting epigenetic changes can have long term effects on gene expression (Bruce *et al.* 2007). A period of water stress at a key time may cause epigenetic changes in the radish plant acclimatising them to the water stress. As a consequence of the changes in physiology due to acclimatisation this may make the plants more resistant to splitting. This could be through changes in physiology which allow the plants to maintain turgor pressure at a level which does not increase susceptibility to splitting or by changing the cell wall components. Again further work is required to investigate this theory.

3.6 Chapter 3 Growth splits: Discussion

The series of experiments (Experiments 3.1 to 3.12) in this section identified several factors which are associated with growth splitting in radish. These experiments also identified several factors which were not associated with splitting despite previous research indicating they might be.

3.6.1 Differences in splitting susceptibility between cultivar

In Experiment 3.1 a difference in splitting susceptibility between cultivars of radish was demonstrated. *Raphanus sativus* 'Rudi' was found to have significantly more (P=0.001) splits at harvest than 'Celesta' or 'Topsi'. This is in keeping with previous research where cultivar has been shown to affect susceptibility to splitting in kohlrabi (Lippert 1999) carrot (Hartz *et al.* 2005; Hole *et al.* 1999) cherry (Demirsoy & Demirsoy 2004) and tomato (Dorais *et al.* 2004). In Experiment 3.2 a significant difference (P=0.031) was again observed in splitting between cultivars. The cultivar 'Celesta' split the least but not significantly less than 'Rudi', 'Rougette' or 'Saxa 2'. The cultivar 'Kaspar' split the most but not significantly more than 'Rudi', 'Rougette' or 'Saxa 2'. As was found in Experiment 3.1, the cultivar 'Celesta' had the lowest number of splits and the greatest yield in terms of total and hypocotyl weight.

In contradiction to results from Experiments 3.1 and 3.3, in Experiment 3.7 splitting rates for 'Celesta' were not significantly lower than 'Rudi' or 'Saxa 2'. In addition, 'Celesta' did not have as great a reduction in splitting when grown under dry conditions as the other two cultivars.

Results from experiments in Chapter 3 would suggest cultivar does have an effect on splitting susceptibility but the results are not consistent. It seems unlikely therefore that breeding for resistance to splitting would prove fruitful.

3.6.2 Periderm thickness

In fruit, cuticle thickness is thought to explain some of the differences in splitting susceptibility between cultivars as Demirsoy (2004) found a negative correlation between

cuticle thickness and splitting in eight cultivars of sweet cherry. In Experiment 3.1, no significant difference (P=0.674) was found in periderm thickness between cultivars of radish 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. In addition, although periderm thickness was not significantly different between cultivars, the thickness of the radish periderm tended (P=0.045) to be greater for the split radishes than the non-split radishes. These results are in accordance with research into splitting in carrots where it has been shown that removing the periderm makes them more resistant to splitting (Hartz *et al.* 2005). These results would suggest that periderm thickness may have an effect on splitting susceptibility but periderm thickness is not the same for all plants of a particular cultivar. Therefore it may be beneficial to select cultivars which tend to have a thinner periderm but other factors will also have an effect on splitting. Understanding the factors other than cultivar which affect periderm thickness is an area which needs further investigation.

3.6.3 Hypocotyl shape

Splitting occurs when mechanical stress exceeds the ability of the tissue to withstand it (Hole *et al.* 1999). Differences in stress within plant tissue may cause it to split depending on the degree of stress and the mechanical strength of the tissue. As stress is more uniformly spread in globes than shapes which deviate from this (Emmons & Scott 1998) shape may be an important factor in determining splitting susceptibility.

Iwata *et al* (2004) showed Japanese radish shape was determined by both genetic and environmental factors. Iwata *et al* (2004) used different soil types to vary environmental growing conditions. The results showed there were significant differences in shape for all varieties and for all soil types and there was no interaction between soil type and variety for most shape characteristics (Iwata *et al.* 2004) suggesting soil type had similar effects on all varieties tested and therefore neither genetics nor environment alone can explain shape but it may be possible to predict the effects of cultivar or environment on shape.

In order to investigate if there is any correlation between susceptibility to growth splitting and hypocotyl roundness, roundness was measured in Experiment 3.1: The effects of radish cultivar on susceptibility to growth splits, Experiment 3.3: Determining the growth stages and growth rate of five cultivars of radishes and Experiment 3.12: Investigating the effects of changes in VWC at Growth Stage 41 on the susceptibility of radishes to growth splits.

In Experiment 3.1, there was no significant ($P=0.21$) difference between cultivars in hypocotyl roundness despite there being a significant difference ($P<0.001$) in splitting between cultivars. *Raphanus sativus* 'Rudi' was found to have significantly ($P<0.001$) more growth splits than 'Celesta' or 'Topsi'. The cultivar 'Rudi' had 43.75% split radishes at harvest compared to 8.33% of 'Topsi' and 2.08% of 'Celesta'. These results suggest there is no relationship between roundness measured by dividing the length of the hypocotyl by the maximum width and growth splitting.

In Experiment 3.3, five cultivars of radish were investigated and significant differences in hypocotyl roundness were observed. As in Experiment 3.1 there was no significant difference in the roundness of the cultivars 'Celesta' and 'Rudi' but there was a difference observed between these two cultivars and 'Rougette' and 'Saxa 2'. The cultivars 'Rougette' and 'Saxa 2' were not significantly different in roundness to each other. The cultivar 'Kaspar' was not found to be significantly different in roundness to any of the other cultivars. However, again as in Experiment 3.1, although a significant difference in growth splitting was observed ($P=0.031$), mean cultivar roundness was not found to be correlated with mean cultivar growth splits. The cultivar 'Celesta' split the least but not significantly less than 'Rudi', 'Rougette' or 'Saxa 2'. The cultivar 'Kaspar' split the most but not significantly more than 'Rudi', 'Rougette' or 'Saxa 2'.

In Experiment 3.12, only one cultivar was grown but under different irrigation regimes. In this experiment, 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. 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. Although the roundness of the radish hypocotyls was consistent between all treatments except T4, the mean number of split radish per pot varied more and therefore there was no correlation between hypocotyl roundness and susceptibility to splitting. The number of radishes with growth splits was significantly lower ($P<0.001$) for plants in T2 and T4 (mean of 1.38 and 1.67 split radish per pot respectively) than T5 (mean of 3.79 split radish per pot) and T5 radishes had significantly fewer ($P<0.001$) splits than radishes from T1 and T3 (mean of 6.5 and 7.5 split radishes per pot respectively).

Differences in the roundness of cultivars may have been missed if some radish cultivars were flatter or more irregularly shaped in general but had a similar maximum width and length ratio.

3.6.4 Mode of failure

The mode of failure depends on the relative strengths of the intercellular bonds and cell walls (Lin & Pitt 1986). In many vegetable crops, root and tuber splitting is thought to occur predominantly due to plasmoptysis as opposed to cellular debonding (Lippert 1999; McGarry 1993). The mode of failure is important as the effects of turgor are thought to depend on the type of splitting which is occurring. When the mode of failure is plasmoptysis higher turgor pressure has been shown to reduce tissue strength but if splitting occurs as a result of cell debonding the opposite is true (Lin & Pitt 1986). As discussed in Chapter 1, previous work by Skok (1941) showed sections of split radishes which appeared to have failed due to plasmoptysis rather than cellular debonding although the mode of failure had not been discussed in the paper. Therefore, in Chapter 3 split radishes were sectioned to verify the mode of failure within radish hypocotyl tissue. In these sections, ruptured cells were again observed along the split surfaces, it was concluded these radishes had split by plasmoptysis. This mode of failure is comparable to other vegetables such as kohlrabi (Lippert 1999) and carrot (McGarry 1993) and it is likely this is due to the limited intercellular space within these types of vegetable tissue. As the

mode of failure within the radish hypocotyl was plasmoptysis turgor pressure is likely to be an important factor in determining splitting susceptibility.

3.6.5 Growth stage

Evidence from other crops suggests growth splitting is affected by growth stage, splitting in carrots has been shown to be affected by crop maturity with splitting mainly occurring later in crop development (Gracie & Brown 2004).

Experiment 3.2 successfully established the growth stages for *Raphanus sativus* 'Rudi'. Experiment 3.3 showed these were relevant to a range of other cultivars of radishes and therefore likely to be applicable to radishes generally. The experiments showed that similar to other crops, splitting in radishes is affected by growth stage however the cause is different. Radishes are unable to split until Growth Stage 41 which occurs midway through the hypocotyl expansion. At this point the exodermis and cortex rupture revealing the periderm. Growth and harvest splits are of the periderm of the radish and the periderm is only fully formed and exposed after Growth Stage 41 therefore it cannot split until this time. In glasshouse grown radishes Growth Stage 41 is usually completed around Day 17.

3.6.6 Growth rate

Growth rate has been suggested by several researchers as a potential explanation for differences in splitting. Latimer (1991) suggested the low splitting rate observed in radishes which were exposed to mechanical leaf damage was a result of slow growth. However, conversely Dowker and Jackson (1977) found a slower growth rate in carrot was correlated with higher levels of splitting. Growth rate has been associated with splitting both in terms of typical growth rate of a cultivar (Lippert 1999) but also in growth rate which is altered as a result of environmental conditions (Latimer 1991). In this thesis the growth rate of radishes was measured as part of a number of experiments both investigating the differences in splitting susceptibility for different cultivars, the differences in susceptibility of radishes grown with different VWC and the interaction between the two. The growth rates were then compared to splitting rates to investigate if there is a

relationship between the two. The results from these experiments are summarised and discussed here.

3.6.6.1 The effects of cultivar on growth rate and growth splitting

In Experiment 3.1 the growth rate of radishes was shown to be affected by cultivar. The cultivar ‘Celesta’ had significantly larger whole weight ($P<0.001$), trimmed weight ($P<0.001$), plant biomass ($P<0.001$), number of leaves ($P<0.001$) and length ($P<0.001$) and width of the hypocotyl ($P=0.008$) at harvest compared to the cultivars ‘Topsi’ and ‘Rudi’. As all the cultivars were sown on the same day, germinated at a similar time and were harvested after the same duration ‘Celesta’ must have grown the most rapidly to reach this larger size. In Experiment 3.1, cultivar was also shown to affect susceptibility to splitting. The cultivar ‘Celesta’ split the least ($P<0.001$) of the three cultivars examined but grew the most rapidly. ‘Rudi’ grew more slowly than ‘Celesta’ and split significantly more. Had only these two cultivars been grown then a similar but opposite conclusion to that of Lippert (1999) would have been made. Lippert (1999) found the rapidly growing cultivar ‘Express Forcer’ was twice more likely to crack than the slower growing cultivar ‘Noriko’ and concluded that cracking in vegetative organs was due to rapid growth. However in Experiment 3.1, despite ‘Celesta’ growing the most rapidly and splitting the least, no correlation was found between rate of growth and splitting. ‘Celesta’ grew faster than ‘Topsi’ yet there were no significant differences in splitting between ‘Celesta’ and ‘Topsi’. There were no significant differences in the size at harvest of ‘Topsi’ and ‘Rudi’ but ‘Rudi’ split significantly more than ‘Topsi’. Therefore, there was no relationship between the rates of growth of a cultivar and how much that cultivar splits during growth, if there was a relationship between growth rate and splitting it would be expected the cultivars which grew at the same rate would split at the same rate. Results from Experiment 3.1 demonstrate how the conclusions of Lippert (1999) cannot be relied upon as they were based on the comparison of cracking rates in just two cultivars of kohlrabi.

Experiment 3.3 showed the rate of hypocotyl expansion was not consistent between cultivars over the growing period. The rate of hypocotyl expansion varied between cultivars prior to Day 15, which is when Growth Stage 41 occurred in this experiment.

After this however, the growth rates were very similar for the hypocotyls of all cultivars. Therefore, the rate of expansion prior to Day 15, or Growth Stage 41, appears to determine time to harvest and could be used by growers to predict harvest date for different cultivars.

As in Experiment 3.1, in Experiment 3.3 no correlation was found between rate of growth and splitting. In this experiment all cultivars were harvested by hypocotyl width, as they would be commercially, rather than on a particular date as in Experiment 3.1. Results from this cultivar experiment demonstrate overall growth rate is not an explanation for the differences in splitting between cultivars in radishes.

In Experiment 3.7 an investigation into the effect of both cultivar and VWC on growth splitting was conducted. Three cultivars of radishes, 'Rudi', 'Celesta' and 'Saxa 2' were grown under two different VWC treatments, Wet and Dry. Each treatment group was harvested when the mean hypocotyl width was of the median commercial size. The cultivars 'Celesta' and 'Saxa 2' were affected to a greater extent by the VWC treatments and had a seven day difference in harvest time between Wet and Dry treatments compared to 'Rudi' which had a five day difference. For all cultivars the radishes which were grown with the Wet treatment had a more rapid hypocotyl expansion rate.

3.6.6.2 The effects of VWC on hypocotyl growth rate and splitting

Experiments 3.5, 3.6, 3.8, 3.9, 3.10 and 3.12 investigated various components of VWC on susceptibility of *Raphanus sativus* 'Rudi' to growth splitting. As part of these experiments, hypocotyl growth rate was recorded and investigated for any relationship with splitting. The main findings from these experiments is summarised in Table 3-112.

Table 3-112 Summary of results from experiments in Chapter 3 which measured hypocotyl growth rate and splitting

Experiment	Effect of VWC on hypocotyl growth rate	Correlation between growth rate and splitting?
3.5	Lower VWC resulted in slower expansion	No correlation
3.6	Dry treatment had slower expansion	Dry treatment split less
3.8	Expansion rate increased with increasing irrigation frequency	No difference in splitting between treatments
3.9	No drought or no drought late in growth result in the greatest hypocotyl expansion rates	No correlation
3.10	Decreases or increases in VWC resulted in decreases or increases in expansion rate respectively	No correlation
3.12	Hypocotyl weight positively correlated to VWC	No correlation

Experiments 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 and 3.12 all showed a decrease in growth rate of the hypocotyl associated with lower VWC. This is in keeping with previous research where it has been shown the usual response of plants to drought is to limit growth (Wilson 1988) and previous research specifically into radishes in which drought conditions were found to reduce or stop cellular division and cellular expansion which would reduce growth rate (Joyce *et al.* 1983). The reduction in growth rate of radishes which are irrigated less suggests the water deficit is resulting in a lower turgor pressure. Turgor pressure is known to regulate both cell division and enlargement in plants (Kirkham *et al.* 1972). Having a water deficit for a period of time would reduce turgor and therefore reduce growth during this period.

In Experiment 3.9 it was found timing of irrigation had an effect on growth rate. Irrigation later in growth and in the case of this experiment, in the fourth and fifth week, appears to

determine how long it takes radishes to achieve a commercial hypocotyl harvest size. There was no relationship between this and splitting.

Experiment 3.10 gave greater resolution to the fluxes in hypocotyl growth rate with changing VWC. For this experiment, there were three treatment groups. W/W received irrigation for the duration of the experiment, W/D received irrigation until Day 18 and D/W received irrigation from Day 19 until harvest. Hypocotyl width was measured at intervals throughout the experiment. On Day 18 when the treatments changed there was no difference in hypocotyl width between treatments. However, by Day 22, early in the second irrigation treatment period, timing of irrigation was having a significant effect on hypocotyl width and therefore growth rate. It appeared the earlier period of drought was having a delayed effect on the hypocotyl width of the plants from the D/W treatment as these were significantly smaller than the W/W treatment. The width of the plants from the W/D treatment was midway between the widths of the other two treatments but not significantly different from either of them suggesting the second drought period was beginning to have an effect on limiting hypocotyl expansion rate. By Day 24 the hypocotyl width was significantly greater for the W/W plants than either of the treatments which had received a period of drying. This suggests the plants from the W/D treatment were responding to water stress as the growth rate of the hypocotyls had slowed. The plants from the D/W treatment were increasing at a steady rate and do not appear to have been limited by the earlier period of water stress. Between Day 24 and Day 28, the rate of hypocotyl expansion was greatest for D/W as there was no significant difference in the width of the W/W and D/W treatments at harvest when previously the W/W plants had been larger than the D/W plants. It was thought this may have been due to compensatory growth and results from this experiment are in accordance with this theory. At harvest W/W and D/W hypocotyls were both significantly ($P<0.001$) larger than the W/D treatment hypocotyls which had decreased in size since Day 24. The decrease in size suggests these plants were experiencing severe water stress and were wilting as a result. There was no relationship observed between growth rates and splitting.

By investigating the effects of timing and duration of a period of drought, Experiment 3.12 showed duration of the period of drying is an important component of yield and growth rate. A negative correlation was found between the duration of the dry period and the trimmed hypocotyl weight at harvest. The results from this experiment would suggest yield is linked to mean VWC. No association was found between growth rates and splitting.

3.6.6.3 The effects of VWC on leaf growth

As well as hypocotyl growth rate, leaf growth was also shown to be affected by VWC. Differences in growth rate of leaves and hypocotyls were observed under the different irrigation regimes in both Experiment 3.6 and 3.7. The usual response to limited water availability is for assimilates to be directed more towards the root than the leaves thus reducing the shoot to root ratio (Wilson 1988). Results from Experiments 3.6 and 3.7 suggest in the case of radishes, which have a swollen hypocotyl and tap root, it appears assimilates are directed to this organ under conditions of drought in a similar way to which they would be towards the taproots and roots in other plants.

In Experiment 3.9 leaf number did not appear to be solely dependent on VWC as there was no clear pattern in relation to mean water content. The plants which received a period of deficit irrigation followed by watering to field capacity had the largest number of leaves followed by the treatment which was irrigated to field capacity for the duration of the experiment. After this, the treatment which received deficit irrigation for the duration of the experiment had the second lowest number of leaves and finally the treatment which received irrigation to field capacity followed by deficit irrigation had the fewest leaves. As was found with radish hypocotyls in Experiment 3.10 as discussed above, it would appear the radish plants which received a period of deficit irrigation followed by a period of irrigation were conditioned to grow under dry conditions and then were able to exceed the growth rate of the radish plants which received irrigation for the duration of the experiment. This is often termed stored or compensatory growth. Similarly, the radishes which were watered to field capacity and then received deficit irrigation had fewer leaves than the radishes which received deficit irrigation for the duration of the experiment

despite receiving more water in total. Again it would appear the plants had been conditioned to grow under environments experienced early in growth and reacted more severely to the decrease in water compared to plants which had experienced it from early in growth. It is thought if water stress develops slowly, osmotic adjustment may occur (Kramer 1983). This enables growth to continue at lower water potentials than would otherwise be possible. Osmotic adjustment has been shown to occur in leaves of wheat plants (Kramer 1983) and may explain the results observed in this experiment.

In Experiment 3.10 there was no significant difference in the number of leaves in the W/D and D/W radish at harvest but these both had significantly ($P<0.001$) fewer leaves than the W/W radish. This is in contrast to the results from Experiment 3.9 where the plants which received a period of drying followed by irrigation had the largest number of leaves at harvest. However, the number of leaves for D/W increased at a faster rate in the final few days compared to W/W and it may be that if the plants had been harvested later then a similar pattern to the previous experiment would have been observed. In contrast to leaf number, the leaf area at harvest was significantly ($P<0.001$) different between all treatment groups. The radishes grown with W/W treatment had on average the greatest leaf area (102.4 cm^2), the radish grown with D/W treatment were second largest on average (67.3 cm^2) and the W/D radish had the smallest leaf area on average (35.7 cm^2). This shows that although there was no significant difference in the number of leaves between W/W and D/W there was a difference in the size of the leaves between treatments and this pattern followed the amount of irrigation the plants received.

3.6.7 Commercial data

The results from Experiment 3.4 and analysis of the commercial data suggest weather had an effect on splitting. Relative humidity and accumulated rainfall during growth were both positively correlated with splitting suggesting radishes are more likely to split with increasing rainfall. Relative humidity and temperature both during growth and at harvest tended to have a negative correlation with splitting suggesting radishes are more likely to split with lower temperatures. It is hypothesised that increased rainfall during growth

would lead to increased hypocotyl water content and turgor pressure. Similarly, high relative humidity may limit transpiration rates and also increase turgor pressure and low temperatures are also thought to increase splitting susceptibility by increasing turgor pressure. High turgor pressure has been shown in carrots (McGarry 1993, 1995) to result in increased splitting susceptibility and low temperatures have been shown to decrease failure force in potatoes (Bourne 1982).

3.6.8 VWC

In keeping with research on the large winter varieties of radishes (Kang & Wan 2005) research in this Chapter 3 showed that when radishes are irrigated to a constant VWC the VWC has an effect on splitting. In Experiment 3.6 and 3.7 splitting rates were greater in radishes which were grown in greater VWCs.

In contradiction to research on the effects of irrigation frequency on winter radishes (Wan & Kang 2005), results from Experiment 3.8 in Chapter 3 show fluctuations in soil water potential during growth had no effect on rates of growth splitting. However, experiments consistently showed timing of water availability during growth did affect growth splitting. Results were similar to those of Sørensen (1997) who found the timing of water stress had an effect on splitting in carrot. Results both from Sørensen (1997) and from this chapter showed plants grown under fully irrigated conditions, split more than plants grown with a period of drought stress mid-growth when rapid expansion was occurring. In Experiment 3.12 it was demonstrated growth splitting could be predicted accurately by measuring the VWC at Growth Stage 41. Experiment 3.2 and 3.3 showed this growth stage can be identified non-destructively in the field. In Experiment 3.12 higher VWC at this point resulted in a higher level of splitting at harvest.

Results from experiments in Chapter 3 although able to find correlations between VWC and splitting were unable to provide an explanation for why a lower VWC during growth or a period of drought prior to Growth Stage 41 might result in a lower susceptibility to splitting. It had been proposed that a higher VWC might lead to an increase in hypocotyl water content or RWC and therefore an increase in turgor. An increase in turgor is thought

to put more stress on cells making them less resistant to splitting as a result of plasmoptysis. In other crops the increase in splitting which is associated with greater water availability is thought to be due to an increase in pressure on the skin from within as a result of water uptake by the vascular system into the tissue (Sekse 1995). Higher pressure within the organ results in the tissue being more susceptible to splitting as less additional force is required to rupture tissue which is already under tension. Plasmoptysis was shown in Experiment 3.1 to be the mode of failure of tissue in a radish hypocotyl. However, in experiments conducted as part of Chapter 3 inconsistent results were found for the relationship between hypocotyl WC and RWC and splitting and no relationship was found to link hypocotyl WC with hypocotyl water pressure in Experiment 3.7 (Table 3-113) suggesting the causes for growth splitting susceptibility may be more complex than just hypocotyl water content and turgor pressure.

Table 3-113 Summary of results linking hypocotyl water content and splitting from experiments in Chapter 3

Experiment	Trend
3.3	Trend (P=0.082) linking higher hypocotyl RWC with higher levels of splitting
3.6	Treatment with higher hypocotyl water content had significantly (P<0.001) more growth splits and radishes which split as a result of impact texture analysis (P<0.001)
3.7	Positive correlation between VWC and hypocotyl WC. Positive correlation between hypocotyl WC and splitting. No relationship found between hypocotyl water pressure and hypocotyl water content.
3.8	No relationship between hypocotyl WC and growth or harvest splitting P=0.478 for growth splits P=0.613 for splits after storage (growth and harvest splits)
3.9	No difference in splitting between treatments. Hypocotyl WC higher for split radishes (93.71% compared to 93.19%) but the difference was not statistically different (P=0.879)
3.10	Negative linear correlation between hypocotyl water content and number of splits (P<0.001)
3.11	Negative linear correlation between hypocotyl water content and number of splits (P<0.001)
3.12	Negative linear correlation between hypocotyl water content and number of splits (P=0.036)

In Experiments 3.10, 3.11 and 3.12 which investigated the effects of timing of water availability on growth, marketable yield was consistently greatest for plants which were given a period of drying from Day 8 to Growth Stage 41. This was because the size of these radishes at harvest was similar to that of radishes which had been irrigated for the duration of the experiment but there were far fewer split radishes.

3.7 Chapter 3 Growth Splits: Conclusions

- Choice of cultivar affects splitting susceptibility
- There is a trend linking greater periderm thickness with increased splitting susceptibility
- VWC has an effect on growth rate
- For all cultivars tested, radishes grown under dryer conditions had a slower rate of growth than radishes grown under greater available water contents
- No correlation was found linking growth rate which is altered as a result of VWC and splitting
- When irrigated to a constant substrate VWC, radishes grown under dryer conditions had a greater marketable yield as they had lower levels of splitting and although they grew more slowly they did eventually achieve the same size
- Growth rate was different for different cultivars but did not correlate with splitting
- No relationship was found between irrigation frequencies and splitting
- Irrigation frequency affected growth rate. Frequent irrigation resulted in the most rapid growth rate and the most infrequent irrigation resulted in the slowest growth rate
- Splitting at harvest can be predicted by the VWC at Growth Stage 41
- Irrigation prior to harvest and more specifically in the final 10 days prior to harvest determines yield at harvest.
- Hypocotyl growth rate is increased by a period of drying from Day 8 to Day 18 which is then alleviated by irrigation until harvest. This is thought to be due to compensatory growth
- Marketable yield was greatest for plants which were given a period of drying from Day 8 to Growth Stage 41. These plants grew at a similar rate to plants which were irrigated for the duration of growth, had a lower level of splitting and tended to be more consistent in size at harvest

The factors which were found to be associated with susceptibility to growth splitting in Chapter 3 are summarised in Figure 3-51.

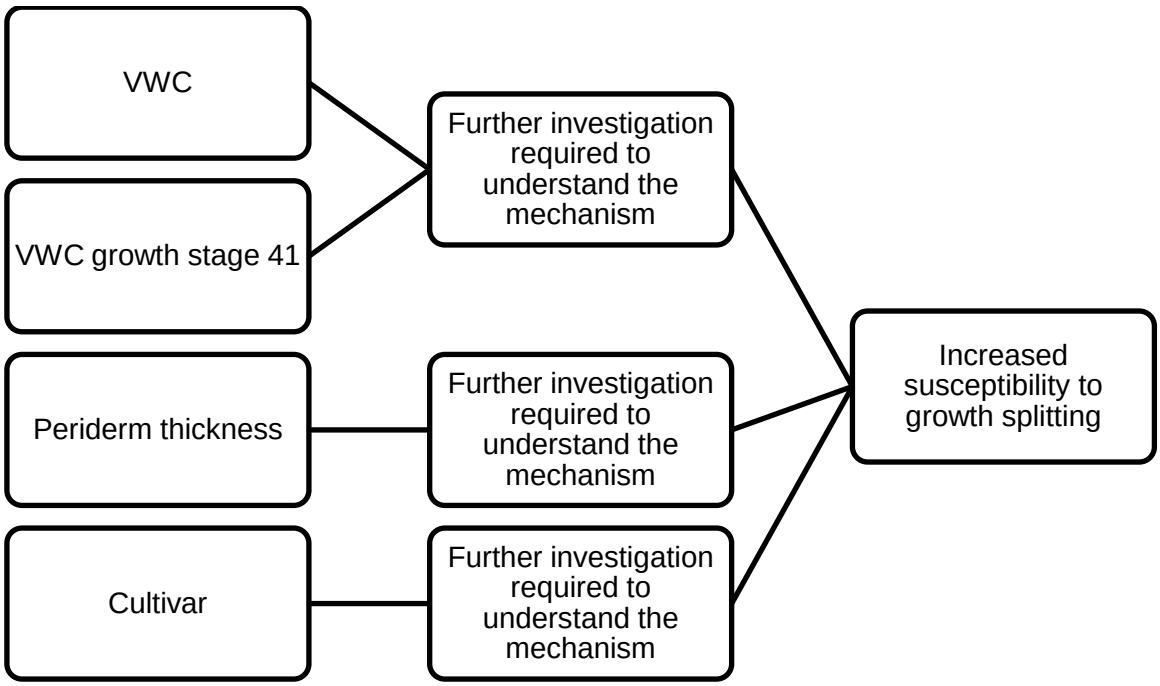


Figure 3-51 Factors which are associated with susceptibility to growth splitting

4. Harvest Splits

4.1 Chapter 4 Harvest splits: Introduction

Hypocotyl splitting in radish can occur during growth, harvest or post-harvest. In Chapter 4 factors affecting splitting susceptibility post-harvest will be investigated.

Commercial data: Much of the research into the causes of splitting has begun by investigating the environmental conditions and agronomic practices of commercially grown produce which correlate with levels of splitting. This then allows future experiments to be conducted to test the observed relationships under controlled environmental conditions. In investigations looking at field grown crops, water potential and temperature have been linked with harvest splitting. Hartz *et al* (2005) found both turgor and air temperature prior to harvest were positively correlated with splitting in carrot suggesting hypocotyls with greater water potentials and harvesting at higher temperatures may result in more splitting. Research is required to investigate the environmental conditions during commercial production which correlate with harvest splitting in radishes.

Relative water content (RWC): Irrigation and water availability during growth may also affect hypocotyl water contents at 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 at harvest, though salinity effects on osmotic potentials can be complex. Post-harvest washing may also increase hypocotyl water content as preliminary experiments have shown radishes are able to absorb 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 radishes.

Temperature: 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 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 led the investigators to conclude that the same mechanism must explain both the effects of temperature and turgor.

The main objective of work carried out in Chapter 4 was to identify some of the factors which effect susceptibility to harvest splitting in radishes. This chapter will begin with Experiment 4.1 an analysis of commercially grown radishes to identify environmental factors which correlate with splitting susceptibility. Then a series of experiments (Experiments 4.3 to 4.7) will investigate the relationships between hypocotyl water content and hypocotyl RWC with splitting. The chapter will then conclude with Experiment 4.8 which is an investigation into the relationship between hypocotyl temperature and harvest splitting.

4.2 Experiment 4.1: Preliminary experiment optimising the method for hypocotyl saturation

4.2.1 Experiment 4.1: Introduction

In order to investigate the relationship between RWC and splitting, a standard method to saturate the radish hypocotyls was required. The method needed to be easily replicable and to not influence any other measurements which were necessary.

Aim:

- The aim of this preliminary experiment was to investigate the differences in water uptake by radish hypocotyls cut into different numbers of sections.

Hypothesis:

1. There will be no difference in the speed of water absorbed by radish hypocotyls cut into different numbers of segments

4.2.2 Experiment 4.1: Materials and method

Radishes grown by G's Growers were purchased from Waitrose. These were weighed using a PCB 2500-2 balance (Kern and Sohn GmbH, Balingen, Germany) then either left whole, cut into halves, quarters or eighths and placed into plastic pots containing approximately 100 mL of dH_2O. Cutting the radishes into sections increased the surface area for water uptake and potentially increased the rate with which saturation occurred.

The pots of radishes were placed into a controlled environment at 2.5°C. The temperature 2.5°C was chosen as it is below 4°C, the temperature at which plant growth stops but above freezing as this may have damaged the cells and affected the amount of water which they could absorb.

The radishes were then weighed 5 times over the subsequent 48 hours. Before weighing, the radishes were all patted dry using paper towel.

4.2.3 Experiment 4.1: Results and discussion

By 48 hours all of the radishes had begun to plateau in weight suggesting they were reaching saturation. The radishes which were cut into eight segments had the greatest and most rapid change in weight (Figure 4-1). Therefore, cutting the radishes into eight segments was chosen as the standard method for calculating RWC for future experiments.

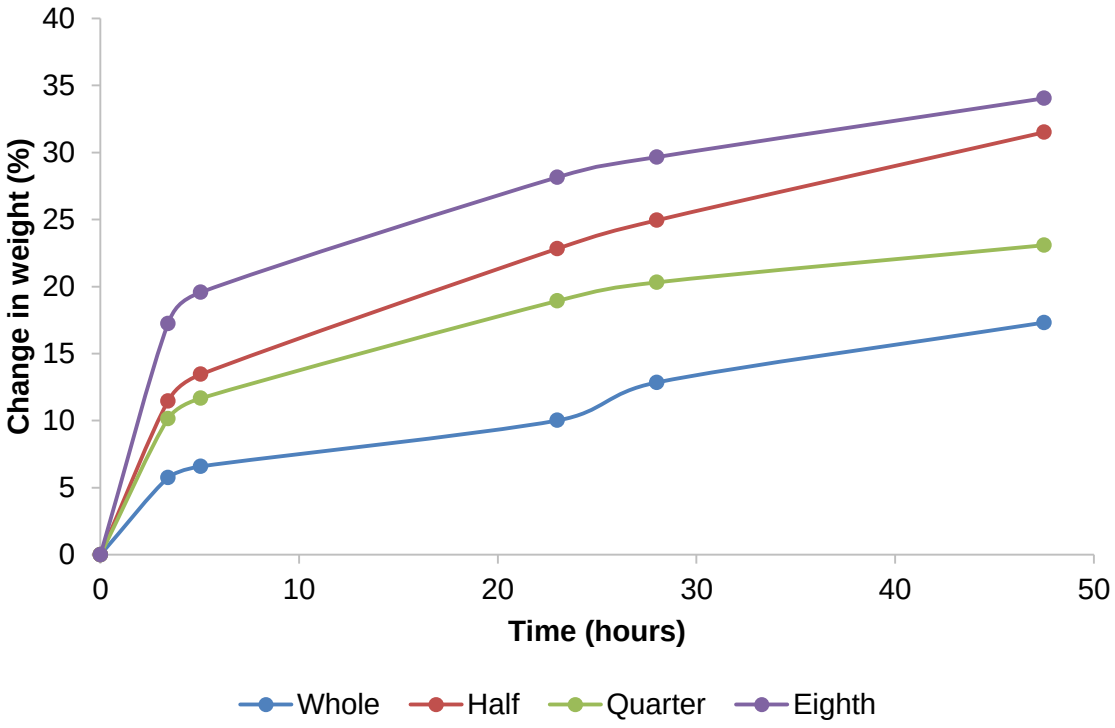


Figure 4-1 Change in weight over time of radish hypocotyls cut into different numbers of segments and placed into dH_2O (n=2)

4.3 Experiment 4.2: Analysis of commercial QA split data

4.3.1 Experiment 4.2: Introduction

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; McGarry 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 water loss as a result 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.

Experiment 4.2 analysed splitting data from G's Growers QA in 2014 for the cultivar 'Celesta' and combined this with additional experiments on splitting susceptibility and measurements of RWC conducted at HAU. By investigating commercial splitting trends and correlating these with weather data, RWC and a standardised test for splitting susceptibility it should be possible to determine if weather appears to affect splitting by changing the RWC of the radish hypocotyl. The factors which appear to be correlated with splitting can then be investigated under controlled conditions in future experiments to determine more conclusively if they have an effect on splitting.

The main objective of the analysis was to investigate commercial splitting trends and correlate these with hypocotyl RWC and weather during growth determine if environmental conditions during growth appear to be related to hypocotyl RWC and splitting.

Aim: To determine if the:

- Amount of splitting observed at a commercial site can be predicted by the weather conditions during growth and harvest of the radishes;
- Weather conditions correlate with the RWC of the radishes;
- RWC of the radishes correlates with the amount of splitting recorded by the QA team at G's Growers
- RWC of the radishes correlates with susceptibility to splitting as a result of impact
- Impact texture analysis can be used to test susceptibility to splitting

Null hypothesis:

1. No significant relationship will be observed between weather parameters and splitting or post-harvest splitting susceptibility in commercially grown radishes
2. There will be no correlation between weather conditions and RWC of the radish hypocotyls
3. RWC of the radishes will not correlate with the amount of splitting recorded by the QA team at G's Growers
4. RWC of the radishes will not correlate with susceptibility to splitting as a result of impact
5. Impact texture analysis is not an accurate way to test susceptibility to splitting

4.3.2 Experiment 4.2: Materials and Methods

Production and delivery: During the 2014 growing season, on 23 occasions, a box of radishes from G's growers, Feltwell, was couriered on the day of harvest to arrive at 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 following standard commercial harvesting procedure but were removed from the harvested batch at an unwashed stage and had not been washed, graded or trimmed prior to transport. For transport the radishes were placed into a clear plastic 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. The batch number was written on the box. This allowed a comparison with the data gathered at G's and the drill date and harvest date to be determined.

Processing: 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 values recorded for the same batch by the QA team at G's Growers. At G's Growers, radishes are assessed after they have been washed and trimmed. The maximum diameter of the radishes was measured and they were weighed. Any radishes which were split were cut into eight pieces and placed in a pot of approximately 100 mL of dH_2O and placed into cold storage at 2.5°C for 48 hours to saturate (as had been determined by Experiment 4.1).

Testing splitting susceptibility: The remaining radishes were tested for susceptibility to splitting using impact texture analysis. 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.

RWC: Additional non-split radishes which were of commercial size, between 18 and 32 mm 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 100 mL of dH_2O 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 100 mL 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 RWC was calculated using the equation (Kirkham 2005):

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

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 (VSN International 2011).

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 batch 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.

All data was analysed using regression, 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.

4.3.3 Experiment 4.2: Results

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 4-1).

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 4-1).

The amount of splitting which was observed on arrival at HAU 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 impact texture analysis was correlated with RWC but the number of radishes recorded as split by G's and the number of radishes which arrived split at HAU were not (Table 4-1).

RWC of the radish hypocotyls was not correlated with any of the weather conditions during growth or at harvest (Table 4-1).

Table 4-1 Correlation matrix showing the relationships (R^2) between splitting (as measured by the quality assessment team at G's, the number which were split on arrival at HAU and the number which split as a result of dropping), RWC and weather (as measured by the Met Office and BADC) during growth for radishes grown in 2014 by G's Growers (n=23)

	GS	**FS**	**DS**	**RWC**	**R**	**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					
R	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 as a result of impact texture analysis, RWC = RWC of the radish hypocotyls, R = 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 4-2).

Table 4-2 Model determined by multiple linear regression and stepwise deletion for the relationship between splitting observed at G's by the quality assessment team and weather conditions measured by the Met Office and BADC.

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), R = 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 4-3).

Table 4-3 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 (%)
T_g[1]	0.364	-0.124	14.13	0.134	38.32
RH_h	0.062	0.0731	80.18	0.0367	18.75
T_h	0.102	-0.245	14.01	0.142	0.003
R	0.062	0.0441	29.23	0.0221	7.75

[1] 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), R = Accumulated precipitation (mm), TSS is the total sum of squares

Using a simple linear correlation showed the number of radishes which were split on arrival at HAU (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 HAU. This may have been as a result of the additional handling and transport which the radishes received on the way to HAU. 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). As expected, the number of radishes which split after dropping was greater than the number of radishes which were split on arrival at HAU. GS and DS were not highly correlated but there was a trend (P=0.091) (Table 4-4).

Table 4-4 Correlations between different split types of radishes grown by G's Growers in 2014

Variables correlated	**P**	**Variance accounted for (%)**	**SE**	**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, SE = standard error

The number of radishes which split after dropping was highly correlated (P<0.001) with hypocotyl RWC (Table 4-5).

Table 4-5 Correlation between the numbers of radishes grown by G's Growers which split when dropped and RWC

Variables		**P**	**Variance accounted for (%)**	**SE**	**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. SE = standard error

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 HAU 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 of 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 4-6).

Table 4-6 RWC and water content (WC) of radishes, grown by G's Growers, which were not split, which were split on arrival at HAU and which split after they were dropped

Split type	n	RWC	WC
Not Split	575	0.888 a[1]	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]Denotes difference at the 5% level, where letters are shared no significant difference is present between values.

4.3.4 Experiment 4.2: Discussion

Analysis of commercially produced radishes was conducted in 2014 to determine if, a) the amount of splitting observed by G's can be predicted by the weather conditions during growth and harvest of the radishes; b) the weather conditions correlate with the RWC of the radishes; c) the RWC of the radishes correlates with the amount of splitting recorded by G's and finally d) 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. In this investigation, the first null hypothesis was rejected because similar to the findings of Hartz *et al* (2005) on carrot, a relationship was observed between some weather parameters and splitting.

There were more splits on arrival at HAU 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 HAU. 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 HAU 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 rejecting the fifth null hypothesis.

The number of radishes which split as a result of impact texture analysis was highly correlated with RWC ($P<0.001$) therefore, the fourth null hypothesis was rejected.

However, in support of the third and second null hypotheses 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 HAU 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. These results indicate the split radishes may have had, had high RWCs at the point of splitting as they still maintained at high water content. However, they may have lost more water than the non-split radishes in the time between splitting and measurement of the RWC as a result of a greater rate of water loss from the split surfaces resulting in a lower RWC at the point of measurement compared to the point at which they split.

The first null hypothesis was rejected as 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 HAU 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 RWC 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.

4.4 Experiments 4.3-4.7: Investigating the effect of hypocotyl water content on the susceptibility of radishes to harvest splits

4.4.1 Introduction: Experiments 4.3-4.7

In Experiment 4.2, relative humidity and accumulated precipitation were observed to be positively correlated with the amount of splitting recorded at G's by the quality control team. Hypocotyl RWC and splitting as a result of impact were also found to be positively correlated. These results suggest a high accumulated precipitation or relative humidity may have resulted in a high hypocotyl RWC which may have made the hypocotyls more susceptible to splitting. Many of the results from Experiment 4.2 were based on observational data therefore Experiments 4.3-4.7 were conducted under controlled conditions to investigate the relationship between hypocotyl water content and splitting susceptibility further.

It is thought the post-harvest hypocotyl water content of radishes may have an effect on splitting susceptibility by affecting the turgor pressure of the cells. In Chapter 3 the mode of failure of split radish hypocotyls was revealed to be plasmoptysis. Higher turgor pressure has been shown to reduce tissue strength and increase tissue failure through plasmoptysis (Lin & Pitt 1986). Hypocotyl water content affects turgor pressure because pressure potential increases as water enters a cell. The increase in the amount of water inside the cell exerts an outward pressure which is opposed by the cell wall. The cell wall is then placed under increased tension. Under increased turgor pressure cells may be more susceptible to splitting because the cell walls are already stressed and consequently more easily ruptured (Kokkoras 1995).

The relationship between turgor pressure and splitting has been reported in a number of root crops: Gracie (2004) found a reduction in turgor pressure caused by partially-lifting carrots reduced splitting susceptibility. The carrots were partially-lifted to sever the fibrous root system then left in the soil over night before harvesting the following morning. These carrots with reduced turgor had a greatly diminished splitting susceptibility when tested with a penetrometer. Further evidence to support turgor pressure affecting splitting susceptibility is provided by the investigation conducted by Konstankiewicz and Zdunek

(2001). They found the compressive strength of the tissue samples decreased with increasing turgor pressure suggesting potato tubers are more susceptible to splitting when they are more turgid (Konstankiewicz & Zdunek 2001). The results obtained by Konstankiewicz and Zdunek (2001) are similar to those of Bajema *et al* (1998) who also found potatoes with lower turgor had higher compressive strength than more turgid potatoes. McGarry (1993) found failure force in the phloem parenchyma tissue of carrots was negatively correlated with both water potential and turgor pressure.

Experiments in this section investigated the effects of hypocotyl water content and water content on splitting susceptibility and how these were linked to hypocotyl water pressure. A series of three preliminary experiments (Experiment 4.3) was conducted to investigate if the radish hypocotyl is able to absorb water through the periderm how rapidly this process occurs. Four experiments (Experiment 4.4-4.7) were then conducted to look at the effects of hypocotyl water content and RWC on splitting of commercially grown radishes and how hypocotyl water content and RWC are relative to hypocotyl water pressure.

Aims:

- Determine if water is absorbed through the radish hypocotyl
- Determine if water is able to pass through the periderm of the radish hypocotyl
- Determine if hypocotyl water content is correlated with splitting susceptibility as a results of impact, compression or puncture
- Determine if hypocotyl RWC is correlated with splitting susceptibility as a result of impact or puncture
- To investigate the relationship between failure force as a result puncture of hypocotyls with and without a periderm
- To investigate the relationship between hypocotyl water content and hypocotyl water pressure
- To investigate the relationship between hypocotyl RWC and hypocotyl water pressure

Null Hypotheses:

1. Water is not absorbed through the radish hypocotyl

2. Water is not able to pass through the periderm of the radish hypocotyl
3. There is no correlation between hypocotyl water content and splitting susceptibility as a results of impact, compression or puncture
4. There is no correlation between hypocotyl RWC and splitting susceptibility as a result of impact or puncture
5. There is no relationship between hypocotyl RWC and hypocotyl water pressure
6. There is no relationship between failure force as a result puncture of hypocotyls with and without a periderm
7. There is no relationship between hypocotyl water content and hypocotyl water pressure

4.4.2 Experiment 4.3: Preliminary experiments: Determining if radishes absorb water through the periderm

4.4.2.1 Experiment 4.3: Introduction

During harvest and post-harvest processing, radish hypocotyls are washed, trimmed and transported by floating in water, a process which can take several minutes. It is unclear firstly if radishes take up water through the periderm of the hypocotyl during this process and secondly, if water is taken up, the rate at which this happens.

In an attempt to answer these two questions, three preliminary experiments were conducted to determine firstly if radishes are able take up water through the periderm of if the surface is too waxy to allow water to penetrate. The mode of water uptake may have implications on methods used in future experiments and could influence recommendations to growers for post-harvest handling if a relationship is found between RWC and splitting. The second and third experiments investigated the rate at which water is lost or absorbed. These experiments will reveal if the post-harvest handling procedure which only takes a few minutes can have an effect on the RWC of the radish hypocotyls.

Aims:

- To determine if water can be taken up through the hypocotyl periderm into the radish hypocotyl.
- To determine the speed at which water is lost or taken up by the radish hypocotyl.

Null hypothesis:

1. There will be no change in water content of the radish hypocotyl.

4.4.2.2 Experiment 4.3: Materials and method

Preliminary Experiment 1 - Water absorption through the hypocotyl periderm: Radishes grown by G's Growers were bought at a local supermarket, Waitrose, having been displayed in a refrigerated display cabinet. Ten radishes were weighed and placed individually in hammocks suspended in beakers of dH_2O. They were positioned so the cut ends at the top and bottom of the radish, where the leaves and roots had been removed, were exposed above the water and some of the periderm was submerged below the surface of the water (Figure 4-2, Figure 4-3). A further ten radishes were weighed and individually placed in 10 beakers with no water in them.

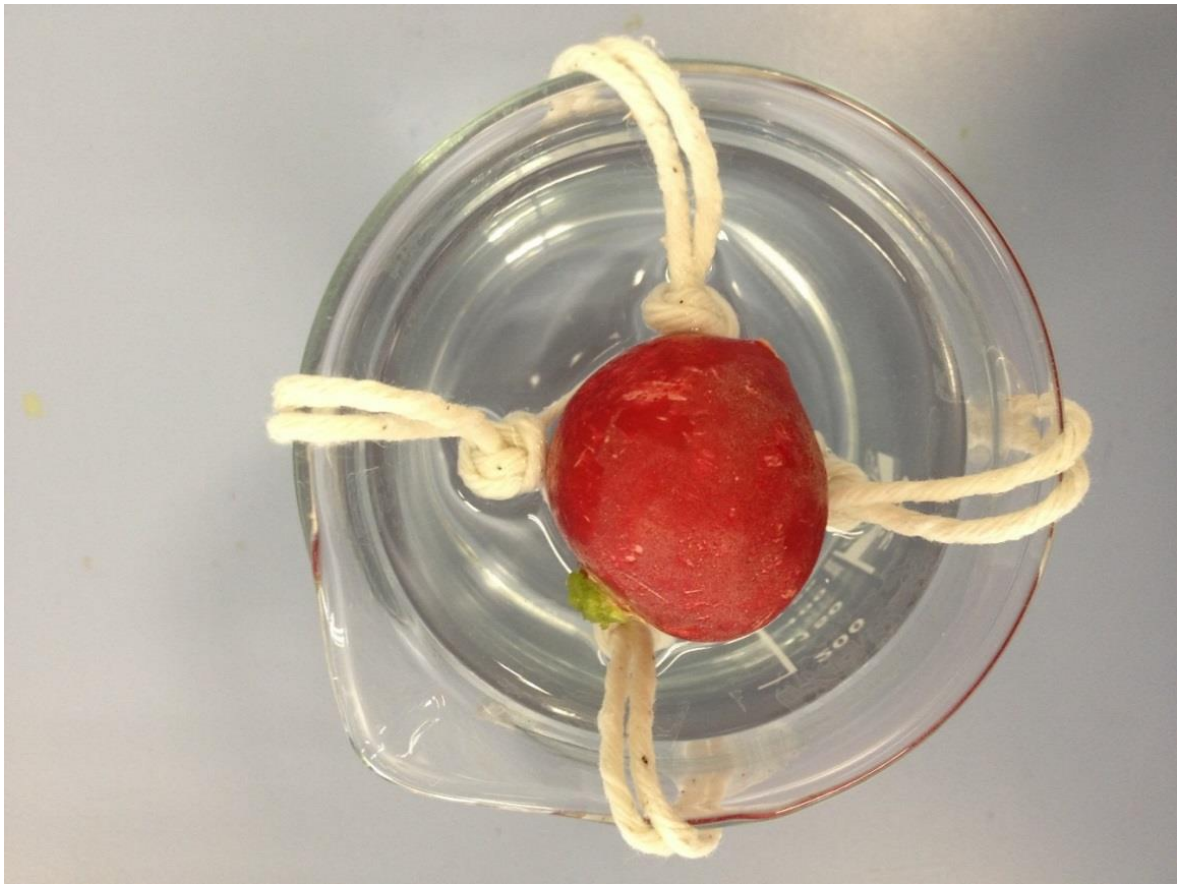


Figure 4-2 Showing the 'hammock' suspending a radish in water. Top view.

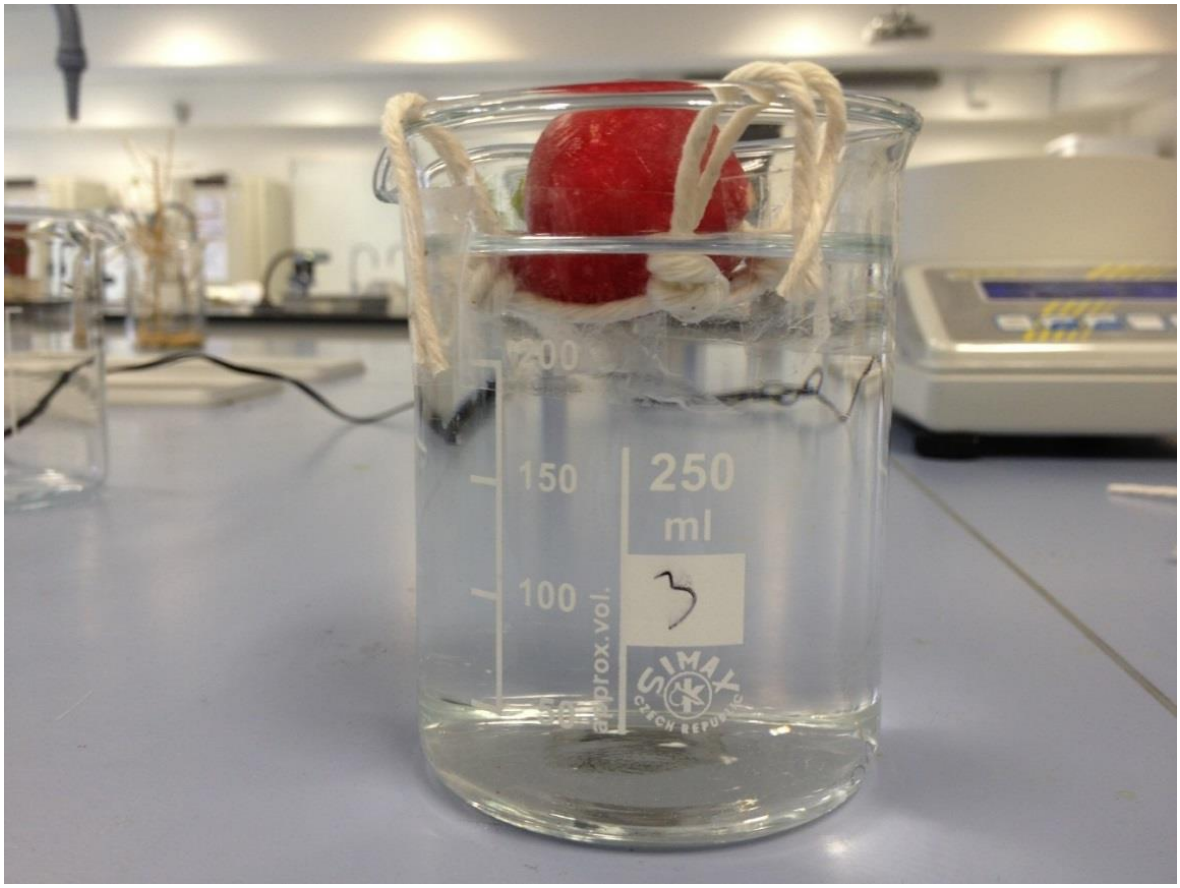


Figure 4-3 Showing the radish suspended in water using a 'hammock'. N.B. the cut ends at the top and bottom were above the surface of the water but the periderm on the side of the radish was submerged. View from the side.

The beakers of radishes were then placed into a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd., Japan) for 24 hours. The chamber was set to 90% relative humidity and the temperature was set to 4°C. This temperature was chosen to minimise plant growth and respiration.

After 24 hours, the radishes were removed from the chamber, dried with paper towel and weighed. The percentage change in weight compared to the initial weight was then calculated and the results were analysed using a Student's T-Test.

Preliminary Experiment 2 – Speed of water uptake/loss for shop bought radishes: Radishes grown by G's Growers were bought at a local supermarket, Waitrose, having been displayed in a refrigerated display cabinet. Seven radishes were individually weighed and placed into seven glass beakers of dH_2O and a further seven radishes were individually weighed placed into seven empty glass beakers. The beakers of dH_2O had

been prepared prior to the start of the experiment and placed in the controlled environment chamber to allow the water to reach 4°C. The beakers of radishes were then placed into a MLR-351H Versatile Environmental Test Chamber (SANYO Electric Co. Ltd., Japan). The chamber was set to 90% relative humidity and 4°C. After five minutes, each radish was individually removed from the chamber, patted dry using paper towel, weighed, and returned to the same glass beaker in the controlled environment chamber. This process was repeated every five minutes for 60 minutes. Each radish was weighed a total of 13 times. The results were then analysed using linear regression.

Skeleton ANOVA:

Table 4-7 Skeleton ANOVA for regression analysis of change in weight of radish hypocotyls placed in water or allowed to air dry

Source of variation	df
Regression	4
Residual	8
Total	12

Preliminary Experiment 3 – Speed of water uptake/loss for fresh radishes: The previous two preliminary experiment in Experiment 4.3 used shop bought radishes which had been transported through the supply chain. Therefore, the conditions following harvest were unknown and may have led to them being more dehydrated than radishes which were freshly harvested affecting the rate of uptake of water. This third preliminary experiment aimed to determine the rate of water uptake of more recently harvested radishes; of greater relevance to the following experiments, Experiments 4.4-4.7 which used freshly harvested and couriered radishes grown by G's Growers. In this preliminary experiment radishes grown by G's Growers were couriered to HAU on the same day as they were harvested for analysis the following day. The radishes had not been washed or sent through quality control. On arrival at HAU they were briefly washed in dH_2O.

Ten radishes were weighed and individually placed into glass beakers of dH_2O and ten radishes were individually placed into empty glass beakers. The beakers of dH_2O had been prepared prior to the start of the experiment and placed in the controlled environment chamber to allow the water to reach 4°C. The beakers containing the radishes were then returned to the controlled environment test chamber. The chamber was set to 90% relative humidity and 4°C. After six minutes, each radish was individually removed from the chamber, patted dry using paper towel, weighed, and returned to the same glass beaker in the controlled environment chamber. This process was repeated every six minutes for 30 minutes. Each radish was weighed a total of six times. The radishes were then placed into an oven and dried at 105°C to a constant weight. Once dry the radishes were again weighed to allow a calculation of their water content to be made. The results were then analysed using linear regression.

Skeleton ANOVA:

Table 4-8 Skeleton ANOVA for the rate of change in weight and water content of radish hypocotyls over 30 minutes

Source of variation	df
Regression	1
Residual	4
Total	5

4.4.2.3 Experiment 4.3: Results and discussion

Preliminary Experiment 1 – Water uptake through the periderm: There was a clear difference in appearance (Figure 4-4) and a significant ($P<0.001$) difference between initial weight and weight after 24 hours (18 df) (Figure 4-5) between the radishes which were placed in empty beakers and radishes which were suspended in hammocks with part of their periderm submerged in water. The radishes which were placed in empty beakers had a mean decrease in weight of 22.7% whereas the radishes which were partially submerged had a mean increase in weight of 5.2% (Figure 4-5).

Figure 4-4 Difference in appearance after 24 hours of shop-bought radishes allowed to air dry (bottom five radishes) or suspended in a hammock with part of their periderm submerged in water (top five radishes).

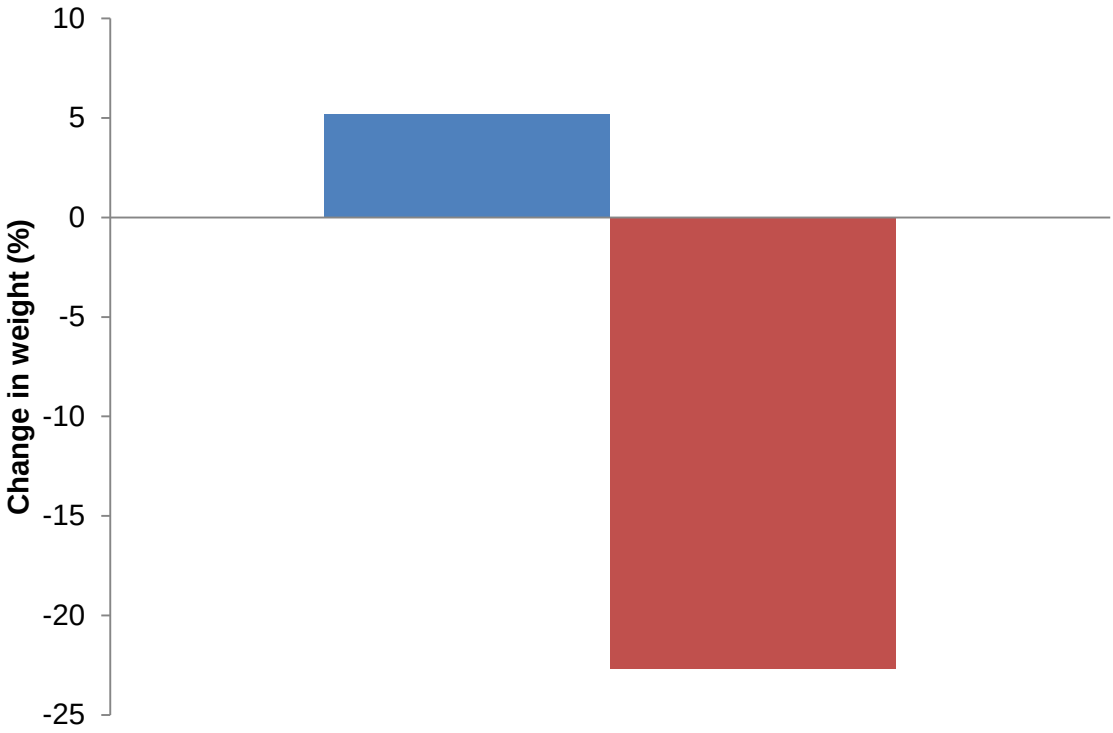


Figure 4-5 Change in weight of radishes placed in a beaker and suspended in a hammock with part of their periderm submerged in dH_2O (blue) or placed in an empty beaker and allowed the air dry (red) for 24 hours (n=10).

As the weight of the radishes which had their periderm in water increased in weight and the radishes which were kept in air decreased in weight, the results from this experiment suggest radishes are able to take up water through the periderm and the null hypothesis is rejected.

Preliminary Experiment 2 – Rate of water uptake/loss for shop bought radishes: Over a 60 minute period there was a significant ($P<0.001$) increase in weight of the radishes which were placed in water and there was a significant ($P<0.001$) decrease in weight of the radishes which were left in the air (Table 4-9, Table 4-10) suggesting the radishes placed in water took up water and the radishes which were placed in air lost water over this time. The results also showed water was lost more rapidly than it was taken up over a 60 minute period, the radishes which were placed in dH_2O had a mean increase in weight of 4.0% whereas the radishes which were in air had a mean decrease in weight of 4.3% (Figure 4-6).

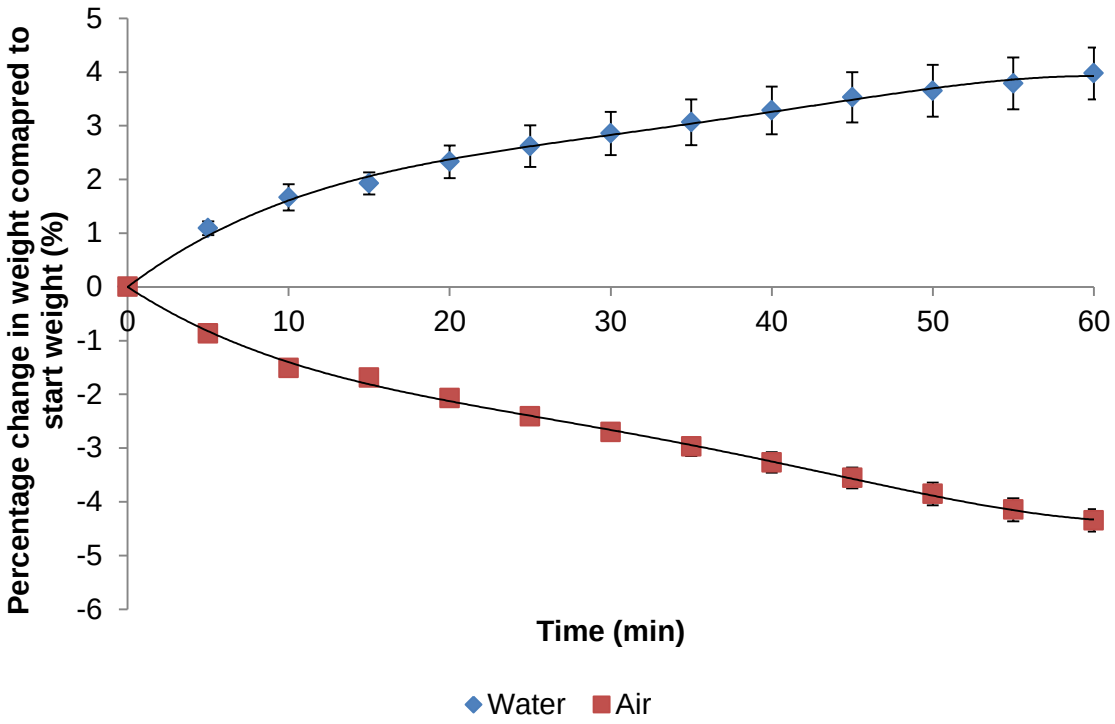


Figure 4-6 Change in weight of radishes placed in dH_2O (blue) or left in air (red) over 60 minutes (n=7). Bars represent standard error.

Table 4-9 Pattern of water uptake by radish hypocotyls over 60 minutes. P<0.001, 99.5% variance accounted for (n=7)

Parameter	Estimate	Standard error	P
Constant	0.0683	0.0742	0.385
Linear	0.2149	0.0187	<0.001
Quadratic	-0.00734	0.00135	<0.001
Cubic	0.0001374	0.0000344	0.004
Quartic	0.000000940	0.000000284	0.011

Table 4-10 Pattern of water loss of radish hypocotyls over 60 minutes. P<0.001, 99.7% variance accounted for (n=7)

Parameter	Estimate	Standard error	P
Constant	-0.0357	0.0620	0.581
Linear	-1.892	0.0156	<0.001
Quadratic	0.00652	0.00113	<0.001
Cubic	-0.0001330	0.0000287	0.002
Quartic	0.000000950	0.000000238	0.004

The pattern of water uptake and loss over time was not linear (Figure 4-6, Table 4-9, Table 4-10). Both groups of radishes changed in weight more rapidly at the start of the 60 minutes and by the end of the 60 minutes had begun to plateau in weight. This suggests when the radishes are being washed and processed commercially they will change in water content over the short period of time which they are being processed for. However, the radishes used for this experiment were shop bought and it may have been several days since they had been harvested. This may have resulted in a decrease in hypocotyl water content and meant they took up water more rapidly than a freshly harvested radish would have done.

Preliminary Experiment 3 – Rate of water uptake/loss for fresh radishes: There was a significant change in weight of the radishes placed in water for 30 minutes (P<0.001) and the radishes left in air for 30 minutes (P=0.006). There was a linear relationship for both the increase and decrease in weight over the 30 minute period. The rate of change in weight for fresh radishes was less rapid than the shop bought radishes in the previous experiment. After 30 minutes, the shop bought radishes in the previous experiment which were left in air had decreased in weight by 2.7% whereas the fresh radishes in this experiment had only decreased in weight by 0.2%. For the radishes which were placed in water, in the previous experiment after 30 minutes, the shop bought radishes had increased in weight by 2.9% whereas the fresh radishes in this experiment had only

increased in weight by 0.9%. The fresh radishes may have taken up water less rapidly because they had a greater RWC compared to shop bought radishes but as this was not measured the hypothesis cannot be tested. It is unclear why the fresh radishes would have lost weight more slowly than shop bought radishes but potentially some radishes are more porous than others. The shop bought radishes would have passed through the more vigorous washing procedure at G's Growers compared to brief hand washing at HAU and may have had more of the periderm removed or scratched.

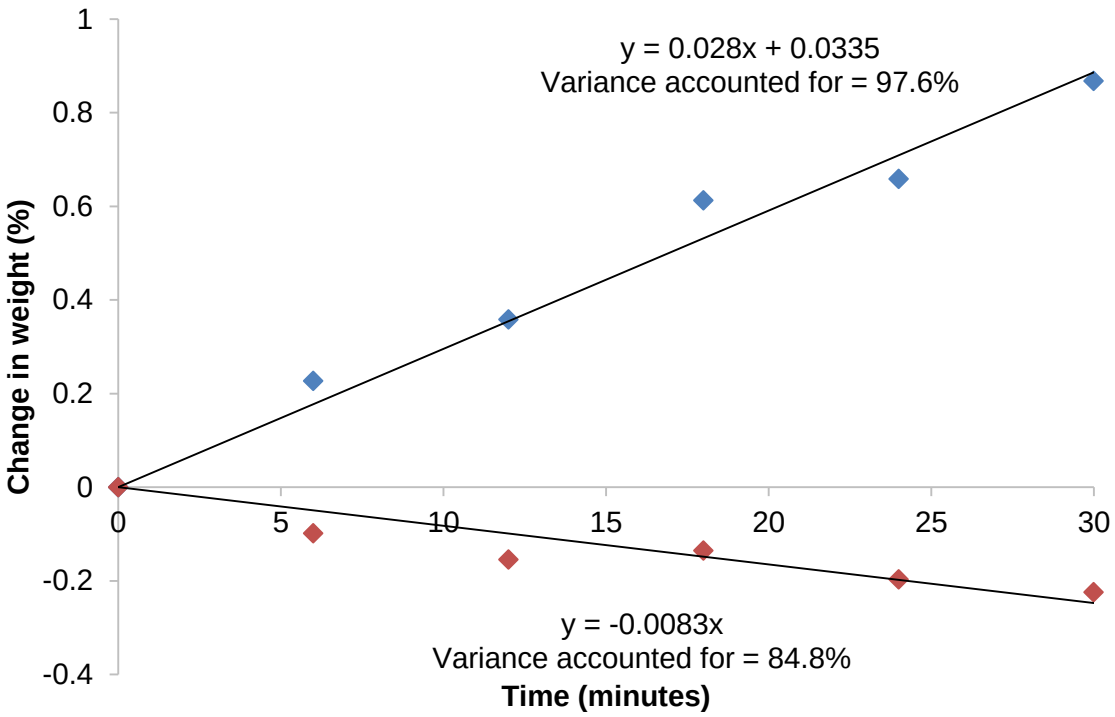


Figure 4-7 Change in weight (%) of fresh radishes placed in dH_2O or allowed to air dry for 30 minutes (n=10)

The radish hypocotyls which were placed in water increased from a mean water content of 97.553 % water to 97.757% water over 30 minutes (Figure 4-8). The radish hypocotyls which were allowed to air dry decreased from 96.863% water content to 96.855% water content over 30 minutes (Figure 4-9). The following experiments in this experiment will test if hypocotyl water content has an effect on splitting susceptibility.

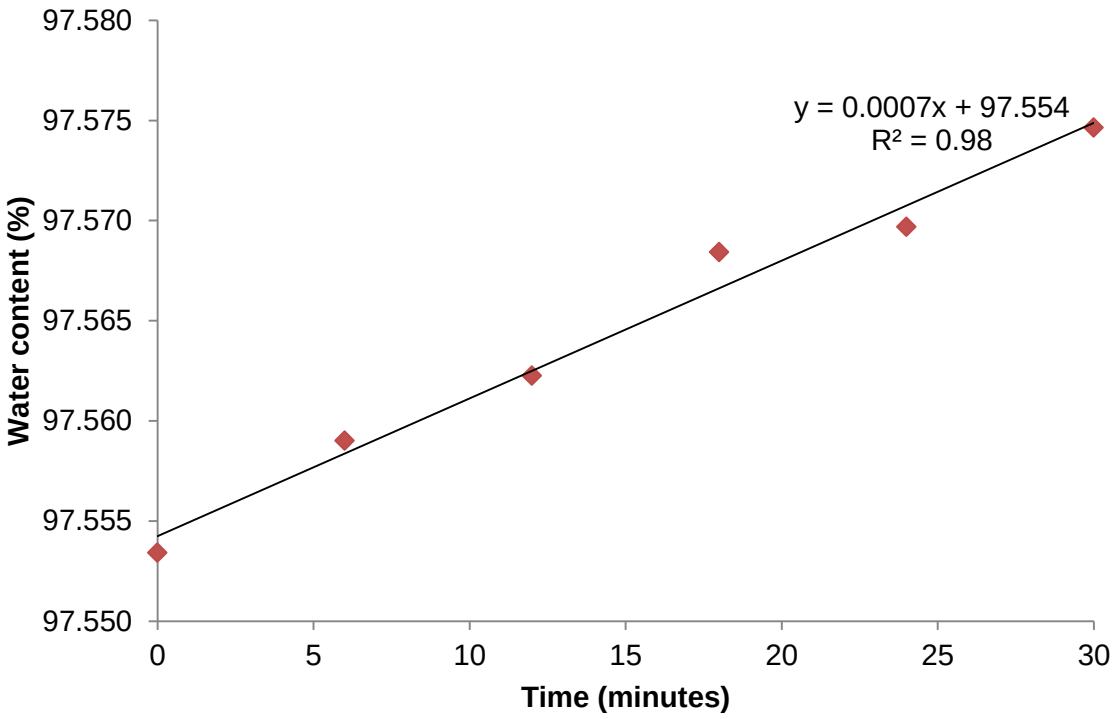


Figure 4-8 Change in water content (%) of fresh radishes placed in water for 30 minutes (n=10)

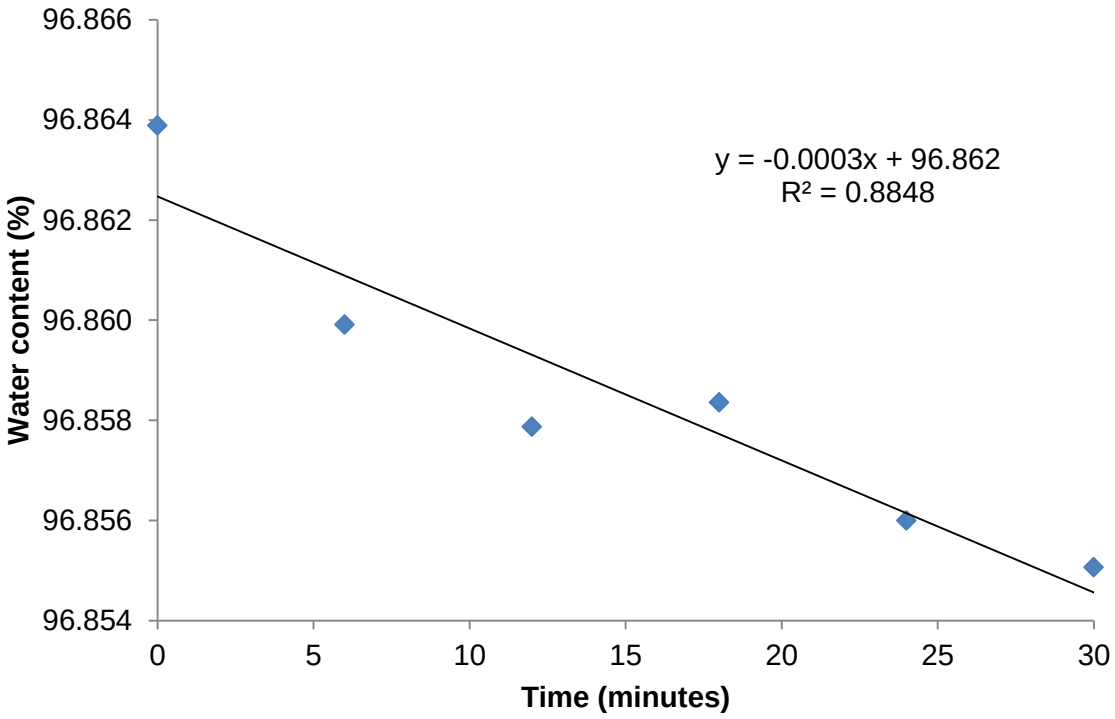


Figure 4-9 Change in water content (%) of fresh radishes allowed to air dry for 30 minutes (n=10)

4.4.2.4 Experiment 4.3: Conclusion

In conclusion, the null hypothesis for Experiment 4.3 was rejected as it was shown water is able to pass through the periderm of the radish hypocotyl.

Radishes in commercial production are only floated for a few minutes but these preliminary experiments showed the hypocotyls were able to take up water over this time period. In addition, water was absorbed most rapidly initially when the radishes were first placed into dH_2O.

Radishes which were more recently harvested were shown to absorb water more slowly than shop bought radishes suggesting the higher the RWC the slower the absorption of water. However, there were other differences in the two groups of radishes which were tested and this result is not conclusive.

Further experiments are required to test if changes in RWC of the magnitude found in this preliminary experiment have an effect on susceptibility to harvest splitting.

4.4.3 Experiment 4.4: Investigating the effect of hypocotyl water content on the susceptibility of radishes to harvest splits

4.4.3.1 Experiment 4.4: Introduction

This experiment was designed to look at the relationship between hypocotyl water content and splitting susceptibility. Splitting susceptibility at different hypocotyl water contents was measured using three different tests: 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 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.

Aim:

- The aim of this experiment was to determine if there is a relationship between hypocotyl water content and splitting susceptibility as a result of impact, puncture and compression.

Null Hypotheses:

1. There is no relationship between hypocotyl water content and splitting susceptibility as a result of impact.
2. There is no relationship between hypocotyl water content and splitting susceptibility as a result of puncture.
3. There is no relationship between hypocotyl water content and splitting susceptibility as a result of compression.

4.4.3.2 Experiment 4.4: Materials and Method

Radishes from G's Growers in Norfolk, England were couriered on the day of harvest to arrive at HAU, Shropshire, England the following morning. Upon arrival radishes were briefly washed in dH_2O to remove soil residue and trimmed to remove any remaining leaf stalks and roots. The radish hypocotyls were then 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. Radishes were removed from the chamber every 2 to 3 days over the following week, weighed and subjected to destructive texture analysis. After texture analysis the radishes were dried to a constant weight at 105°C to calculate the water content at the point of analysis. The chamber was set to 90% relative humidity and achieved a mean relative humidity of 83.5% ranging from 62.0% to 100.0%, the temperature was set to 4°C and achieved a mean temperature of 4.5°C ranging from 2.5°C to 7.4°C. The variations in temperature and relative humidity are thought to have been due to the opening and closing of the chamber to remove samples.

Puncture: Puncture tests were performed 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 1 mmS^{-1} and the test distance was 16 mm. 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.

Impact: Impact tests were performed using the method described in Chapter 4.2. The drop height was 1.4 m 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.

Compression: Uniaxial compression tests were performed using a P/75 probe fitted to a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England). The pre-set apple compression test was used. The test speed was 0.05 mmS^{-1} and the test distance was 16

mm. 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 first peak was recorded as the failure force of the radish hypocotyl.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Either simple or polynomial regression was used to analyse the data. As the data for compression and puncture both the response variable and explanatory variables contained continuous data, therefore simple or polynomial regression was appropriate to use to estimate their relationship. The data for the results from impact contained continuous explanatory variables and interval data as the response variable. In this case it was also appropriate to use polynomial regression for analysis.

Skeleton ANOVA:

Table 4-11 Skeleton ANOVA for the three types of texture analysis conducted as part of Experiment 4.4

	df		
Source of variation	**Impact**	**Compression**	**Puncture**
Regression	3	1	1
Residual	66	66	35
Total	69	67	36

4.4.3.3 Experiment 4.4: Results and discussion

Impact:

There was a significant (P<0.001) increase in the number of radishes which split as a result of impact as hypocotyl water content increased. 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 4-10).

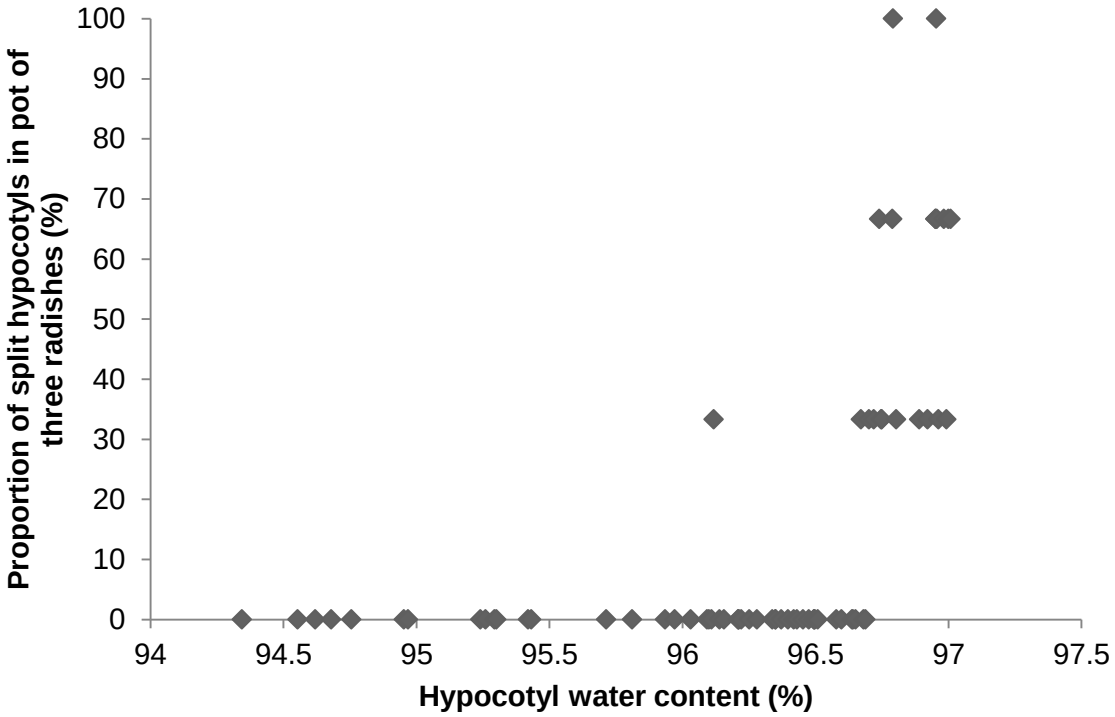


Figure 4-10 Results from regression analysis of the percentage of split radish hypocotyls in a sample of three which split as a result of dropping down a 1.4 m tube onto an aluminium plate at different hypocotyl water contents (n = 70).

Table 4-12 Parameters and their estimates for the regression analysis of the percentage of split radish hypocotyls in a sample of three which split as a result of dropping down a 1.4 m tube onto an aluminium plate at different hypocotyl water contents (n = 70)

Parameter	Estimate	Standard error	P
Constant	-15790749	360861	<0.001
Linear	441260	100425	<0.001
Quadratic	-3468	786	<0.001
Cubic	0	-	-
Quartic	0.0635	0.0143	<0.001

In this experiment the propensity of radish hypocotyls to split due to impact as a result of dropping from a height of 1.4 m was found to increase at hypocotyl water contents over 96.5%. This would appear to be the point at which the cells walls of radish hypocotyls are under enough stress that when the additional stress of being dropped from a fixed height of 1.4 m, the tissue fails and the radish splits. There was a significant linear relationship between failure force due to puncture and hypocotyl water content again suggesting that there may be a particular amount of stress which the hypocotyl cells are able to withstand before they fail. As the amount of stress increases due to increased water content the added amount of stress the cell walls are able to withstand as a result of puncture force decreases.

Puncture: In this experiment, a relationship was observed between hypocotyl water content and splitting susceptibility as a result of both impact and puncture, therefore, the first and second null hypotheses were rejected. Results from this experiment suggest hypocotyls are more susceptible to splitting as a result of impact and damage as a result of puncture at high hypocotyl water contents.

There was a significant ($P<0.001$) negative linear correlation between the puncture force (kg) and the hypocotyl water content (%) which explained 70.6% of the variance. This relationship was expressed by the equation:

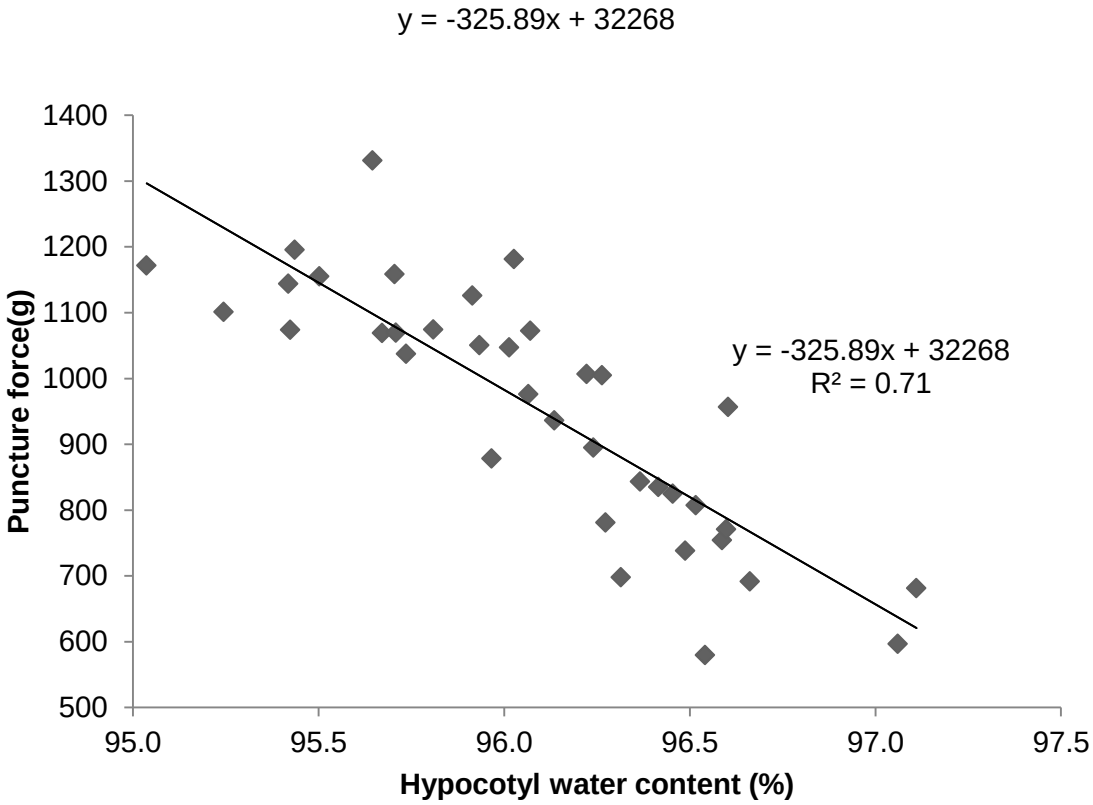


Figure 4-11 The force (g) required to puncture the periderm of radishes at different radish hypocotyl water contents (%)

Compression: No relationship between hypocotyl water content and the failure force due to compression was observed (P=0.391) (Figure 4-12). Some radishes had not split at the maximum load of 35 kg. The third null hypothesis was not rejected because no relationship was observed between hypocotyl water content and splitting susceptibility as a result of compression. However, this may have been as a result of faults in the methodology of this investigation rather than there being no relationship. It was considered the test speed was too slow, resulting in the radishes being squeezed and the hypocotyls leaking water during the compression analysis. This would have meant the hypocotyls were not at the measured water content when and if they eventually split and therefore no meaningful conclusions can be drawn from the results. The association between hypocotyl water content and failure force as a result of compression needs further work to establish if there is a connection.

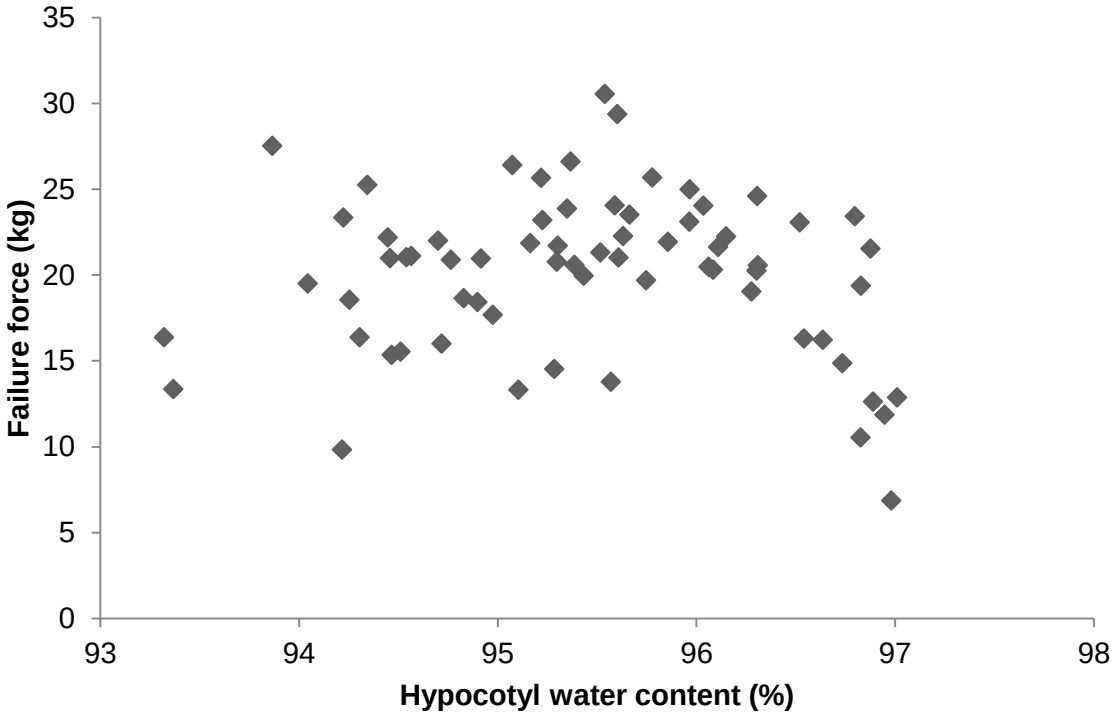


Figure 4-12 Failure force (kg) of radish hypocotyls at different water contents (%) as a result of compression (n=68)

Overall, a relationship was observed between hypocotyl water content and splitting susceptibility as a result of both impact and puncture, therefore, the first and second null hypotheses were rejected. There was no relationship with compression but this may have been due to faults with the methodology used rather than a lack of relationship. The methodology will be improved in further experiments.

In Experiment 4.4, hypocotyl water content was used to indicate the amount of stress which cells in the hypocotyl may be under. However, the RWC of the radishes may be a more accurate way to indicate the amount of stress which they are under. Water content calculates the proportion of water compared to dry matter, whereas the RWC determines the water content compared to the potential maximum water content. Maximum water content would be the most stress the cells could be placed under as a result of water uptake. Knowing how close the radishes were to this point could be beneficial and should be investigated further.

In conclusion, results from Experiment 4.4 suggest hypocotyls are more susceptible to splitting as a result of impact and damage as a result of puncture at high hypocotyl water contents.

4.4.4 Experiment 4.5: Investigating the effect of hypocotyl water content on the susceptibility of radishes to harvest splits with improved methodology

4.4.4.1 Experiment 4.5: Introduction

In the previous experiment, Experiment 4.4, the results from the investigation into the relationship between hypocotyl water content and splitting susceptibility due to compression were inconclusive. The methodology used in this experiment was modified with the aim to determine more conclusively if there is a relationship between hypocotyl water content and splitting susceptibility as a result of compression.

In this experiment, Experiment 4.5, in addition to measuring hypocotyl water content, measurements of hypocotyl RWC were made for impact and puncture analyses. This was done to determine if RWC has a greater correlation with splitting susceptibility than hypocotyl water content.

Aim: The aims of this experiment were to determine or confirm if there is a relationship between:

- Hypocotyl water content and splitting susceptibility due to impact
- Hypocotyl RWC and splitting susceptibility due to impact
- Hypocotyl water content and failure force due to puncture
- Hypocotyl RWC and failure force due puncture
- Hypocotyl water content and failure force due to compression

Null hypotheses: There will be no significant relationship between:

1. Hypocotyl water content and splitting susceptibility due to impact
2. Hypocotyl RWC and splitting susceptibility due to impact
3. Hypocotyl water content and failure force due to puncture
4. Hypocotyl RWC and failure force due puncture
5. Hypocotyl water content and failure force due to compression

4.4.4.2 Experiment 4.5: Materials and method

For this experiment, the protocol from Experiment 4.4 was refined. As in Experiment 4.4, all radishes were grown and harvested by G's Growers and then couriered to HAU on the day of harvest for testing the following day. The radishes were placed into a controlled environment set to 4°C as soon as they arrived and until analysis was carried out. At the point of analysis all radishes were considered commercially viable by the examiner. The mean temperature in the controlled environment for the duration of storage of this experiment and the following two experiments 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 100% and a minimum of 99.1%.

Splitting susceptibility at different water contents was measured using three different tests, impact, puncture and compression using the methods described above for Experiment 4.4 with modification. As before, all radishes used for analysis were of a commercial size.

To ensure there was a range of hypocotyl water contents and RWCs, the radishes which were to be used for the puncture, compression and pressure tests, were divided into three groups:

1. Fresh (tested 1 day post-harvest)
2. Saturated for 1 day (tested 2 days post-harvest)
3. Air dried for 1 day (tested 2 days post-harvest)

For saturation the radishes were placed into pots of approximately 100 mL of dH_2O to increase the hypocotyl water content. The radishes which were air dried were also placed into empty plastic pots.

Impact: Impact analysis was performed on 30 radishes, 10 which were fresh, 10 which had been saturated for one day and 10 which had been air dried for one day. The radishes were tested using the method described previously other than each radish was an experimental unit, not a group of three radishes.

Puncture: Puncture tests were performed on 45 radishes, 15 which were fresh, 15 which had been saturated for one day and 15 which had been air dried for one day. The radishes were tested using a TA.HD.plus texture analyser (Stable Micro Systems, Surrey,

England). As in Experiment 4.4, the texture analyser was fitted with a P/2 cylindrical probe, the test distance remained 16 mm but the test speed was increased to 2 mmS^{-1}. Compared to Experiment 4.4 the test speed was doubled as the increased speed enabled more tests to be conducted in the same amount of time.

Compression: Uniaxial compression tests were performed on 45 radishes, 15 which were fresh, 15 which had been saturated for 1 day and 15 which had been air dried for 1 day. The radishes were tested using a P/75 probe fitted to a TA.HD.plus texture analyser (Stable Micro Systems, Surrey, England). The test speed was 2 mmS^{-1} and the test distance was 25 mm. This is a slight modification of the method used for Experiment 4.4. The test speed was increased from 0.05 mmS^{-1} to 2 mmS^{-1} to allow a greater number of samples to be processed and to minimise the amount of water lost from the hypocotyl during compression. A preliminary experiment was conducted to optimise the test speed. As no clear pattern was observed relating the test speed to failure force (Table 4-13) the fastest speed was chosen.

Table 4-13 Test speed and mean failure force for freshly harvested and 1 week post-harvest radishes (n=5)

Test speed (mm/sec)	Failure force (kg) Fresh radishes	Failure force (kg) 1 week old radishes
0.5	21.51	27.53
1	18.80	20.73
2	19.24	22.62

In addition liquid had been observed to be lost from the radishes at the slower speed making it impossible to determine exactly the water content at the point of failure.

For compression texture analysis, the test distance was also increased from 16 to 25 mm. This was because a number of radishes did not fail with the shorter distance and it was felt an increased test distance, in addition to the increased speed, would reduce the likelihood of this occurring.

RWC: Prior to testing, each radish was weighed. After testing, the radishes which had been subjected to compression analysis were dried at 68°C to a constant weight and weighed to enable a calculation of water content (WC) to be made.

After impact and puncture analysis the radishes were saturated using the same method as described in Experiment 4.1. Radish hypocotyls were cut into 8 segments and placed into individual pots of approximately 100 mL of dH_2O. The pots were then placed into controlled environment storage at 2.5°C for 48 hours. The radish segments were then dried at 68°C to a constant weight and the RWC for each radish was calculated. The radishes which had undergone compression testing were too damaged allow saturation for a calculation of RWC to be made.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Linear regression was used to estimate the relationship between the failure force due to compression or puncture of the radish hypocotyls and the hypocotyl water content or RWC. This method of analysis was selected as the response variable and explanatory variables both contained continuous data. When the response variable consisted of presence/absence data, as in the case of the impact texture analysis, and there were unequal group sizes, a Two-sided T-test was used.

Skeleton ANOVA:

Table 4-14 Skeleton ANOVA for the three types of texture analysis conducted as part of Experiment 4.5

		df	
Source of variation	**Impact**	**Compression**	**Puncture**
Regression	1	1	1
Residual	28	43	43
Total	29	44	44

4.4.4.3 Experiment 4.5: Results and discussion

Impact: Hypocotyl water contents ranged from 96.0% to 97.5% giving a range of RWCs from 0.85 to 0.97.

The radishes which split as a result of dropping had significantly (P=0.009) greater hypocotyl RWCs than the radishes which did not split. Therefore the second null hypothesis was rejected. The first hypothesis was partially rejected because there was a non-significant trend (P=0.057) suggesting the radishes which split also had a greater hypocotyl water content than those which did not split (Table 4-15).

Table 4-15 Mean water content and RWC of radishes which split and did not split as a result of being dropped from a height of 1.4 m

	Mean water content (%)	**Mean RWC**
Split (n=9)	96.8	0.93
Non-split (n=21)	96.6	0.90
P	0.057	0.009
SEM split	0.142	0.008
SEM non-split	0.061	0.006

Compression: The range in hypocotyl water contents which were used for compression texture analysis ranged from 95.9% to 97.7%.

The fifth hypothesis was rejected because a significant (P<0.001) relationship was observed between hypocotyl water content and failure force due to compression. Failure force as a result of compression was negatively correlated with hypocotyl water content (P<0.001) (Figure 4-13) suggesting radish hypocotyls are more susceptible to splitting due to compression at high hypocotyl water contents. When correlated using linear regression, 22.4% of the variance in failure force due to compression was accounted for by hypocotyl water content.

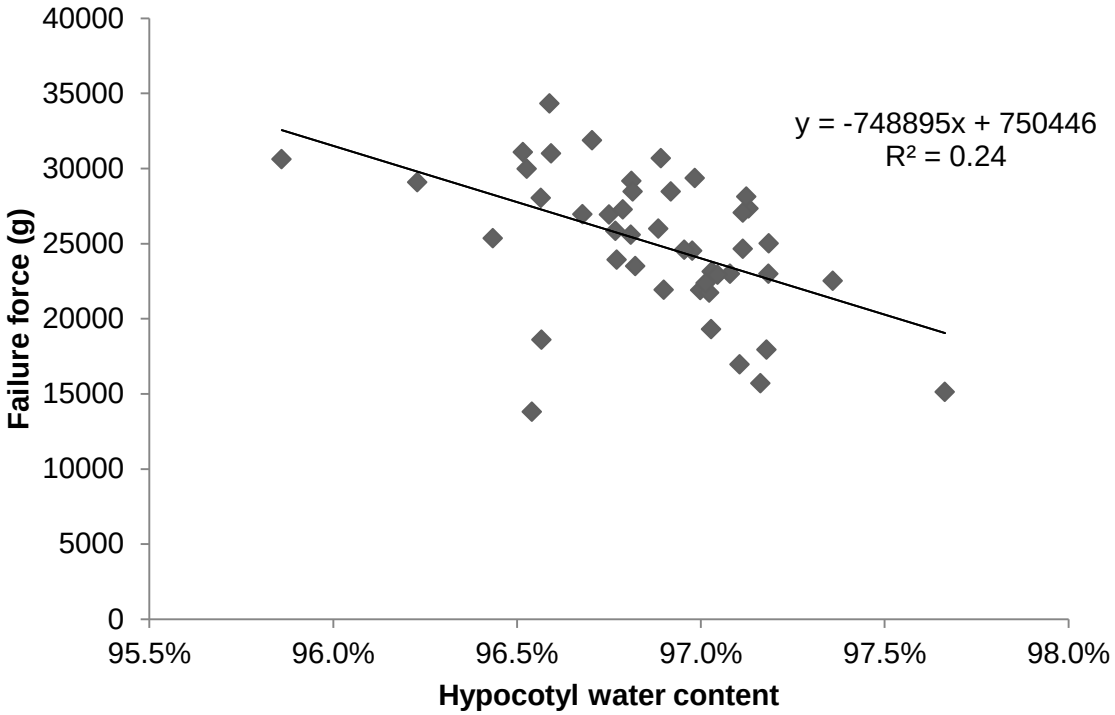


Figure 4-13 Failure force of radish hypocotyls as a result of compression at different hypocotyl water contents (n=45)

Puncture: The range in hypocotyl water contents which were used for puncture texture analysis ranged from 96.0% to 97.1% and the range in hypocotyl RWCs was from 0.83 to 0.94.

The third null hypothesis was rejected because hypocotyl failure force as a result of puncture was negatively correlated with hypocotyl water content (P=0.004) (Figure 4-14). When hypocotyl periderm failure force was correlated with hypocotyl water content, 15.7% of the variance in failure force was accounted for by hypocotyl water content.

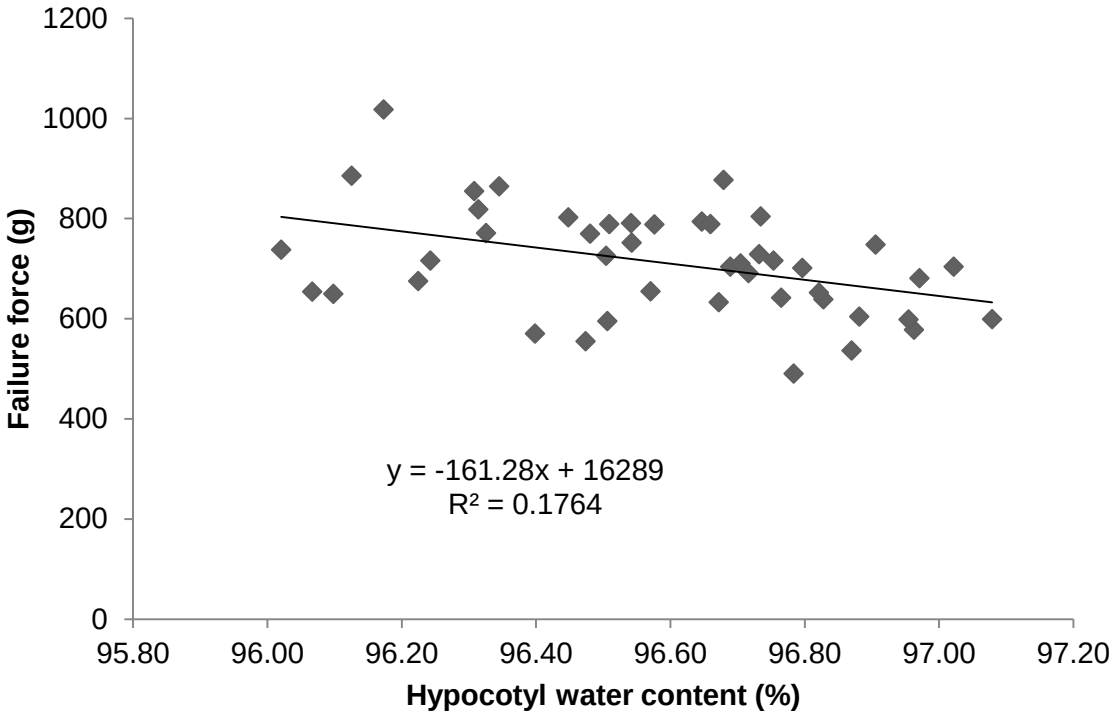


Figure 4-14 Hypocotyl failure force as a result of puncture at different hypocotyl water contents (n=45)

The fourth null hypothesis was also rejected because a significant relationship was found between hypocotyl RWC and failure force due puncture (P=0.025) (Figure 4-15). When hypocotyl periderm failure force was correlated with hypocotyl RWC, 9.1% of the variance in failure force was accounted for by hypocotyl RWC.

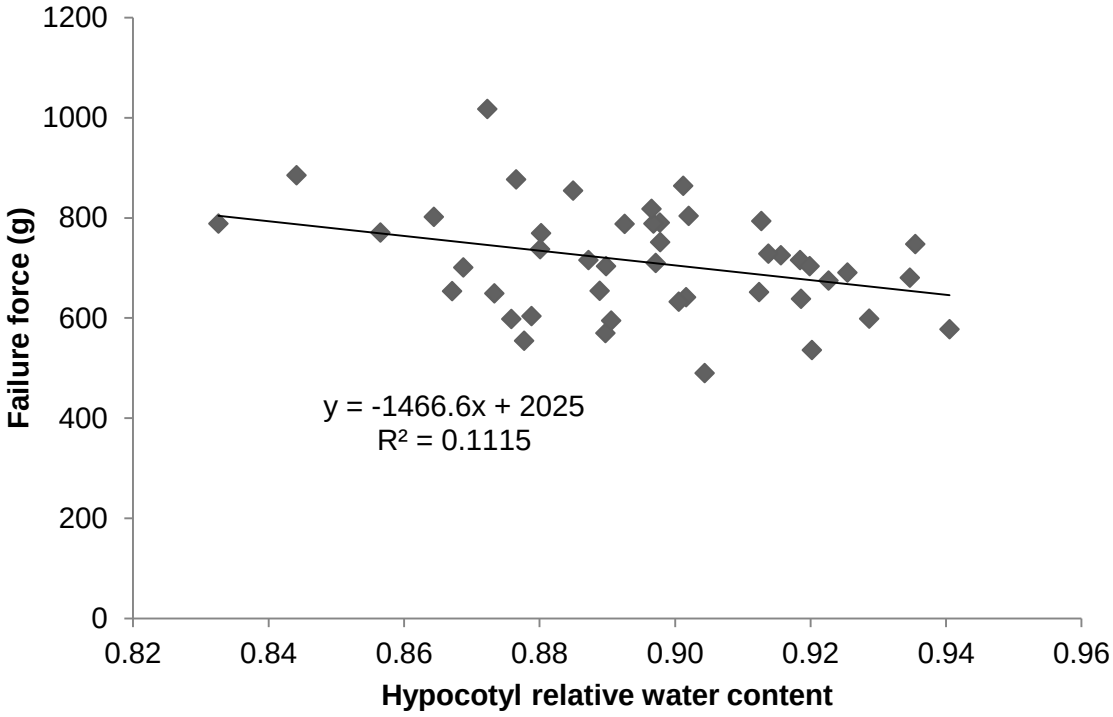


Figure 4-15 Hypocotyl failure force as a result of puncture at different hypocotyl RWCs (n=45)

In conclusion the first null hypothesis was partially rejected as the radishes which split as result of impact tended (P=0.057) to have a higher water content than those which did not split. The second null hypothesis was rejected as the radishes which split as result of impact had a significantly higher (P=0.009) water content than those which did not split. This result suggests RWC is a better predictor of susceptibility to splitting as a result of impact than hypocotyl water content.

The third null hypothesis was rejected as there was a significant (P=0.004) relationship between hypocotyl water content and splitting. Radishes with higher hypocotyl water contents were more susceptible to splitting as a result of puncture. The fourth null hypothesis was also rejected as there was a significant (P=0.025) relationship between hypocotyl RWC and splitting. Radishes with higher hypocotyl RWC were more susceptible to splitting as a result of puncture. Hypocotyl water content had a stronger relationship with hypocotyl failure as a result of puncture than hypocotyl RWC because hypocotyl

water content explained more of the variance in splitting (15.7%) than RWC (9.1%). Hypocotyl water content therefore appears to be a more accurate indicator of splitting susceptibility as a result of puncture.

The fifth null hypothesis was also rejected as there was a significant ($P<0.001$) relationship between hypocotyl water content and failure force as a result of compression. Radishes with greater hypocotyl water contents were shown to be more susceptible to splitting as a result of compression.

4.4.5 Experiment 4.6: Investigating the effect of hypocotyl water content and periderm on the susceptibility of radishes to harvest splits

4.4.5.1 Experiment 4.6: Introduction

In Experiment 3.1 a trend was observed linking periderm thickness with splitting susceptibility of different radish cultivars. A thicker periderm was linked to a greater splitting susceptibility. In this experiment (Experiment 4.6) failure force as a result of puncture both with and without periderm was investigated to determine how periderm strength relates to the failure force due to puncture. No correlation between the forces required to puncture the radish with and without the periderm would suggest there is no relationship between periderm strength and cortex strength, and either the cortex or the periderm could determine splitting susceptibility in different radishes. If the two are correlated and neither has a higher puncture force, this suggests it is either the strength of the cortex which is determining the splitting susceptibility and the periderm is stretching until the tissue under it fails or both the cortex and the periderm have the same strength. If the two are correlated and the periderm has a higher puncture force, this suggests the periderm has determined the splitting susceptibility but the genotypic and environmental factors affecting the strength and composition of the periderm were also likely to be determining the strength and composition of the cells in the cortex.

In this experiment the diameter of the radish hypocotyls was recorded for compression texture analysis. It is hypothesised that the size of the radish hypocotyl may affect how resistant it is to splitting as a result of compression. Potentially larger radishes have a greater number of cells if the cell size is similar within different sized radishes. A greater number of cells may increase the capacity of the hypocotyl for compression. If each cell has a similar amount it is able to compress, having a greater number of cells will result in a greater cumulative amount the hypocotyl is able to compress before it fails.

In an attempt to make the relationship between hypocotyl water content and hypocotyl RWC with splitting susceptibility clearer, this experiment aimed to have a greater range in hypocotyl water and RWCs compared to Experiment 4.5, this was achieved by allowing the hypocotyls longer to air dry or absorb water.

Aim:

- Investigate the role of the periderm in splitting as result of puncture
- Investigate if a greater degree of variance in failure force as a result of compression can be accounted for if there is a greater range in hypocotyl water contents and RWCs
- Investigate if a greater degree of variance in failure force as a result of compression can be accounted for if there is a greater range in hypocotyl water contents.
- Investigate if the size of the radish hypocotyl has an effect on splitting susceptibility as a result of compression.

Null hypotheses:

1. There is no significant relationship between puncture failure force with and without a hypocotyl periderm
2. There is no relationship between the failure force of the periderm as a result of puncture and hypocotyl water content
3. There is no relationship between the failure force of the periderm as a result of puncture and hypocotyl RWC
4. There is no relationship between failure force as a result of compression and hypocotyl water content
5. There is no relationship between hypocotyl diameter and failure force as a result of compression

4.4.5.2 Experiment 4.6: Materials and method

This experiment attempted to create a greater range in the water content and RWC of the radish hypocotyls compared to Experiment 4.5. Therefore the radishes were allowed to air dry or take up water for a greater amount of time. Again the radishes were divided into three groups before testing but this time they were:

1. Fresh (tested 1 day post-harvest)
2. Saturated for 6 days (tested 7 days post-harvest)
3. Air dried for 3 days (tested 4 days post-harvest)

Compression and puncture texture analyses were carried out and then the hypocotyl water content and RWC were measured as described previously for Experiment 4.5. Twenty radishes from each group were used for texture analysis giving a total of 60 radishes for puncture analysis and 60 for compression texture analysis. The diameter of each radish which underwent compression texture analysis was measured before analysis.

In addition to the puncture analysis with the periderm, texture analysis was also performed on the same radishes with the periderm removed. The first analysis was performed with the periderm intact and then the radish hypocotyls were rotated by 90°, an area of periderm approximately 5 mm^2 was shaved and the texture analysis was repeated on the shaved area. The periderm which was removed was retained for saturation and weighing and drying to calculate the hypocotyl water content and RWC.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Linear regression was used to estimate the relationship between the failure force due to compression or puncture of the radish hypocotyls and the hypocotyl water content or RWC. This method of analysis was selected as the response variable and explanatory variables both contained continuous data.

Skeleton ANOVA:

Table 4-16 Skeleton ANOVA for hypocotyl texture analysis at different hypocotyl water contents and RWCs

	df	
Source of variation	**Compression**	**Puncture**
Regression	1	1
Residual	58	58
Total	59	59

4.4.5.3 Experiment 4.6: Results and discussion

Compression: Experiment 4.6 failed to achieve a greater range in water contents compared to Experiment 4.5. A similar range of values was observed in this experiment but at higher water contents compared to RWC 1. Water contents ranged between a maximum of 98.5% and a minimum of 96.7%.

The fourth null hypothesis was rejected because a significant (P=0.016) negative correlation was observed between hypocotyl failure force due to compression and hypocotyl water content (Figure 4-16). The variance in failure force due to compression accounted for by hypocotyl water content was 8.1%. This result suggests radishes increase in susceptibility to splitting as a result of compression as hypocotyl water content increases. However, as the variance accounted for was relatively low this suggests there are other factors involved.

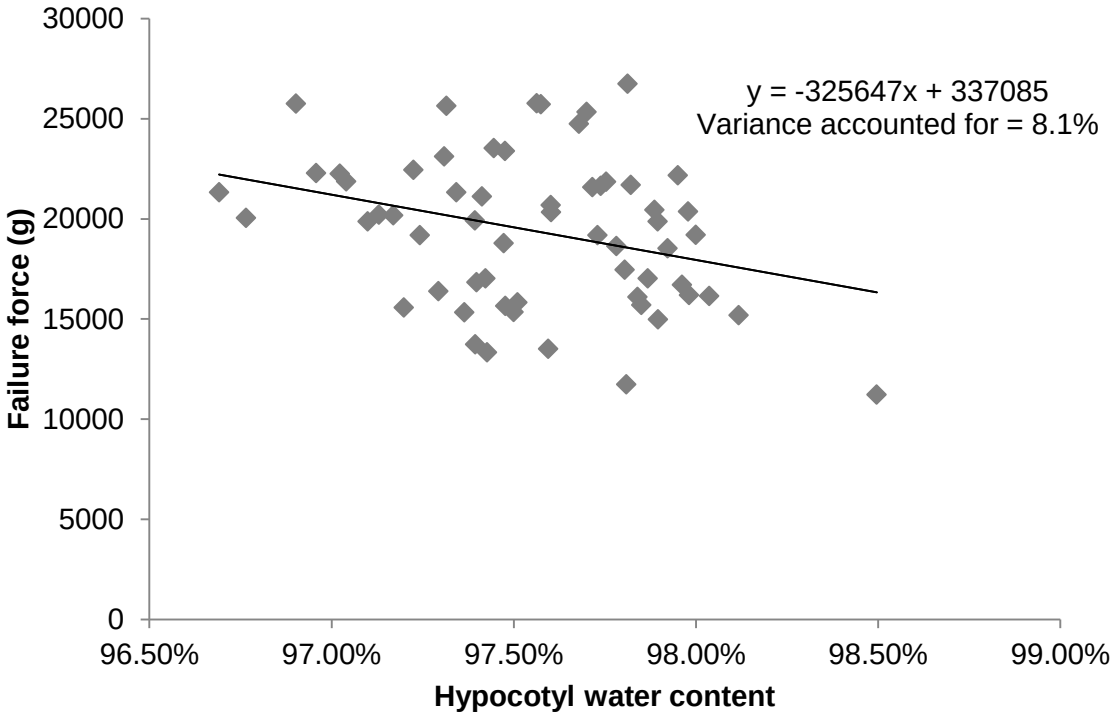


Figure 4-16 Hypocotyl failure force (g) due to compression at different hypocotyl water contents

When hypocotyl water content (%), diameter (mm) and days post-harvest were all included in a multiple linear regression with failure force due to compression there was a significant relationship (P<0.001) and 60.0% of the variation in failure force was accounted for (Table 4-17). As there was a significant relationship between hypocotyl diameter and failure force due to compression the fifth null hypothesis was rejected.

Table 4-17 Model determined by multiple linear regression and stepwise deletion for the relationship between hypocotyl failure force due to compression, water content (%) (WC), diameter (mm) and days post-harvest (DHP).

	Model fitted	**P**	**Variance accounted for**
Failure force (g)	WC + diameter +DPH	<0.001	60.0%

Table 4-18 Parameters and their estimates for the linear regression between failure force = WC + diameter + DPH

Parameter	**Estimate**	**SE**	**P**
Constant	307113	86320	<0.001
Water content	-322919	88616	<0.001
Width	1019	117	<0.001
DPH	-547	134	<0.001

There was a negative parameter estimate for water content therefore as hypocotyl water content increases the failure force due to compression decreases. Consequently, radish hypocotyls are more susceptible to splitting at higher hypocotyl water contents. As discussed previously, this is thought to be as a result of cells with a greater water content being having cells walls which are under a greater amount of stress and therefore requiring less additional force for them to fail.

There was a positive parameter estimate for diameter suggesting larger hypocotyls are more resistant to splitting than smaller ones. This may be because larger radishes have a greater number of cells. If the cell size is similar within different sized radishes there must be a greater number of cells within a larger radish hypcotyl. A greater number of cells may increase the capacity of the hypocotyl for compression. If each cell has a similar amount it is able to compress, having a greater number of cells will result in a greater cumulative amount the hypocotyl is able to compress before it fails.

There was a negative parameter estimate for days post-harvest suggesting as time since harvest increases, the radish hypocotyls become less resistant to splitting. This may be due to senescence. Potentially the structures within the cell walls begin to degrade over time making them less resistant to splitting.

Puncture:

Periderm

The first null hypothesis was rejected because failure force of the radish hypocotyl due to puncture with and without the periderm were correlated ($P<0.001$) (Figure 4-17). The percentage variance accounted for in the break force of the hypocotyl without a periderm accounted for by the break force of the hypocotyl with a periderm was 21.2 %. This relationship would suggest the genotypic and environmental factors which are involved in determining the strength of the periderm and cortex cells are linked. This result suggests that although there was a relationship between the strength of the periderm and cortex cells and some of these are likely to be related other factors were also likely to be involved in determining strength. The failure force due to puncture was greater for hypocotyls with a periderm than without a periderm suggesting in general the periderm is adding strength to the hypocotyl and determines the failure force of the hypocotyl as a result of puncture. This is in keeping with the results from Experiment 3.1 where there was a trend linking the periderm thickness of the radish hypocotyl with splitting susceptibility.

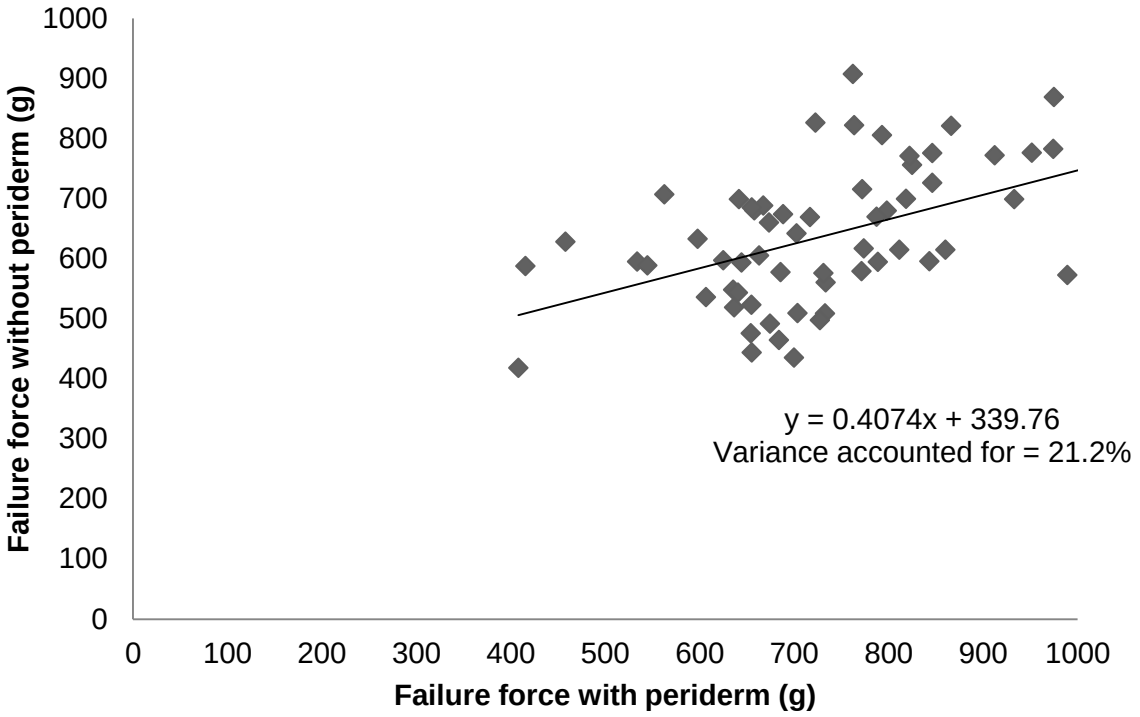


Figure 4-17 Failure force due to puncture of radish hypocotyls with and without a periderm (n=60)

RWC

This experiment successfully achieved a greater range in RWCs than Experiment 4.5. A range in RWCs from a maximum of 0.92 to a minimum of 0.75 were observed.

The third null hypothesis was rejected because the hypocotyl puncture force both with ($P=0.002$) and without a periderm ($P<0.001$) were correlated with RWC. The percentage variance accounted for in the hypocotyl failure force by RWC in hypocotyls with a periderm was 13.7% and without a periderm was 32.8%. These results suggest the splitting susceptibility of the hypocotyl periderm is less affected by the hypocotyl RWC than the cells in the cortex. However, the hypocotyl RWC was calculated for the whole of the hypocotyl and as the periderm was only a small proportion of this, the results are more likely to reflect the water content of the cortex than the periderm. It may be that as the periderm cells have a different role and constitution to the cortex cells the RWC does not change proportionally for both cell types. As the periderm tends to have a higher failure force than the cortex this means the periderm dictates the failure force of the hypocotyl. If

the measurement of RWC does not accurately indicate the RWC of the periderm this could explain why there is less variance accounted for by RWC in the failure force due to puncture with the periderm compared to without.

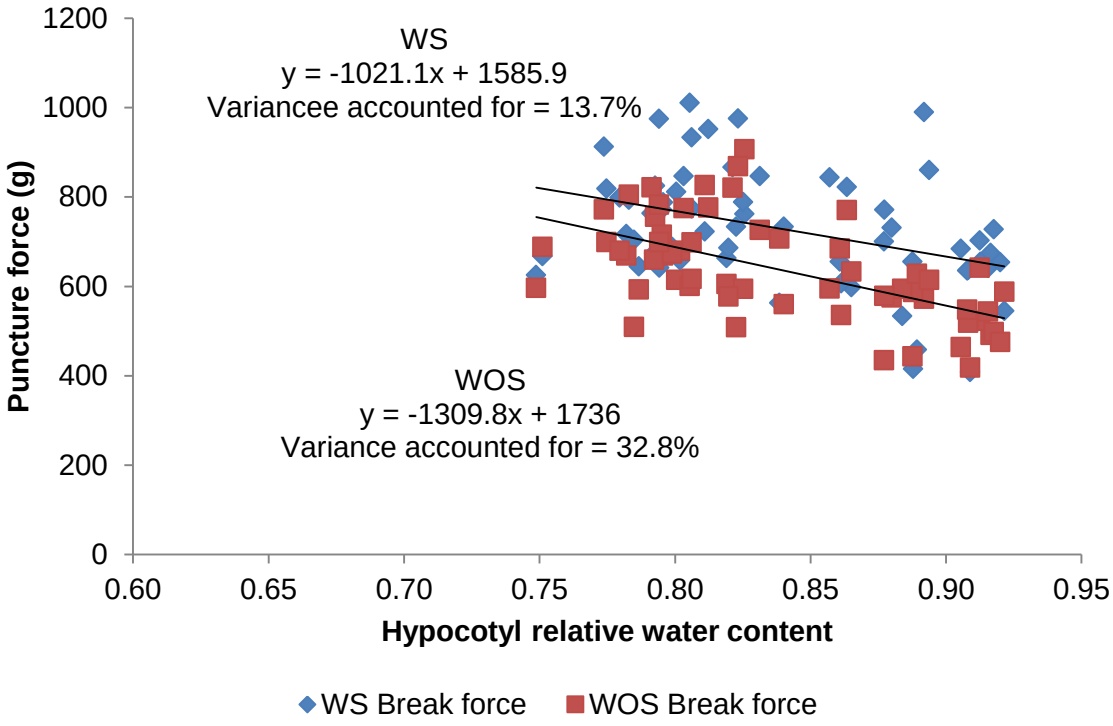


Figure 4-18 The failure force of radish hypocotyls both with (WS) and without (WOS) a periderm as a result of puncture at different RWCs (n=60)

When hypocotyl RWC and days post-harvest were both included in a multiple linear regression with hypocotyl failure force due to puncture (with periderm) there was a significant relationship (P=0.002) and 17.0 % of the variation in failure force was accounted for (Table 4-19). This was greater than the variance in failure force accounted for by RWC alone suggesting the time since harvest has an effect on splitting susceptibility.

Table 4-19 Model determined by multiple linear regression and stepwise deletion for the relationship between hypocotyl failure force due to puncture, RWC and days post-harvest (DPH).

	Model fitted	P	Variance accounted for
Failure force (g)	RWC +DPH	0.002	17.0%

Both RWC and DPH have a negative parameter estimates (Table 4-20) suggesting as they increase failure force due to puncture decreases. This suggests the radish hypocotyls become more susceptible to splitting as a result of puncture at higher RWCs and as time since harvest increases.

Table 4-20 Parameters and their estimates for the linear regression between failure force = RWC + days post-harvest (DPH)

Parameter	Estimate	SE	P
Constant	1055	392	0.009
RWC	-309	500	0.539
DPH	-18.2	10.0	0.075

Water content

This experiment successfully achieved a greater range in water contents than Experiment 4.5, a range in water contents from a maximum of 97.9% to a minimum of 95.8% were observed.

The second null hypothesis was rejected because hypocotyl puncture force both with (P=0.005) and without a periderm (P=0.004) were correlated with water content. The percentage variance accounted for in the hypocotyl failure force by water content in hypocotyls with a periderm was 11.4% and without a periderm was 12.0%. Both of these figures are less than the variance accounted for by the RWC with or without a periderm

suggesting RWC is a better indicator of splitting susceptibility due to puncture than water content. This is thought be because RWC gives a proportion of the maximum potential water content and therefore potentially an indicator of the stress which the cells are under, whereas water content is just a percentage.

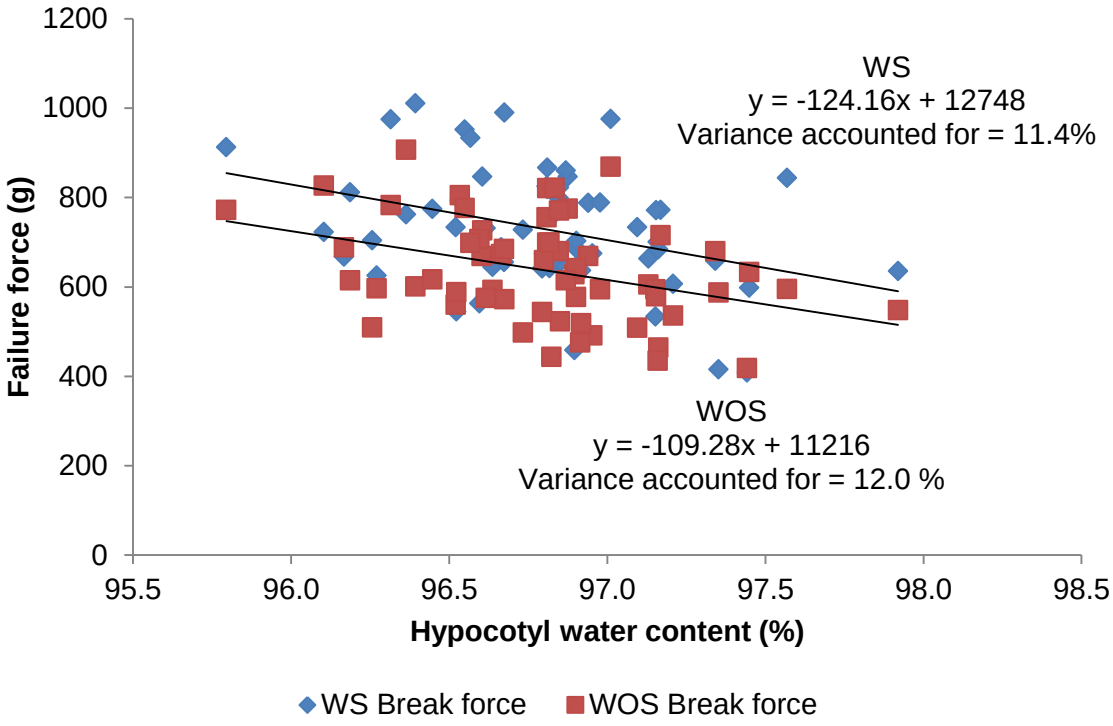


Figure 4-19 The failure force of radish hypocotyls both with (WS) and without (WOS) a periderm as a result of puncture at different water contents (n=60)

When hypocotyl water content (%) and days post-harvest were both included in a multiple linear regression with hypocotyl failure force due to puncture (with periderm) there was a significant relationship ($P<0.001$) and 23.1% of the variation in failure force was accounted for (Table 4-19).

Table 4-21 Model determined by multiple linear regression and stepwise deletion for the relationship between hypocotyl failure force due to puncture, water content (%) (WC) and days post-harvest (DPH).

	Model fitted	P	Variance accounted for
Failure force (g)	WC +DPH	<0.001	23.1%

Table 4-22 Parameters and their estimates for the linear regression between failure force = water content (WC) + days post-harvest (DPH)

Parameter	Estimate	SE	P
Constant	9627	3951	0.018
Water content	-91.2	40.9	0.030
DPH	-19.53	6.21	0.003

Both water content and days post-harvest have negative parameter estimates (Table 4-22). This suggests the radish hypocotyls become more susceptible to splitting as a result of puncture at higher water contents and as time since harvest increases. This is in keeping with the results from RWC where time since harvest also increased splitting susceptibility and as RWC increased splitting susceptibility increased. As hypocotyl RWC and water content are correlated (Figure 4-20) this result would be expected.

There was a significant correlation ($P<0.001$) between hypocotyl water content and RWC but only 21.3% of the variance in RWC is accounted for by water content. This is likely to be because RWC takes into consideration the potential maximum water content, which would be affected by physiological factors such as cell wall elasticity, in addition to the water content at the time of analysis whereas the water content just calculates the proportion of water compared to dry matter. Results in this experiment have shown RWC is a better indicator of splitting susceptibility than water content.

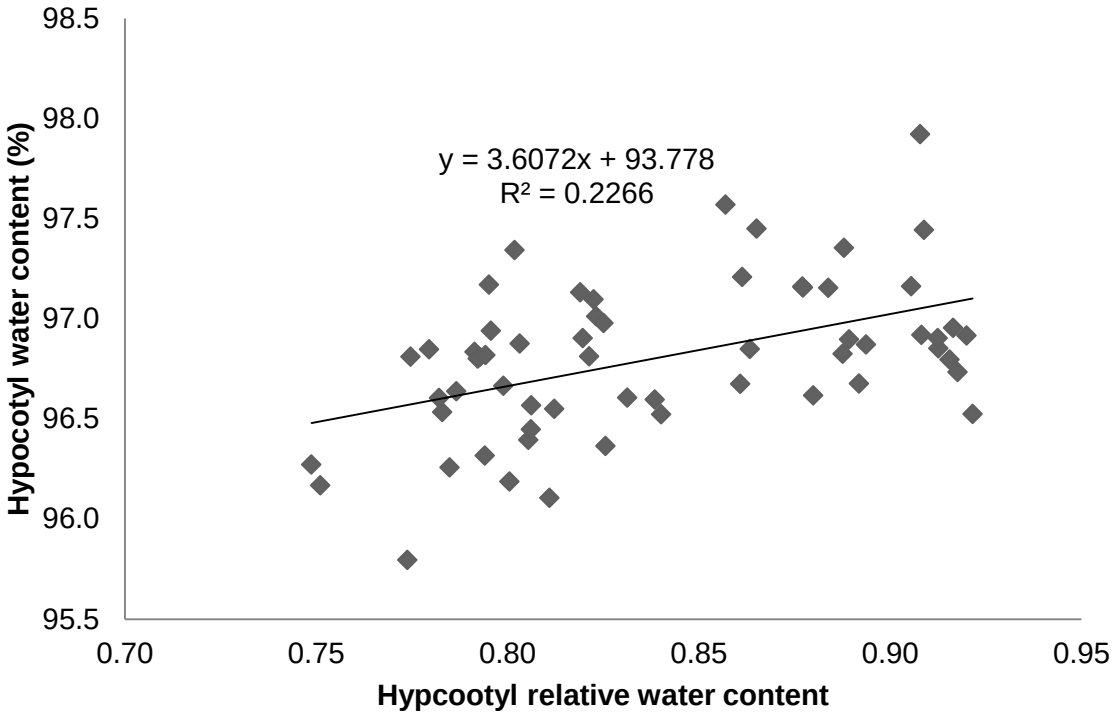


Figure 4-20 Relationship between hypocotyl water content and hypocotyl relative water content

In conclusion the first null hypothesis was rejected as there was a correlation ($P<0.001$) between failure force due to puncture both with and without the periderm. The failure force with the periderm tended to be greater than the failure force without. The second and third null hypotheses were rejected as there was a correlation both between the failure force of the periderm as a result of puncture and hypocotyl water content ($P=0.005$) and RWC ($P=0.002$). As in Experiment 4.5 it was again found that failure force decreased with increasing RWC and water content. The fourth null hypothesis was rejected as there was a correlation ($P=0.016$) between compression failure force and hypocotyl water content. As in Experiment 4.5 failure force decreased with increasing water content. The fifth null hypothesis was also rejected as there was a correlation ($P=0.001$) between hypocotyl failure force as a result of compression and hypocotyl diameter. Failure force was found to increase with increasing hypocotyl diameter.

A correlation between the time since harvest and splitting susceptibility was also observed. As days post-harvest increased, the failure force decreased suggesting splitting susceptibility increased with time.

4.4.6 Experiment 4.7: Investigating the effect of hypocotyl water content on the susceptibility of radishes to harvest splits and how this relates to hypocotyl water pressure

4.4.6.1 Experiment 4.7: Introduction

In the previous experiment, Experiment 4.6 there was a correlation between the time since harvest and splitting susceptibility. As days post-harvest increased, the failure force decreased suggesting splitting susceptibility increased with time. As the radishes which had undergone different treatments to create differences in water contents were tested on different days there may have been an effect on the results of the experiment. Therefore, to remove the effects of this confounding factor, in this experiment all radishes were tested on the same day.

In previous experiments it has been hypothesised the increase in splitting susceptibility at higher hypocotyl water contents and RWCs is a result of increased water pressure. In this experiment hypocotyl water pressure was tested using a pressure chamber.

Aim: To determine if:

- More of the variance in failure force as a result of compression and puncture are accounted for by RWC and water content when the texture analysis is all performed on the same day.
- Hypocotyl RWC and water content are correlated with hypocotyl water pressure

Null hypotheses:

1. There will be no significant correlation between hypocotyl failure force as a result of compression and water content
2. There will be no significant correlation between hypocotyl failure force as a result of puncture and water content
3. There will be no significant correlation between hypocotyl failure force as a result of puncture and RWC
4. There will be no significant correlation between hypocotyl water content and hypocotyl water pressure

5. There will be no significant correlation between hypocotyl RWC and hypocotyl water pressure

4.4.6.2 Experiment 4.7: Materials and method

In Experiment 4.7 all radishes underwent texture analysis on the same day. As in previous experiments, to create a range of water contents three treatments were used:

1. 1 day saturated (tested 2 days post-harvest)
2. 1 day closed container (tested 2 days post-harvest)
3. 1 day open container (tested 2 days post-harvest)

Compression and puncture texture analyses were carried out and hypocotyl water content and RWC were measured as described previously for Experiments 4.5 and 4.6. Twenty radishes from each group were used for texture analysis giving a total of 60 radishes for puncture analysis and 60 for compression texture analysis.

Pressure: The water potential (bar) of 20 radish hypocotyls per treatment was measured.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Linear regression was used to estimate the relationship between the failure force due to compression or puncture of the radish hypocotyls and the hypocotyl water content or RWC. Linear regression was also used to estimate the relationship between hypocotyl water content and RWC with hypocotyl water pressure. Linear regression was selected as the response variable and explanatory variables both contained continuous data.

Skeleton ANOVA:

Table 4-23 Skeleton ANOVA for compression and puncture texture analysis and hypocotyl water pressure at different hypocotyl water contents and RWCs

	df		
Source of variation	**Compression**	**Puncture**	**Pressure**
Regression	1	1	1
Residual	43	43	18
Total	44	44	19

4.4.6.3 Experiment 4.7: Results and discussion

Compression: Despite having the shortest treatment times to create a range in hypocotyl RWCs this experiment had a greater range in hypocotyl water contents than Experiment 4.5 and Experiment 4.6. A 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.

When a correlation matrix between the force required to split the radish and the WC and diameter of the radish was conducted, both the size of the radish and its water status were correlated with failure force, therefore the first null hypothesis was rejected. As has been observed and discussed previously, there was a negative correlation between failure force and hypocotyl water content suggesting failure force decreased as water content increased. There was a positive correlation between diameter and failure force (Table 4-24) suggesting larger radishes are more resistant to damage from compression, the potential reasons for this have been discussed previously.

Table 4-24 Correlation matrix (R^2) for failure force due to crushing, water content (WC) and hypocotyl diameter (n=55); (PPMCC critical value for n=60 = 1.671 at P=0.05; 2.390 at P=0.01; 2.660 at P=0.005)

	Diameter (mm)	**WC**	**Failure force (kg)**
Diameter (mm)	1		
WC	0.180	1	
Failure force (kg)	0.527	-0.337	1

When both diameter and hypocotyl water content were included in a multiple linear regression with failure force there was a significant relationship (P<0.001) and 66.1% of the variation in failure force was accounted for. This is a greater amount of the variance in failure force due to compression than has been explained in previous experiments suggesting testing the radishes on different days as had been done previously had introduced variability.

Table 4-25 Model determined by multiple linear regression and stepwise deletion for the relationship between failure force due to compression and hypocotyl diameter and water content

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

Puncture:

RWC

Again despite having the shortest treatment times to create a range in hypocotyl RWCs this experiment had the greatest variety in hypocotyl RWCs compared to Experiment 4.5 and 4.6. The range in RWC which was observed at the time of texture analysis was between a maximum of 0.98 and a minimum of 0.76. The third hypothesis was partially rejected because there was a non-significant trend (P=0.058) between hypocotyl RWC and puncture force with 6.0% of the variance being accounted for (Figure 4-21). These results would suggest radishes may be less resistant to splitting as a result of puncture at higher RWCs. The variance in failure force accounted for by RWC in this experiment was lower than has been observed in previous experiments. The reasons for this are unclear but potentially the hypocotyls were not fully saturated or dried prior to calculation of the RWC.

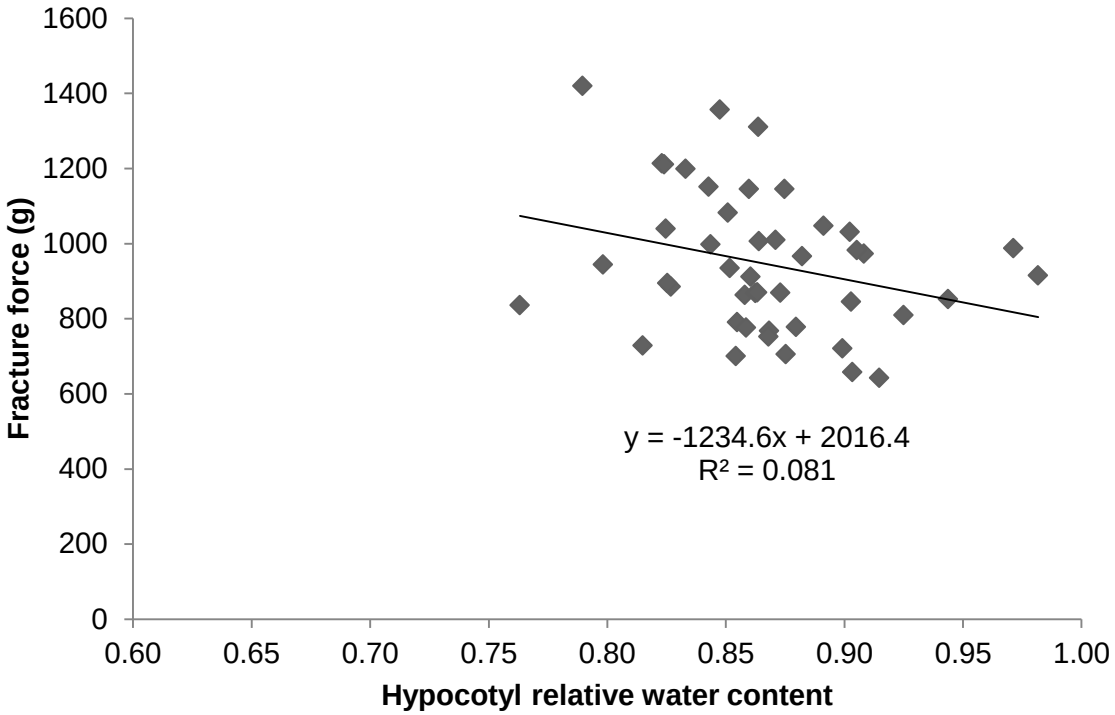


Figure 4-21 The force required to puncture the hypocotyl of radishes at different hypocotyl RWCs (n=55)

Water content

A 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. The second null hypothesis was rejected because there was a negative linear correlation ($P<0.001$) between water content (WC) and puncture force with 44.4% of the variance being accounted for (Figure 4-22). As observed in previous experiments, these results would suggest radishes are less resistant to splitting as a result of puncture at higher water contents. A greater amount of the variance in failure force as a result of puncture has been explained in this experiment compared to previous experiments where texture analysis was performed on different days. As with compression, this would suggest testing the radishes on different days as had been done previously introduced variability.

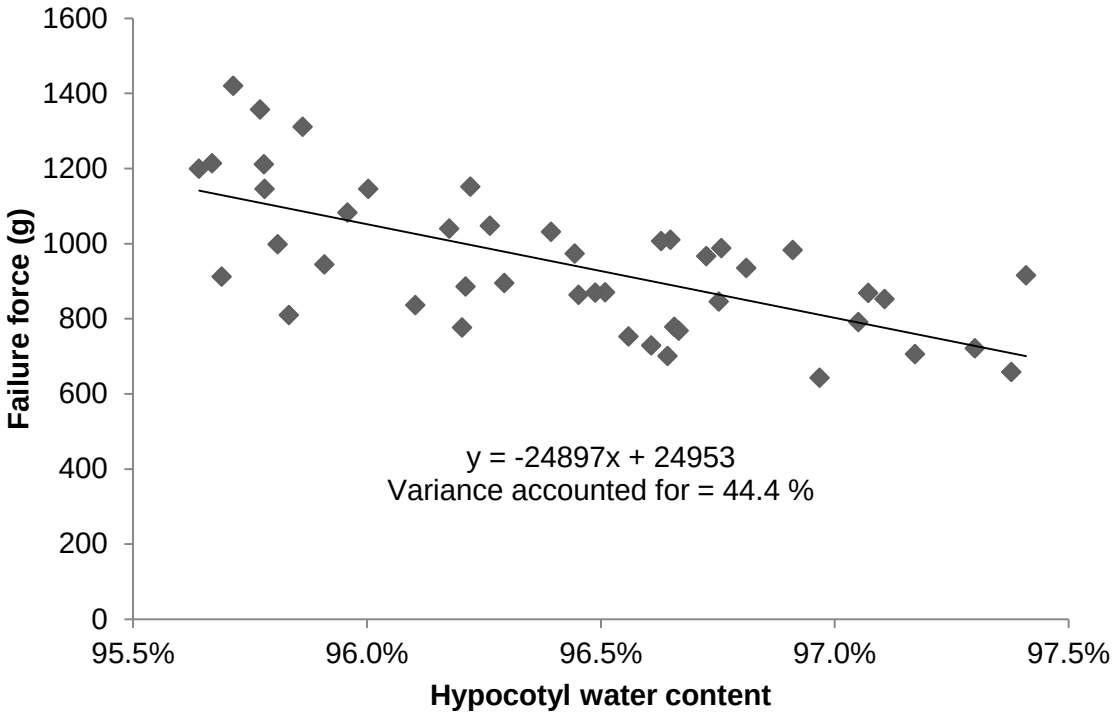


Figure 4-22 The force required to puncture the hypocotyl of radishes at different hypocotyl water contents (n=55)

Hypocotyl water pressure:

Water content

The fourth null hypothesis was rejected because a significant (P=0.034) negative linear relationship was observed between hypocotyl water content and hypocotyl water pressure, with 18.4% of the variance in hypocotyl water pressure being accounted for by hypocotyl water content. This would suggest as has been hypothesised in previous experiments, at higher water contents the cells within the radish hypocotyl are under increased pressure.

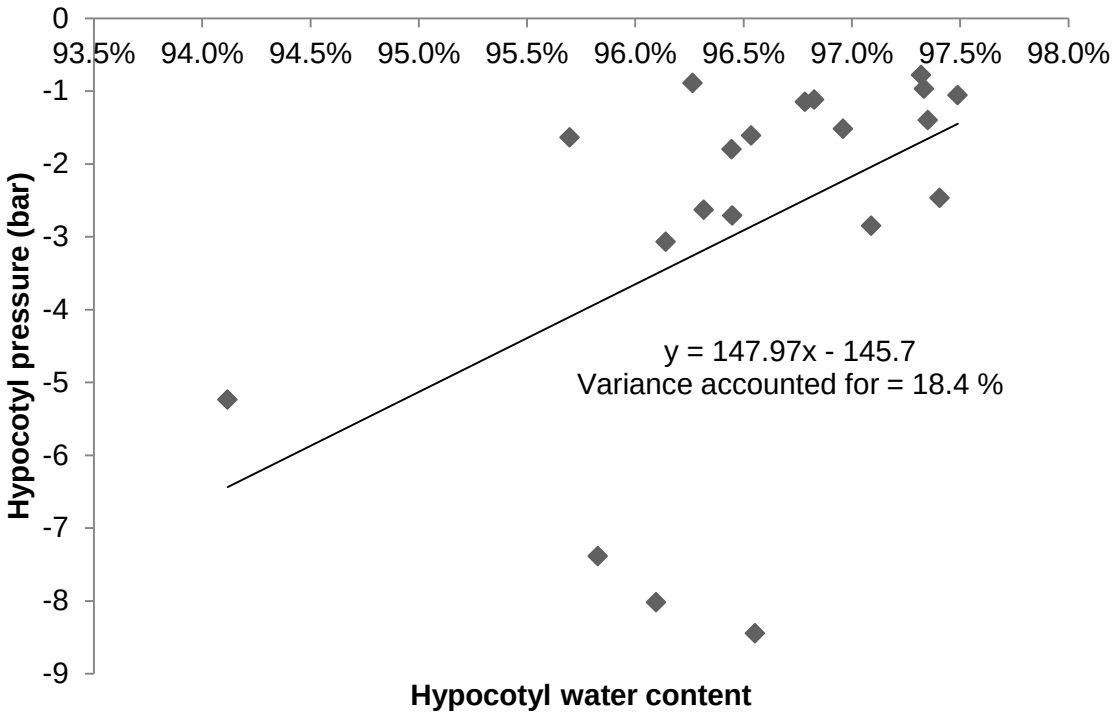


Figure 4-23 Linear correlation between hypocotyl pressure (bar) and hypocotyl water content (%)

RWC

The fifth null hypothesis was rejected because there was a significant (P=0.002) linear correlation between hypocotyl water pressure and hypocotyl RWC (Figure 4-24). The variance in hypocotyl water pressure accounted for by RWC was 40.2%. This is greater than the variance in hypocotyl water pressure which was accounted for my water content suggesting RWC is a more accurate indicator of hypocotyl water pressure. This is thought to be because RWC is a measure of the proportion of the maximum potential water content and therefore potentially an indicator of the stress which the cells are under, whereas water content is just a percentage.

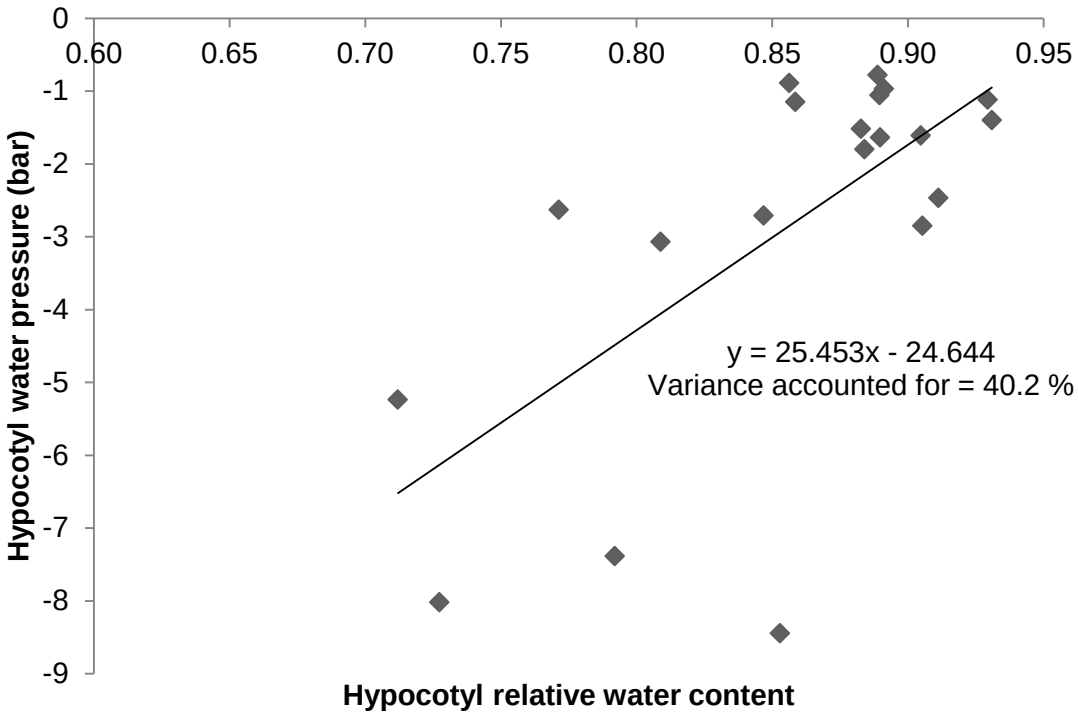


Figure 4-24 Linear correlation between hypocotyl pressure (bar) and hypocotyl RWC

In conclusion the first null hypothesis was rejected as a significant (P<0.001) correlation between hypocotyl failure force as a result of compression and water content was observed. As with Experiment 4.6 hypocotyl failure force as a result of compression was found to decrease with increasing water content. Again as in Experiment 4.6 a correlation was also observed between hypocotyl failure force as a result of compression and hypocotyl diameter. Failure force was found to increase with increasing hypocotyl diameter.

The second null hypothesis was rejected as there was a significant (P<0.001) correlation between hypocotyl failure force as a result of puncture and water content. As with the results from Experiments 4.4-4.6 the failure force as a result of puncture decreased with increasing water content.

The third null hypothesis was partially rejected as a trend (P=0.058) was observed correlating hypocotyl failure force as a result of puncture and RWC. As in Experiments 4.5 and 4.6 the failure force as a result of puncture decreased with increasing RWC.

The fourth and fifth null hypotheses were rejected as there was a significant correlation between hypocotyl water content (P=0.034) and RWC (P=0.002) with and hypocotyl water pressure.

4.4.7 Experiments 4.3 to 4.7: Discussion

In Experiment 4.2, relative humidity and accumulated precipitation were observed to be positively correlated with the amount of splitting recorded at G's by the QA team. Hypocotyl RWC and splitting as a result of impact were also found to be positively correlated. These results suggest a high accumulated precipitation or relative humidity may have resulted in a high hypocotyl RWC which may have made the hypocotyls more susceptible to splitting. The results in Experiment 4.2 were made from observational data and Experiments 4.3-4.7 were conducted under controlled conditions to investigate the relationship between hypocotyl water content and splitting susceptibility further.

It was demonstrated the radish hypocotyl was able to absorb water through the periderm as had been hypothesised in Experiment 4.2. As a consequence, the first two null hypotheses were rejected as preliminary experiments showed:

1. Water was able to pass through the periderm of the radish hypocotyl
2. There was change the water content of the radish hypocotyl over time

As had been shown in observational studies in Experiment 4.2, results in Experiment 4.3-4.7 also showed a significant relationship between hypocotyl water content and RWC. Splitting susceptibility as a result of impact was again shown to increase at higher water contents. Furthermore, results in Experiments 4.3-4.7 also showed this relationship was true for compression and puncture, two other likely causes of splitting in radish hypocotyls in commercial production. Therefore, the third null hypothesis was rejected because significant correlations were observed between hypocotyl water content and splitting susceptibility as a result of impact, compression and puncture.

The fourth null hypothesis was also rejected because significant correlations were found between hypocotyl RWC and splitting susceptibility as a result of impact and puncture. Hypocotyl RWC tended to explain more of the variance in failure force as a result of puncture and impact than hypocotyl water content and was thought to be a better indicator or splitting susceptibility. The RWC of radishes which underwent compression texture analysis could not be measured as the radish hypocotyls were too damaged after the texture analysis.

The fifth and sixth null hypotheses were rejected as significant relationships were observed between hypocotyl water content and hypocotyl water pressure, and hypocotyl RWC and hypocotyl water pressure. RWC was observed to explain more of the variance in hypocotyl water pressure than hypocotyl water content suggesting RWC is a better measure of hypocotyl water pressure.

One explanation of the observed responses is that post-harvest radish hypocotyl water content may affect splitting susceptibility by affecting the turgor pressure of the cells. In Experiment 3.1 the mode of failure of split radish hypocotyls was revealed to be plasmoptysis and when the mode of failure is plasmoptysis higher turgor pressure has been shown to reduce tissue strength (Lin & Pitt 1986). Hypocotyl water content affects the turgor pressure because pressure potential increases as water enters a cell. As water passes through the cell wall and cell membrane, it increases the total amount of water present inside the cell. The increase in the amount of water inside the cell exerts an outward pressure which is opposed by the cell wall. The cell wall is then placed under increased tension. Under increased turgor pressure cells may be more susceptible to splitting because the cell walls are already stressed and consequently more easily ruptured (Kokkoras 1995).

Evidence of water status having an effect on splitting in other crops comes from Gracie (2004). Gracie (2004) found a reduction in turgor pressure caused by partially-lifting carrots reduced splitting susceptibility. The carrots were partially-lifted to sever the fibrous root system then left in the soil over night before harvesting the following morning. These carrots with reduced turgor had a greatly diminished splitting susceptibility when tested with a penetrometer. Further evidence to support turgor pressure affecting splitting susceptibility is provided by the investigation conducted by Konstankiewicz and Zdunek (2001). They found the compressive strength of the tissue samples decreased with increasing turgor pressure suggesting potato tubers are less susceptible to splitting when they are more turgid (Konstankiewicz & Zdunek 2001). The results obtained by Konstankiewicz and Zdunek (2001) are similar to those of Bajema *et al.* (1998) who also found potatoes with lower turgor had higher compressive strength than more turgid

potatoes. McGarry (1993) found failure force in the phloem parenchyma tissue of carrots was negatively correlated with both water potential and turgor pressure.

In conclusion:

- Water is absorbed through the periderm by the radish hypocotyl
- Water is able to pass through the periderm of the radish hypocotyl
- There is a correlation between hypocotyl water content and splitting susceptibility as a results of impact, compression or puncture
- There is a correlation between hypocotyl RWC and splitting susceptibility as a result of impact or puncture
- There is a relationship between hypocotyl RWC and hypocotyl water pressure
- There is a relationship between failure force as a result puncture of hypocotyls with and without a periderm
- There is a relationship between hypocotyl water content and hypocotyl water pressure

4.5 Experiment 4.8: Investigating the effect of hypocotyl temperature on the susceptibility of radishes to harvest splits

4.5.1 Experiment 4.8: Introduction

Experiment 4.2 showed temperatures both during growth and at harvest were negatively correlated with splitting suggesting lower temperatures may result in an increased susceptibility to splitting. In support of this finding, there is evidence within the literature that temperature affects splitting susceptibility (Bourne 1982; Bajema *et al.* 1998; Kokkoras 1995).

Following harvest, radishes are handled in a controlled environment therefore if temperature has a significant effect on splitting there is the potential to reduce the prevalence of post-harvest splitting by changing the temperature.

In Experiment 4.8 impact texture analysis was selected to investigate the relationship between temperature and splitting susceptibility. This method of texture analysis was chosen due to its relevance to commercial post-harvest practices. 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.

Aim:

- The aim of this experiment was to investigate the relationship between hypocotyl temperature and splitting susceptibility as a result of impact at a range of temperatures.

Null hypothesis:

1. There is no significant relationship between splitting susceptibility as a result of impact and temperature.

4.5.2 Experiment 4.8: Materials and method

Radishes from a commercial grower in Norfolk, England were couriered on the day of harvest to arrive at HAU, Shropshire, England the following day. Upon arrival 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 80 mm (Weller Packaging, Lichfield, UK). The bags of radishes were placed into baths of water at the required temperature (Figure 4-25). The temperatures of the five baths were set to 5, 10, 20, 30 and 40°C respectively. The experimental unit was one radish hypocotyl and 20 replicates were used for each temperature. An additional three radishes were placed in grip seal bags at each temperature to enable the temperature of the hypocotyls to be measured. This was done by inserting a temperature probe into the radish hypocotyls (Figure 4-26).

Figure 4-25 Water bath set to 40°C containing radishes in grip seal bags prior to texture analysis

Figure 4-26 Measuring the temperature of a radish hypocotyl in a water bath using a temperature probe

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 (Figure 4-26). Impact tests were performed as previously described, by dropping the radishes down a 1.4 m 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.

Statistical analysis: All data was analysed using GenStat for Windows 15th Edition (VSN International 2011).

Linear regression was used to analyse the data as the response variable and explanatory variables both contained continuous data.

Table 4-26 Skeleton ANOVA for hypocotyl RWC at harvest

Source of variation	df
Regression	1
Residual	3
Total	4

4.5.3 Experiment 4.8: Results

The five different water baths set to 5, 10, 20, 30 and 40°C resulted in radishes with mean temperatures of 6.0, 11.0, 23.7, 29.6 and 38.7°C respectively.

Radish splitting susceptibility as a result of impact was found to have a strong (R^2=0.82) negative linear correlation with temperature (P=0.035). The greatest amount of splitting, 70% was observed at the lowest temperature, 6.0°C, and the least amount of splitting, 0%, was observed at the highest temperature, 38.7°C. The variance in splitting susceptibility accounted for by temperature was 75.5%. These results suggest radishes are more susceptible to splitting at lower temperatures (Figure 4-27).

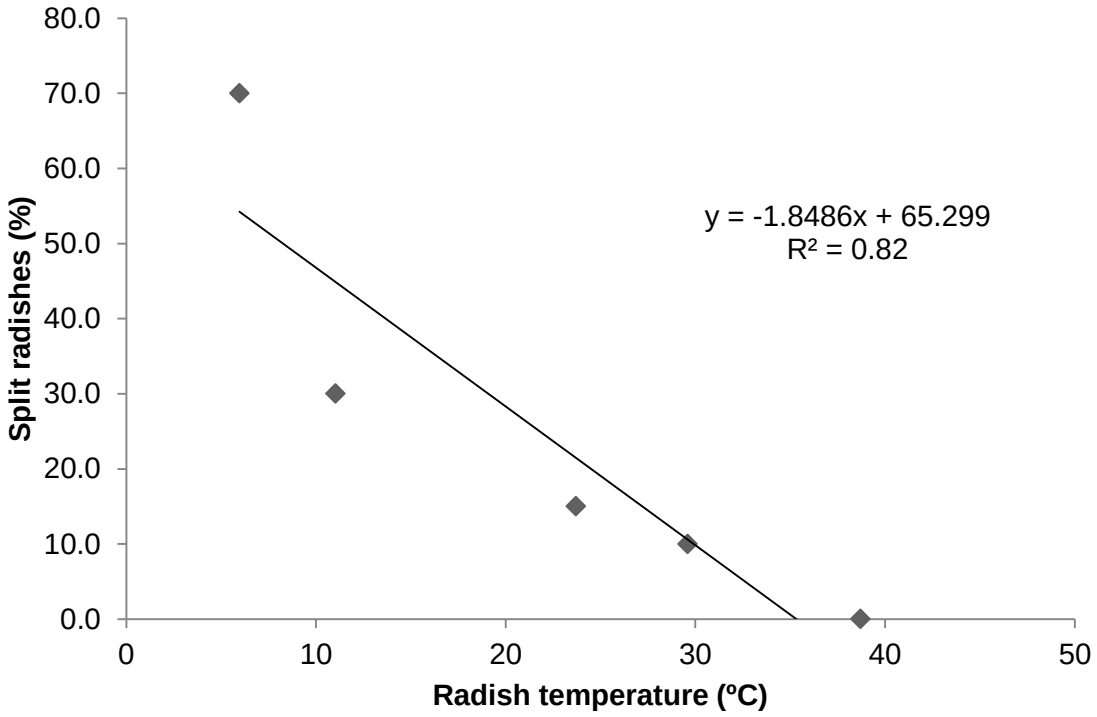


Figure 4-27 Percentage of radishes which split as a result of impact at different temperatures (n=20).

These results are in keeping with previous research into the effects of temperature on failure force (Bourne 1982). Under commercial conditions, radishes are stored and packed between 2 and 5°C which is slightly lower than the lowest temperature, 6.0°C, used in this investigation. Using the model S = -1.85T + 65.30, which described the linear relationship

between temperature (T) and percentage splitting (S) in this investigation, it is predicted that at 5°C, 56.1% of the radishes which were dropped would split, and at 2°C, 61.6% of the radishes would split. These results suggest, in terms of reducing splitting susceptibility it may be preferable to store and process radishes at warmer temperatures and then to chill them after handling to limit the consequences on shelf life and respiration.

4.5.4 Experiment 4.8: Discussion

The null hypothesis was rejected because results from this experiment showed increased splitting susceptibility at lower temperatures. In a review of the effects of temperature on a range of fruits and vegetables, Bourne (1982) also showed for the majority of crops tested, increased temperature was associated with decreasing firmness and Bajema *et al* (1998) found decreasing temperature resulted in significantly decreasing failure strain and tissue toughness in potato. Bajema *et al* (1998) also investigated the effects of turgor and a similar pattern was observed with increasing turgor resulting in decreasing failure strain and tissue toughness. 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. Kokkoras (1995) also found an effect of temperature on tissue stress within carrots and concluded the effect was due to associated changes in turgor making the tissue for susceptible to damage. They proposed low temperatures cause an increase in cellular turgidity by causing differences in contraction between the vacuole content, which is predominantly water, and the cytoplasm and cell wall. It is thought the cytoplasm and cell wall may contract to a greater extent than the vacuole at low temperatures causing an increase in turgor pressure (Kokkoras 1995).

Results from Experiment 4.7 linked radish hypocotyl water pressure with splitting susceptibility and it may be true that temperature affects water pressure and turgidity within the radish hypocotyl as has been suggested by Kokkoras (1995). However, this was not tested as part of this experiment so requires further investigation to determine the veracity of the statement.

In conclusion, to reduce susceptibility to splitting growers should store and handle radishes at as warm a temperature as is possible whilst maintaining shelf life and other quality attributes. As there is a linear relationship between temperature and splitting susceptibility, any increase in temperature would be beneficial in terms of reducing susceptibility to splitting.

4.6 Chapter 4 Harvest splits: Discussion

The main objective of work carried out in this chapter was to identify some of the factors which affect post-harvest splitting susceptibility in radishes. This chapter began with analysis of commercially grown radishes to identify environmental factors which correlate with splitting susceptibility. It was found relative humidity and temperature at harvest were correlated with the amount of splitting recorded by the quality control team at G's and the hypocotyl RWC was found to correlate with splitting susceptibility as a result of impact when analysed at HAU.

Following the results from the analysis of commercially grown radishes, a series of experiments were conducted to investigate the relationships between hypocotyl water content and hypocotyl RWC with splitting as a result of impact, compression and puncture. The aim of these experiments was to investigate if the results from the commercially grown radishes could be replicated under controlled experimental conditions. In support of the results from the commercial data, it was observed that splitting susceptibility as a result of impact, puncture and compression was increased at higher water contents. For impact and puncture texture analysis, the relationship between RWC and splitting susceptibility was also investigated. RWC tended to explain more of the variance in splitting susceptibility as a result of puncture or impact than water content. It was postulated that the explanation for increased splitting susceptibility at higher water contents and RWCs was due to the cells being under a greater amount of stress and therefore less additional stress was required to cause failure. To explore this hypothesis further, an experiment was conducted to investigate the relationship between hypocotyl RWC and water content with hypocotyl water pressure. In these investigations, it was shown that hypocotyls had a higher water pressure at higher water contents and RWCs. RWC was found to have a greater correlation with hypocotyl water pressure than water content; this is thought to be why RWC tended to explain more of the variance in splitting susceptibility than water content. RWC was a better indicator of splitting susceptibility and had a greater correlation with hypocotyl water pressure. This is not surprising as RWC is a measure of the water content of the tissue as a proportion of the total possible water

content whereas water content is a simple percentage. Results from these experiments support the hypothesis that the increase in splitting susceptibility which was observed at higher RWCs was due to a greater water pressure within the hypocotyl putting the cells under increased stress. These results are supported by the literature as Hartz *et al* (2005) found turgor prior to harvest was positively correlated with splitting in carrot and, similarly, McGarry (1993, 1995) found failure force in carrot tissue was negatively correlated with tissue turgor and water potential.

The chapter then concluded with an investigation into the relationship between hypocotyl temperature and harvest splitting. This showed radish hypocotyls were more susceptible to splitting at lower temperatures a finding that is in keeping with results from similar investigations involving other crops such as apple, cherry, carrot, beet and pea (Bourne 1982); 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 lead to the conclusion that the same mechanism must explain both the effects of temperature and turgor. The mechanism for differences in splitting susceptibility at different temperatures may be similar to that of RWC in radishes but as this was not investigated it cannot be verified.

4.7 Chapter 4 Harvest splits: Conclusions

- Water is able to be taken up by the hypocotyl through the periderm
- Water can be gained or lost by the periderm in minutes
- Higher hypocotyl water content results in higher susceptibility to splitting as a result of impact
- Higher hypocotyl water content results in higher susceptibility to splitting as a result of puncture
- Higher hypocotyl water content results in higher susceptibility to splitting as a result of compression
- Higher hypocotyl RWC results in higher susceptibility to splitting as a result of impact
- Higher hypocotyl water content results in higher susceptibility to splitting as a result of puncture
- RWC tends to correlate better with splitting susceptibility as a result of impact and puncture than water content
- Hypocotyl water content is correlated with hypocotyl water pressure
- Hypocotyl RWC is correlated with hypocotyl water pressure
- Hypocotyl RWC has a better correlation with hypocotyl water pressure than hypocotyl water content
- Radishes with larger hypocotyl diameters are more resistant to splitting
- As days since harvest increase splitting susceptibility as a result of puncture and compression increase
- Splitting susceptibility as a result of impact reduces with increasing temperature

The factors which were found to be associated with susceptibility to growth splitting in Chapter 4 are summarised in Figure 4-28.

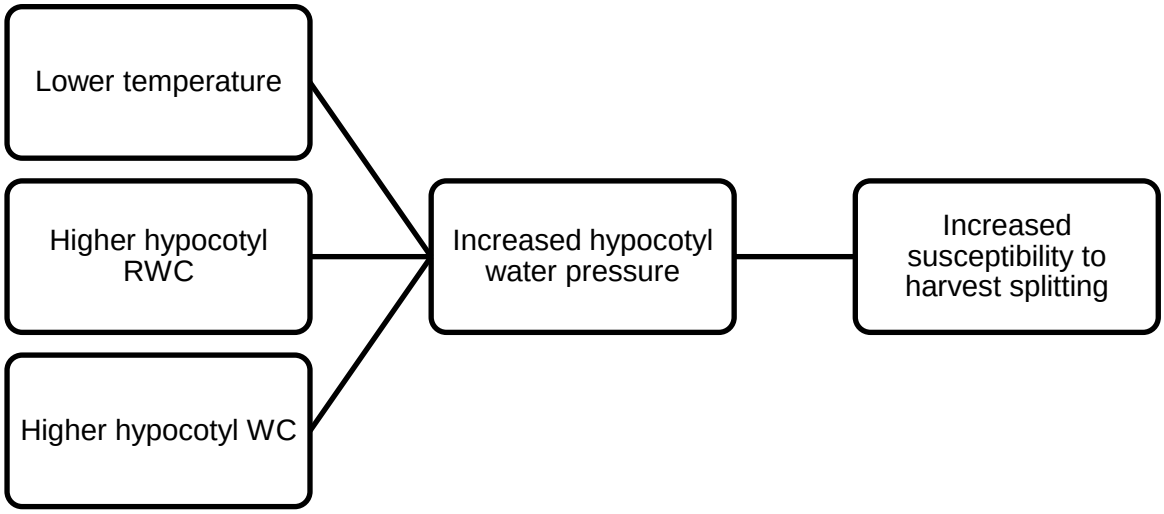


Figure 4-28 Factors which have been associated with susceptibility to harvest splitting in Chapter 4.

5. Summary of main findings

The main objectives of this thesis were to investigate the potential of reducing splitting in radishes, while maintaining acceptable yields. The aim was to achieve this by understanding the factors which affect splitting and the underlying mechanisms behind susceptibility to splitting. The prevalence of splitting could then be minimised by manipulating conditions during growth, harvest or post-harvest. These objectives were achieved by dividing the research into two principal studies: growth splits (Chapter 3: Growth Splits, investigated splitting which occurs during growth); and harvest splits (Chapter 4: Harvest Splits focused on splitting which occurs during and post-harvest). In Chapter 3, the effects of cultivar were investigated, the growth stages of radishes were established, quality control data from commercially grown radishes was investigated for trends correlating growth splits with weather conditions and the relationship between VWC and splitting was investigated. In Chapter 4, commercial quality control data was again analysed for relationships between weather data and harvest splitting, further to this, the effects of hypocotyl water content on harvest splits was investigated as was the effect of hypocotyl temperature on harvest splitting susceptibility. At the end of Chapter 3 and Chapter 4 there was a discussion of the findings relating solely to growth or harvest splitting. To avoid repetition, Chapter 5 will focus on the theme which is common to both Chapter 3 and Chapter 4, hypocotyl turgor pressure.

In Experiment 3.1 split radishes were sectioned to verify the mode of failure within radish hypocotyl tissue. In these sections, ruptured cell walls were observed, therefore it was concluded these radishes had split by plasmoptysis. This result was in keeping with previous work by Skok (1941) who showed sections of split radishes which appeared to have failed due to plasmoptysis rather than cellular debonding. This mode of failure is comparable to other vegetables such as kohlrabi (Lippert 1999) and carrot (McGarry 1993) and may result from the limited intercellular space within these vegetable tissues.

Results from Chapter 4 demonstrated harvest splitting susceptibility is increased at higher hypocotyl WC, RWC and water pressure. As the mode of failure within the radish hypocotyl was plasmoptysis, it is thought the increased susceptibility to splitting at higher hypocotyl WC, RWC and water pressure was a result of reduced tissue strength resulting from increased turgor pressure. At higher pressure, tissue is more susceptible to plasmoptysis as less additional force is required to rupture cell walls which are already under tension. These findings are in accordance with results from other vegetable crops such as carrot (McGarry 1993, 1995) where higher turgor pressure has also been shown to result in increased splitting susceptibility.

In Experiment 4.8 susceptibility to splitting was also shown to increase with decreasing temperature. The mechanism for this is also thought to be due to turgor pressure as a result of low temperatures causing differences in contraction between the vacuole content, which is predominantly water, and the cytoplasm and cell wall. It is thought the cytoplasm and cell wall may contract more than the vacuole at low temperatures causing an increase in turgor pressure (Kokkoras 1995). This theory was not tested in experiments conducted as part of Experiment 4.8 and requires further investigation to test its validity.

Results from Chapter 3 are not conclusive in terms of the effects of hypocotyl water pressure on susceptibility to growth splitting. In Experiment 3.4, it was shown that weather conditions were correlated with levels of splitting in commercially grown radishes. Results from the analysis of commercial QA data showed relative humidity and rainfall both had exclusively positive parameter estimates when correlated with splitting suggesting radishes were more susceptible to splitting with increasing rainfall and relative humidity. It was hypothesised that the radishes which experienced higher levels of rainfall during growth had higher hypocotyl turgor pressure as a result of absorbing water by osmosis from the soil through the periderm into the hypocotyl. Greater water availability in the soil may also result in greater water uptake by the vascular system into the tissue, increasing turgor pressure and placing an increased pressure on the skin from within (Sekse 1995). The radishes which experienced higher relative humidity may have had higher hypocotyl

turgor pressure as a result of lower transpiration rates resulting in less water being lost from the hypocotyl.

Similar to the results from Experiment 3.4, in Experiments 3.6 and 3.7, it was shown different cultivars of radishes grown under conditions of constantly higher VWC all had a higher rate of splitting compared to radishes grown under conditions of constantly lower VWC. In Experiment 4.3 it was demonstrated that radishes are able to absorb water through their periderm hence the higher rates of splitting observed in radishes grown in conditions of greater VWC could have been due to these radishes having a relatively higher turgor pressure within the hypocotyl resulting in the periderm being under higher levels of stress and more susceptible to splitting compared to radishes grown under conditions of lower VWC. However, inconsistent results were found for the relationship between hypocotyl WC and RWC and splitting and no relationship was found to link hypocotyl WC with hypocotyl water pressure in Experiment 3.7. This may be because the underlying mechanism behind susceptibility to growth splitting is more complex than hypocotyl WC and water pressure putting increased stress on the cell wall making it less resistant to splitting. During growth radishes are developing and responding to the environmental conditions they are exposed to. For instance it is known water availability can have an effect on cellular composition and in particular lignin biosynthesis (Lee *et al.* 2007). 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. As lignin is a constituent of cell strength this may have an effect on susceptibility to splitting. Lignin levels were not measured in these studies and this area requires further investigation.

There may also be problems with the method used to measure hypocotyl WC and RWC of split radishes because the split surface may have resulted in greater water loss compared to radishes without a split surface. If radishes with a higher hypocotyl water content split more readily but then also lose water at a greater rate as a result of this split, measuring the hypocotyl water content at the point of harvest will not be a true representation of the hypocotyl water content at the point of splitting. As it is impossible to predict growth

splitting it would be difficult to measure the hypocotyl water content at the point of splitting to determine if this is correlated with growth splitting.

Results from experiments conducted as part of this thesis suggest it may be more practical for growers to reduce post-harvest splitting susceptibility by optimising post-harvest handling. If radishes which have been split as a result of growth splits can be removed at harvest, harvest splitting can be minimised by reducing turgor pressure. This should prevent supermarket rejections.

5.1 Overall conclusions

- Radishes split by plasmoptysis
- Substrate VWC affects growth splitting
- Radishes are more susceptible to splitting as a result of high VWC at Growth Stage 41
- Water can be absorbed by the hypocotyl post-harvest
- High RWC and WC are linked to high water pressure
- Increasing hypocotyl WC, RWC or water pressure increases susceptibility to harvest splitting
- Decreasing temperature increases susceptibility to harvest splitting

5.2 Adoption and field application

Field grown radishes are not routinely irrigated in the UK and accurately controlling the VWC according to the growth stage of the crop could be challenging under field conditions where there is always a chance of rainfall at any point during radish growth. This may limit the successful application of a period of drying prior to Growth Stage 41 in radishes. It is not thought it would be commercially viable to grow radishes under polytunnels which tend to be used exclusively for higher value crops such as strawberries in the UK. The climate of the UK is, however, changing and the total summer precipitation has decreased in most parts of the UK, typically by between 1 to 40% since 1961 (Street 2007).The trend is towards warmer wetter winters and hotter drier summers with projected 50% less precipitation in the summer months and up to 30% more precipitation in the winter months by 2100. The impacts of these projected changes are expected to increase the temporal and spatial demand for irrigation around 20% by 2020 and around 30% by 2050 due to longer dry periods in summer (Street 2007). Therefore, in future if radish crops do become routinely irrigated there is scope for reducing growth splitting by introducing a period of drying prior to Growth Stage 41 which is easily and non-destructively identifiable in the field.

Under current agronomic practices, harvest splitting in radishes could be reduced by reducing the turgor pressure at key points in handling identified as when the produce is most susceptible to damage. Reducing the turgor pressure could either be achieved by reducing the hypocotyl water pressure or by increasing temperature. In experiments conducted as part of this thesis, small changes in hypocotyl water content resulted in large changes in splitting susceptibility therefore it may be possible to reduce the water content of the radishes to a level which is commercially acceptable but which is also less susceptible to harvest splitting. Alternatively, radish hypocotyls were shown to be able to absorb water through the periderm post-harvest so it may be possible to re-hydrate the radishes prior to packing. As a linear relationship was shown between hypocotyl temperature and susceptibility to harvest splitting any increase in temperature during handling would decrease the susceptibility to splitting.

5.3 Critical review of methodologies

The experimental designs and number of replicates used in all applied experiments (Chapters 3 & 4) were generally acceptable for quantifying the effects of different factors on growth and harvest splitting. Where faults in methodology were identified, improvements were made and further investigation conducted.

Methodology from research in this thesis can be applied to similar research into splitting in other crops. In addition some of the equipment designed for use in experiments has already been used for other pieces of research assessing lettuce leaf rib RWC and cracking.

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