POSTHARVEST BERRY SPLIT AND ABSCISSION IN 'THOMPSON SEEDLESS' AND 'WALTHAM CROSS' TABLE GRAPES

BY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

SUMMARY

Postharvest berry split and abscission are prevailing physiological disorders that negatively impact on the quality of table grapes exported from South Africa. Inferior grape quality due to these disorders results in a considerable decline in consumer confidence in the branded product, which leads to a drop in demand, and consequently, lower prices. Since information concerning postharvest factors influencing postharvest berry split and abscission is limited, the search for reliable methods to adequately control these problems remains elusive. In an attempt to obtain the required information, the influence of harvest temperature, harvest maturity, perforated liners, field heat removal prior to packing, delay periods before and after packing, storage duration and the elevation of storage temperature on the development of berry split and abscission in 'Thompson Seedless' (*Vitis vinifera* Linnaeus) table grapes was investigated. Changes in abscission related factors during berry development, and the influence of pre-and postharvest ethylene inhibitors on the development of berry abscission in 'Waltham Cross' table grapes, was also studied.

Berry split was aggravated by packing 'Thompson Seedless' grapes at high pulp temperatures of approximately 30°C, especially if the grapes were packed in non-perforated bags. The incidence of berry split could be reduced by between 80 and 90% by packing grapes in perforated instead of non-perforated liners. Perforated bags also reduced levels of SO_2 damage. However, due to significantly more moisture loss from grapes in perforated bags, compared to non-perforated bags, the risk of higher fruit and stem desiccation and softer berries existed. Optimum size and density of perforations needs to be determined to reduce berry split without excessive loss of moisture from the grapes, and SO_2 gas from the air space surrounding the product. The influence of harvest temperature and liner type on berry abscission was not conclusive. Advanced maturity increased grape resistance to berry split. However, grapes harvested too mature were prone to stem desiccation and the development of *Botrytis* decay. The occurrence of berry abscission also appeared to increase with advanced harvest maturity. Consequently, to ensure optimal post-storage quality, 'Thompson Seedless' grapes should be harvested as soon as horticultural maturity has been reached, which appears to be at approximately 18°Brix.

Field heat removal for 1.5 hours at 19°C prior to packing had no beneficial or adverse effect on berry split and abscission. Delay periods prior to packing aggravated berry abscission, but did not influence berry split significantly. Grapes delayed for 12 hours showed a significant increase in berry abscission and *Botrytis* decay, compared to grapes delayed for only 3 or 8 hours.

Considering that the absence of fungal decay is the most important quality prerequisite in table grapes, it is of vital importance to pack grapes with as short a delay period as possible. Grapes packed in non-perforated liners and delayed for different durations after packing, before the onset of forced-air cooling (FAC), showed significant differences regarding the incidence of berry split. Grapes delayed for 18 hours had significantly higher levels of berry split directly after the delay period, compared to grapes delayed for 6 or 12 hours. No significant difference in berry abscission occurred between grapes delayed for different periods. To minimise the amount of berry split, FAC should be applied as rapidly as possible after the packing of grapes in non-perforated liners.

Two storage related factors significantly influenced the incidence of berry split in 'Thompson Seedless' grapes during cold storage significantly, *viz.* the duration of storage at -0.5°C, and the increase in temperature after low temperature storage. Berry split increased almost linearly with prolonged storage at -0.5°C. An elevation of storage temperature from -0.5°C to 10°C any time during the cold storage period, further aggravated the split problem. Consequently, the reduction of berry split in 'Thompson Seedless' table grapes during cold storage requires (a) the shortest possible cold storage period, and (b) good temperature management throughout distribution, from initiation of cooling until the final point of sale.

The grape berry abscission potential, as quantitatively indexed by the measurement of the fruit removal force (FRF), showed significant changes during berry development of 'Waltham Cross' table grapes, from 27 to 111 days after full bloom (DAFB). This showed that at certain stages of fruit growth, 'Waltham Cross' grapes are more prone to berry abscission. At 27 DAFB, when the berries had an average diameter of 6.6mm, the grape bunches showed a significantly higher potential for berry abscission, compared to grapes sampled at a later stage. 'Waltham Cross' has inherently straggly bunches with bare shoulders. Therefore, any abscission during berry development will aggravate the problem. Consequently, it is of vital importance that any adverse factors such as moisture stress be avoided, especially during the period when 'Waltham Cross' grapes appear to be very susceptible to berry abscission. Of all parameters measured, moisture loss showed the best correlation with abscission. Grapes harvested with total soluble solids (TSS) of 12.3°Brix, 83 DAFB, had a significantly higher abscission potential than grapes harvested more mature. Therefore, by harvesting 'Waltham Cross' grapes at optimum maturity, at a TSS of approximately 16.4°Brix, berry abscission can be reduced to a great extent. It was evident that at veraison, the metabolism of grape berries changes drastically, and additional to the rapid increase in sugars and the rapid decrease in acidity, a decrease in FRF occurs.

Preharvest sprays of ReTain™ (a derivative of aminoethoxyvinylglycine), which inhibits ethylene synthesis, showed no promise as a means to reduce postharvest berry abscission. A postharvest treatment with EthylBloc® (1-methylcyclopropene), which inhibits ethylene action, only reduced berry abscission during one season.

OPSOMMING

Die fisiologiese defekte korrelbars en los korrels wat algemeen voorkom tydens opberging van sekere tafeldruif-kultivars, het 'n negatiewe invloed op tafeldruiwe wat uitgevoer word vanaf Suid-Afrika. Minderwaardige kwaliteit as gevolg van hierdie defekte het 'n aansienlike afname in verbruikers-vertroue tot gevolg wat aanleiding gee tot 'n ooreenkomstige afname in aanvraag en prys van die produk. Inligting rakende na-oes faktore wat die voorkoms van korrelbars en los korrels beïnvloed is beperk, en geen gewaarborgde metode bestaan om hierdie twee defekte volkome te beheer nie. In 'n poging om dié gewenste inligting te bekom, is ondersoek ingestel na die effek van oes-temperatuur, oes-rypheid, geperforeerde sakke, veldhitte verwydering voor verpakking, vertragingsperiodes voor en na verpakking, tydsduur van opberging, en die verhoging van die opbergingstemperatuur, op die voorkoms van korrelbars en los korrels by 'Thompson Seedless' (*Vitis vinifera* Linnaeus) druiwe. Daar is ook ondersoek ingestel na veranderings in afsnoering verwante faktore tydens korrel-ontwikkeling, en die invloed van voor- en na-oes toedienings van etileen inhibeerders op die ontwikkeling van los korrels by 'Waltham Cross' tafeldruiwe.

Korrelbars is vererger deur 'Thompson Seedless' met hoë pulptemperature van ongeveer 29.5°C te verpak, veral indien dit in 'n nie-geperforeerde sak verpak is. Die voorkoms van korrelbars kon tussen 80 en 90% verminder word deur 'Thompson Seedless' druiwe in geperforeerde sakke te verpak, in plaas van nie-geperforeerde sakke. Geperforeerde sakke het ook SO_2 skade op die druiwe verminder. Tog, as gevolg van betekenisvol meer vogverlies vanaf druiwe in geperforeerde sakke as vanaf druiwe in nie-geperforeerde sakke, bestaan die risiko van meer stingel-uitdroging en minder ferm korrels indien druiwe in geperforeerde sakke verpak word. Optimale grootte en digtheid van perforasies moet bepaal word om korrelbars te verminder, maar sonder oormatige vogverlies vanaf die druiwe en oormatige verlies aan SO_2. Die invloed van oes-temperatuur en sak-tipe op los korrels was nie oortuigend nie. Gevorderde oes-rypheid het die druif se weerstand teen korrelbars verhoog. Daarteenoor was druiwe wat té ryp geoes is, meer gevoelig vir stingel-uitdroging en *Botrytis* bederf. Dit wil ook voorkom of die voorkoms van los korrels toeneem met gevorderde rypheid. Dus, om optimum kwaliteit na opberging te verseker, moet 'Thompson Seedless' geoes word sodra hortologiese rypheid bereik word, wat blyk om by 'n totale opgeloste vaste stof-inhoud (TOVS) van ongeveer 18°Brix te wees.

Veldhitte verwydering voor verpakking, vir 1.5 uur by 19°C, het geen effek gehad op die voorkoms van korrelbars en los korrels nie. 'n Vertragingsperiode voor verpakking het die los

korrel-probleem vererger, alhoewel dit geen betekenisvolle invloed op die voorkoms van korrelbars gehad het nie. Druiwe wat vir 12 uur voor verpakking vertraag is, het betekenisvol meer los korrels en *Botrytis* bederf getoon, in vergelyking met druiwe wat slegs 'n vertragingsperiode van 3 of 8 uur ondergaan het. Aangesien die afwesigheid van bederf die belangrikste kwaliteits-vereiste vir tafeldruiwe is, is dit van kardinale belang om druiwe so gou as moontlik na oes te verpak. Druiwe, verpak in nie-geperforeerde sakke, wat vir verskillende periodes vertraag is voor geforseerde-lug verkoeling, het betekenisvolle verskille getoon betreffende die voorkoms van korrelbars. Druiwe vertraag vir 18 ure voor verkoeling, het betekenisvol meer korrelbars getoon, soos gemeet onmiddellik na die vertragingsperiode, in vergelyking met druiwe wat slegs vir 6 of 12 ure vertraag was. Geen betekenisvolle verskille in los korrels het voorgekom tussen druiwe wat verskillende vertragingsperiodes ondergaan het nie. Om korrelbars te verminder, moet geforseerde-lug verkoeling so gou as moontlik na verpakking van druiwe in nie-geperforeerde sakke toegepas word.

Twee opbergings-verwante faktore beïnvloed die voorkoms van korrelbars by 'Thompson Seedless' druiwe tydens koelopberging, naamlik die tydsduur van opberging by -0.5°C, asook 'n styging in temperatuur vanaf -0.5°C tot 10°C. Korrelbars het feitlik liniêr toegeneem met verlengde opberging by -0.5°C. 'n Styging in temperatuur vanaf -0.5°C tot 10°C op enige tydstip gedurende die koelopbergingsperiode, het korrelbars verder vererger. Dus, om korrelbars by 'Thompson Seedless' tydens opberging tot die minimum te beperk, moet die tydsduur van opberging so kort as moontlik wees, en moet die koue ketting regdeur die distribusie-proses gehandhaaf word, vanaf inisiëring van verkoeling tot en met die uiteindelike verkoop van die produk.

Die afsnoerings-potensiaal van druiwe, soos kwantitatief geïndekseer is deur meting van die vrug-verwyderings-vermoë (VVV), het betekenisvol verander gedurende korrel-ontwikkeling van 'Waltham Cross' tafeldruiwe, vanaf 27 tot 111 dae na volblom (DNVB). Dit het getoon dat 'Waltham Cross' druiwe by sekere stadiums van vrug-groei meer gevoelig is vir korrel afsnoering. By 27 DNVB, wanneer die korrels 'n gemiddelde deursnee van 6.6mm gehad het, het die druiwe 'n betekenisvolle hoër potensiaal vir afsnoering getoon, in vergelyking met druiwe wat op 'n latere stadium getoets is. 'Waltham Cross' is inherent geneig tot yl trosse met kaal skouers, gevolglik sal enige afsnoering tydens korrel-ontwikkeling die probleem vererger. Dus is dit van kardinale belang dat enige nadelige faktor, soos byvoorbeeld vogstres, vermy moet word, veral gedurende periodes wanneer dit wil voorkom of 'Waltham Cross' baie vatbaar is vir korrel afsnoering. Van al die parameters wat gemeet is, het vogverlies die beste korrelasie met korrel afsnoering getoon. Druiwe wat 83 DNVB, by 'n TOVS van 12.3°Brix geoes is, het 'n

betekenisvol hoër potensiaal vir korrel afsnoering getoon, in vergelyking met druiwe wat ryper geoes is. Dus, deur 'Waltham Cross' druiwe by optimum rypheid te oes, by 'n TOVS van ongeveer 16.4°Brix, kan korrelbars in 'n groot mate verminder word. Tydens *veraison*, wanneer die metabolisme van die druiwe drasties verander, was daar gepaardgaande met die drastiese toename in TOVS en die drastiese afname in totale titreerbare sure (TSS), ook 'n afname in VVV.

Voor-oes bespuitings met ReTain™, wat etileen sintese inhibeer, het geen potensiaal getoon om los korrels by 'Waltham Cross' te verminder nie. 'n Na-oes behandeling met EthylBloc®, wat etileen werking inhibeer, het slegs korrel afsnoering in een van die seisoene effens verminder.

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CONTENTS

1. LITERATURE REVIEW : FRUIT SPLITTING

1.1 INTRODUCTION

1.1.1 Overview

One of the most wide-spread physiological disorders that limits the production and marketing of soft, fleshy and blemish-free fruit is the cracking of the skin and splitting of the underlying flesh. Fruit species in which this is a significant horticultural problem are cherries (Christensen, 1975), grapes (Meynhardt, 1964a), tomatoes (Reynard, 1960), citrus (Garcia-Luis *et al.*, 1994), prunes (Mrozek and Burkhardt, 1973), nectarines (Fogle and Faust, 1976) and apples (Faust and Shear, 1972). Cracking and splitting also occurs extensively in grain crops, e.g. rice (Lague and Jenkins, 1991a,b) and in vegetable crops such as sweet potatoes (Lutz *et al.*, 1949), pepper (Johnson and Knavel, 1990) and carrots (McGarry, 1993).

Cracking and splitting prior to harvest (Meynhardt, 1964a), while the fruit is still attached to the plant, is of considerable economic importance to growers, because it leads to significant commercial losses by reducing both quality and yield. The cracking and splitting of detached fruit during postharvest handling (Mohsenin, 1972) and in cold storage (Mezzetti, 1959; Ryall and Harvey, 1959; Goode *et al.*, 1975) is also of major concern.

The presence of cracks or splits alters the structural integrity and lowers the mechanical strength of the fruit (Lague and Jenkins, 1991a). When subjected to mechanical stresses, cracks or splits produce lines of weakness along which the fruit is more likely to undergo further damage. Cracks and splits account for excessive crushing of soft, fleshy fruits in harvesting containers and loss of fruit juices (Reynard, 1960). It can also facilitate rapid moisture loss and excessive shrivelling, which lowers fruit quality and storage life (Meyer, 1944; Mezzetti, 1959; Goode *et al.*, 1975). Pre-harvest chemical sprays or insects may further damage the cracked or split fruit (Shear, 1971). Fruit with cracks are also more susceptible to chemical injury during postharvest washing to remove chemical residues (Fisher, 1937a,b) or during postharvest fumigation, as in SO_2-damage of grapes (Laszlo and Saayman, 1991). The problem of cracked or split fruit is often further exacerbated by invasion of decay-causing fungi through the damaged sections of the skin and by fruit abscission (Considine, 1981a). Cracking or splitting of fruit normally increases with maturity. Therefore, producers of susceptible cultivars tend to harvest their fruit too early to avoid or reduce the amount of cracked fruit (Opara *et al.*, 1997). This usually results in the marketing of fruit of inferior taste, non-uniform quality, and that is

under-coloured. All these secondary quality defects associated with cracked or split fruit, lead to further financial losses.

Fruit cracking in the apple cultivar 'Stayman Winesap' can exceed 90% during some seasons (Byers *et al.*, 1990), and therefore this cultivar is declining in importance in some fruit growing areas (Marini, 1991). In crack-susceptible sweet cherry cultivars and in regions where rain occurs near harvest, crop losses due to fruit splitting can exceed 75%, and may lead to the loss of the entire crop during some seasons (Davenport *et al.*, 1972; Harrington *et al.*, 1978).

Cracking and splitting of grape berries during cold storage is a major problem in South African table grape production. The severity of damage varies with the cultivar. The cultivars 'Thompson Seedless', 'Sunred Seedless', 'Red Globe', 'Queen of the Vineyard' and 'Flame Seedless' are especially susceptible. According to Capespan, a company marketing South African produced grapes on the export markets, the packed cartons affected by this disorder during the 1999 season were approximately 20.8% (T. Olivier, Pers. Comm.). The total export value of cartons affected and at risk from splitting was estimated to be over R11.4 million. Inferior grape quality due to berry cracking and splitting usually results in a considerable decline in consumer confidence and a corresponding drop in demand for and price of the product. This can lead to even further financial losses on highly competitive markets overseas.

1.1.2 Terminology

Swift *et al.* (1974) distinguished between cracking and splitting of grape berries. Cracking has been defined as fine superficial fractures in the fruit skin. Cracks normally involve rupture of only the cuticle and epidermal tissue. Splitting is an extreme form of cracking in which the cracks penetrate deep into the flesh. In splitting the cuticle, epidermis, sub-epidermis and many of the outer pericarp cells are ruptured (Swift *et al.*, 1974).

The rate at which excessive turgor pressure builds up, determines whether the grape berry skin will crack or split (Swift *et al.*, 1974). A slow increase in turgor pressure may result in cracking, while a rapid increase may cause splitting. In the berries anatomically examined, suberization was present in the cells beneath cracks, but was not evident in the cells around splits. No penetration of micro-organisms into the berry through the fine cracks was detected, possibly because of the suberization of exposed cell walls combined with the high phenolic content in subepidermal cells. Splitting, on the other hand, induced berry deterioration. As no suberization took place around the split to seal off the damaged cells from the rest of the pericarp, micro-organisms could penetrate and establish in the pericarp tissue. On some berries, splitting

occurred in the region of cracks, but it is still uncertain whether splitting is more likely to occur in the region of cracks than in regions where cracks are absent.

In apples, lenticel or cuticle cracking is characterized by the presence of minute superficial cracks on the fruit surface, followed by the gradual peeling off of the skin in patches, giving the affected areas a russeted appearance (Schrader and Haut, 1938; Meyer, 1944; Shutak and Schrader, 1948; Taylor and Knight, 1986). Skin cracking is prevalent in the calyx region of the fruit (Pilgaard, 1957). Some of these cracks heal by cork formation with a light deposit of suberin on the cell walls (Schrader and Haut, 1938). Unhealed cracks are responsible for excessive shrivelling of apples during storage (Meyer, 1944). Skin cracks normally develop perpendicular to the axis of the apple, but, if insect or a similar injury is present, the cracks generally develop concentrically around the injured spot (Schrader and Haut, 1938; Shutak and Schrader, 1948). The presence of skin cracks is often associated with the development of extensive russeting (Skene, 1965).

In the apple cultivar 'Golden Russet', fruit splitting consisted of deep (up to 40.3mm) and wide (up to 20mm) equatorial furrows containing easily detached cork tissue, and occurred mainly as stem-end splitting (Proctor and Lougheed, 1980). Verner (1935) divided splits into those originating in regions of the fruit skin with physical defects (such as russet and scar lesions), and those originating in or near the stem depression.

1.1.3 Measurements of cracking and splitting

Assessment of the susceptibility of fruit to cracking and splitting by means of a reliable and objective method, would be useful to determine the susceptibility of new cultivars during breeding, or for evaluating existing cultivars during growth and maturation and under different management conditions (Opara *et al.*, 1997). Byers et al. (1990) induced water absorption and cracking of detached 'Stayman Winesap' apples in the laboratory by submerging fruit in non-ionic and anionic surfactant-water solutions. Within 24 hours, both water uptake and fruit cracking increased linearly with increasing concentrations of X-77 surfactant solution. The submerging of apples in X-77 surfactant solution to predict the potential for the fruit to crack under field conditions, was suggested by the authors. However, Opara (1993) showed that some types of field cracking, such as stem-end splits or internal ring cracks were not formed following laboratory immersion tests.

For cherry fruit cracking, a laboratory procedure to determine cracking sensitivity was developed by Verner and Blodgett (1931). Fifty cherries, free of blemishes, were immersed in distilled

water under controlled temperature conditions for a period of 10 hours. After two hour intervals, all cracked cherries were counted and discarded. A cracking index, expressing the cracking intensity, was determined by multiplying the number of cracked cherries at each time of recording by a weighting factor, which was highest for the earliest recordings and least for the last recordings. The cracking index was calculated as the percentage of the maximum reading obtainable. This method assumed, without much supporting evidence, a close relationship between the cracking index and the susceptibility to cracking under natural conditions in the field (Christensen, 1996). Some results can be misleading, since the conditions of fruit wetting by immersion in distilled water in the laboratory differ from the natural orchard conditions.

Considine and Kreidemann (1972) developed an objective method for estimating the internal turgor pressure required for grape berry splitting (critical turgor pressure) in order to determine a cultivar's resistance to splitting. Excised berries were sorted according to density to provide fruit of uniform sugar content, and hence of osmotic potential. The osmotic potential of the juice of fruit from part of each sample was determined by refractometry, while fruit from the remainder of the sample was dewaxed in chloroform and divided into 10 samples that were placed into sucrose solutions which usually ranged from 0 to 3 MPa in osmotic potential. A record was kept of the number of berries which split at each level of osmotic potential, and the critical turgor pressure was determined as the arithmetic difference between the osmotic potential of the juice and the initial osmotic potential of the solution which caused 50% of the berries to split (referred to as the P_{50} of the fruit). The critical turgor pressure was approximately 15atm in grape cultivars prone to splitting and 40atm in resistant cultivars.

To observe the mechanical behaviour of the grape berry skin under conditions that simulate the natural process occurring in the vineyard, to obtain reliable information on the berry's resistance to splitting and to determine turgor pressure, Bernstein and Lustig (1985) developed a hydrostatic method and suitable instrumentation for these measurements on the whole intact berry. The technique involves the application of measurable pressure and the injection of known quantities of distilled water into the fruit through fine hypodermic needles inserted through the pedicel into the approximate centre of the berry. This "Injection tester" measures the normal turgor pressure of the berry and the burst strength of the berry skin. During this test, the pressure and the volume of injected water are measured. The method is based on the pressure balance principle. When external pressure, generated by the injection tester is raised slowly, there is no flow of water into the berry through the hypodermic needle. Only when the external pressure exceeds the internal turgor pressure of the berry, water will enter the berry. The initial turgor pressure of the berry is equal to the external pressure measured just prior to an

inflow of injected water into the berry. As the external pressure is further increased, burst pressure may ultimately be reached (Bernstein and Lustig, 1985). The injection tester was used to simulate splitting in the vineyard and tests carried out on various cultivars resulted in splitting patterns which duplicated those occurring naturally in the vineyard in form and in location (Lustig and Bernstein, 1985).

1.2 TYPES OF CRACKING AND SPLITTING

Grape berries exhibit three different, well-defined types of fractures, viz circular or semi-circular fractures at the pedicel end of the berry, similar fractures formed next to or around the suberized style remnant at the apical end of the berry, or length wise fractures along the sides of the berry (Meynhardt, 1964a). The semi-circular fractures that develop at the apex of the berry near the remnant of the style is usually characteristic for berries of 'Queen of the Vineyard'. Split in 'Alphonse Lavallée' usually starts near the pedicle and extends longitudinally to the apex of the berry. The cultivar 'Thompson Seedless' usually exhibits both longitudinal and circumferential ring fractures (Meynhardt, 1964a).

Studies on plums (Uriu *et al.*, 1962) showed that apical and lateral splitting are two independent phenomena, as irrigation affected the two processes differently. The ripeness level had a great influence on the development of lateral splits, while apical splits appeared immediately after irrigating in areas with severe drought, regardless of the degree of ripeness. When soil moisture conditions were favourable throughout the period of fruit growth, very few apical splits occurred. Similar results were recorded on sweet cherries (Christensen, 1972b) which showed that the occurrence of apical cracks did not correlate with the variety's susceptibility to more severe lateral cracking. Some varieties were susceptible to apical cracking and others to stem-end cracking.

Meynhardt (1964a) suggested a possible relationship between splitting and the vascular strands (axial and peripheral). The axial vascular strand forms the axis of the berry and stretches from the pedicel to the remnant of the style. The peripheral vascular strands branch off from the axial strand a small distance below the weak connection of the style remnant with the axial vascular strand. At the pedicel attachment, the axial and peripheral vascular strands are connected again. Thus, the vascular strands are more dense around the pedicel and style remnant ends. According to Meynhardt (1964a), there is probably a higher concentration of water in the tissue around these junctions of vascular strands. Therefore, splitting can possibly develop when an excess inflow of water occurs around the apical or distal part of the berry. The poor connection

of the axial vascular strand with the remnant of the style, may cause it to break off at this point. Furthermore, the remnant of the style, being lignified, is less viscoelastic than the surrounding tissue. Stresses developing in this region may result in splitting around or adjacent to the rigid style remnant, due to uneven stretching.

In sweet cherries, the same three types of cracking as in grapes are prevalent : circular or semicircular cracks around the stem end, similar fine cracks at the apical end, and the most injurious kind, in the form of long, irregular, and often deep splits on the sides of the cherry fruit (Christensen, 1972b). Histochemical studies by Glenn and Poovaiah (1989) showed that the area next to or around the stylar scar was devoid of a cuticle and that a greater penetration of solute, which contained $^{45}Ca^{2+}$, occurred at the stylar remnant. This implied that water penetration may also be greater in this region. Glenn and Poovaiah (1989) suggested that this may contribute to the susceptibility of the fruit apex to splitting. The importance of the style remnant as a site of water penetration may be even greater since water-drops normally form at the fruit apex during rainfall and persist for some time after the rain has stopped (Verner and Blodgett, 1931).

In the grape cultivar 'Alphonse Lavallée', which is prone to longitudinal splitting, the peripheral vascular strands are superficial (Meynhardt, 1964a). In 'Waltham Cross', which is relatively resistant to splitting, the peripheral strands are typically deep-seated. The longitudinal splits in 'Alphonse Lavallée' run parallel to the peripheral, vascular strands. Thus, Meynhardt (1964a) suggested that the location of the split may be due to the shallow peripheral strands which occur under a weak subepidermal cell-layer system. Verner (1935) indicated that for apples there is a correlation between the location of cracks and the incidence and size of peripheral vascular strands.

Sawada (1931) proclaimed that the type of cherry cracking is mainly determined by fruit shape. He reasoned that cracking occurs primarily at right angles to the curvature of the fruit, and noted that other fruits and vegetables, such as grapes, plums, cabbage and carrots, are affected in the same manner. Considine and Brown (1981) use the 'theory of shells' to explain the orientation of fractures. The fracture patterns are due to fruit shape and the presence of a relatively inextensible core. Fruit of the cultivar 'Thompson Seedless' are elongated, and in the case of a prolate spheroid the 'theory of shells' predicts that hoop stresses will be greater than longitudinal stresses. This results in the formation of longitudinal fractures. The occurrence of even a small indentation at the insertion of the pedicel, indicates the presence of a relatively inextensible core tissue and this is probably the reason for the circumferential ring fractures.

The surface of the grape skin contains an average of 16 lenticels (Swift *et al.*, 1973). The grape skin is reinforced in the region of lenticels, which probably makes the lenticel more rigid and less viscoelastic than the surrounding tissue (Considine, 1982). Therefore, the presence of lenticels may cause rupturing of the grape skin (Considine and Brown, 1981).

Verner (1935) and Teaotia and Singh (1970) noted that incipient cracks in apple fruit originated at hypertrophied lenticels, and that this may be caused or promoted by greatly retarded transpiration, accompanied by an excessive supply of water to the regions of hypertrophy. The proliferation that constitutes lenticel hypertrophy, possibly decreases the extensibility of the adjacent peripheral cell layers and lowers their mechanical resistance to being torn apart. It is therefore probable that the lenticels make the weakest point at which rupture would begin, whenever peripheral tissue strain becomes sufficiently excessive.

Various researchers (Jenkins and Storey, 1955; Schmid, 1960, 1961; Cropley, 1968) observed star-shaped cracks in the skin of apple fruit, sometimes on the side of the fruit, but more frequently near the calyx end. In severely affected fruit, the cracks which developed deeply, usually healed, resulting in severely scarred fruit (Jenkins and Storey, 1955). Star cracking is associated with infection by certain virus diseases (Opara et al., 1997).

1.3 FACTORS INFLUENCING CRACKING AND SPLITTING

1.3.1 General

The incidence of cracking and splitting are erratic, causing great losses in some years, seasons, climatic regions and orchards, and almost none in others (Tetley, 1930; Verner, 1935, 1938; Teaotia and Singh, 1970). Cracking and splitting in a wide range of fruits, such as apple, peach, grape and cherry, occurs as a result of excessive water absorption by the fruit, either directly through the fruit skin by osmosis (Bohlmann, 1962), or by way of the root system and vascular tissue (Meynhardt, 1956; Sekse, 1995a; Webster and Cline, 1994a). Peet (1992) concluded that tomato fruit cracking occurs when there is a rapid net influx of water and solutes into the fruit at the same time that ripening and/or other factors are reducing the strength and elasticity of the fruit skin. Considine and Kriedeman (1972) attributed splitting to the development, under conditions of high water availability and low evaporative demand, of a high hydrostatic pressure in the fruit (turgor pressure) in excess of the tensile strength of the cell walls. Mrozek and Burkhardt (1973) identified 23 factors believed to be associated with the cracking of apple, cherry, tomato, prune and avocado fruit. Water, high humidity, rain and fruit maturity were

common factors in the cracking and splitting of all the fruit kinds. According to Opara *et al.* (1997), the factors associated with fruit cracking and splitting can be divided into genetic or internal factors, which account for cultivar differences, and external or environmental factors, which influence the degree of splitting within susceptible cultivars. The cause of cracking and splitting is probably due to the interaction of several of these factors.

1.3.2 Biotic

Early researchers on apple fruit cracking suggested that the disorder is caused by the fungus *Coniothecium chomatosporum* (Evans, 1907; Kirk, 1907; Van der Bijl, 1914) or by virus infection (Jenkins and Storey, 1955; Fischer, 1955; Schmid, 1960, 1961). Results obtained by Schmid (1960, 1961, 1963) indicated that certain apple cultivars were resistant to virus-induced cracking while other cultivars were particularly susceptible. Research by Goodwin (1929) showed that the coniothecium disease was an after effect, and that fruit splitting could be due to general debility of the tree. Goodwin (1929) found apple and pear fruit cracking to be associated with severe scab and blotch. Fawcett and Lee (1926) reported that splitting in citrus fruit is most often associated with diseased tissues, such as lesions. These diseased tissues absorb water exceptionally when the water supply is abundant, and cause rupture due to abnormal swelling. In cherries, Powers and Bollen (1947) could find no correlation between cracking and different kinds of micro-organisms. According to Opara *et al.* (1997), no recent research associated cracking and splitting in fruits with viral or fungal diseases.

1.3.3 Skin abnormalities

Structural deformities on the fruit skin may arise from physiological disorders, diseases, insects, or mechanical injury (Fisher, 1937a,b; Schrader and Haut, 1938; Shutak and Schrader, 1948; Walter, 1967; Teaotia and Singh, 1970). Many authors (Verner, 1935; Gardner and Christ, 1953; Stiles *et al.*, 1959; Walter, 1967; Skene, 1982; Proctor and Lougheed, 1980) believed that the occurrence of skin abnormalities, such as sunburn and russet, contribute to a higher incidence of fruit splitting. Gardner and Christ (1953) observed an eightfold increase in the number of incipient cracks and splits that developed on severely russeted samples, compared to smooth-skinned samples. The russeted areas showed increased skin permeability to water vapour. Verner (1935) noted that apples with some abnormality such as sunburn or russet, were more susceptible to cracking than sound fruit. During one season, 88% of the fruit cracks were directly associated with russeted skin, sunburn or scab spots. The remaining 12% were most often on the sound cheek of fruit surfaces that were most exposed to sunlight. Cracking appeared to be independent of the skin abnormalities themselves, but the abnormalities rendered weak portions of the fruit that are more susceptible to cracking than the normal

portions when environmental influences are favourable for cracking.

Meynhardt (1964b) reported that splitting of grape berry tissue adjacent to or through cork tissue is common to most cultivars. The incidence of cork tissue may be due to spray damage, sun scorch, abrasions, wind injury or other factors. The cork tissue is relatively rigid. The absorption of excess water under particular circumstances normally causes the berry skin to stretch in order to accommodate enlargement of the pericarp tissues. Due to the relatively rigid cork tissue, an uneven stretching of the berry skin may result in rupturing of the berry skin next to or through the cork tissue. Considine and Brown (1981) confirmed that the presence of rigid bodies such as scleroid masses or heavily cutinised areas of damaged tissue may cause rupturing of the grape skin.

1.3.4 Genetic factors

It is generally accepted that berries of the grape cultivars 'Waltham Cross', 'Almeria' and 'Barlinka' seldom if ever split. It is also true that berries of 'Alphonse Lavallée', 'Sultana' and 'Queen of the Vineyard' are very susceptible to splitting. The distinct differences in susceptibility which occur between grape cultivars, indicates a significant genetic contribution (Lang and Düring, 1990). Zielinski (1964) investigated the genetic resistance to fruit splitting in 38 sweet cherry cultivars by immersing fruit in distilled water at 20 ± 2°C. He found that some cultivars were five to seven fold more resistant to fruit splitting than others.

Highly significant varietal differences were found in the incidence of radial, concentric and total tomato fruit cracking (Gill and Nandpuri, 1970). Reynard (1951) and Young (1959) identified genes in tomato plants that determine radial cracking, as well as genes that control radial crack resistance in tomato fruit. Crack-resistant genes were associated with pink fruit colour, high number of fruits per plant, low average number of locules per fruit, small fruit diameter and determinate plant growth habit (Young, 1959). Prashar and Lambeth (1960) noted that resistance is not controlled by the same gene in all the cultivars. Reynard (1960) found that radial and concentric cracks in tomato are governed by separate gene systems. Through cross-breeding, crack-resistant tomato cultivars have been produced (Frazier, 1947; Reynard, 1960). Crack-resistant cultivars of many other crops, including cherry (Christensen, 1983), apricot (Layne, 1991a) and nectarine (Layne, 1991b), have been developed by breeding.

1.3.5 Environmental factors

1.3.5.1 Rainfall, irrigation and soil moisture

Meynhardt (1957) showed that spray irrigation could increase the incidence of berry splitting in certain grape cultivars when the relative humidity is high. Berry splitting was caused, amongst others, by absorption of water directly through the skin of the fruit. Bohlman (1962) reported that peach, apple and cherry fruit tend to crack more easily when they come into contact with rain or mist or when immersed in water. Christensen (1972a) and Sawada (1931) attributed cherry cracking solely to water absorption through the fruit skin. However, several authors (Trought and Lang, 1991; Webster and Cline, 1994a; Meynhardt, 1956) found fruit crack in protected environments where rain was not allowed to fall on the fruit surface. These authors suggested that water uptake through the root systems may be of greater importance in causing fruit splitting than had been realised before.

Many authors (Frazier, 1947; Bohlman, 1962; Kelperis, 1962; Walter, 1967; Peet, 1992; Sekse, 1995a) suggested that the major factor causing preharvest splitting of various fruits was a sudden marked increase in soil moisture content, due to rain or late irrigation, shortly before maturity, especially if growth had been preceded by a long period of drought. Graebner (1920) found that periods of drought resulted in the development of strengthening tissues, which normally appeared first in the xylem and phloem. These strengthened tissues probably lost their ability to divide and their capacity to expand. If water supply was greatly increased after a dry period, the meristematic tissues quickly resumed growth, but not the strengthened tissues. This difference in growth rate between the rigid strengthened and flexible meristematic tissues could result in excessive tensions and failure of the rigid tissue. Reed and Crabill (1915) found that skin cracking of apple occurred very rarely in dry seasons but normally after late rains that followed drought. Fluctuations in soil moisture from low to high induced more cracks on tomato fruit than a change from a medium to high soil moisture (Niiuchi *et al.*, 1960). Proctor and Lougheed (1980) attributed cracking of 'Golden Russet' apple to fluctuating water supply in the early growing season. Irving and Drost (1987) reported that water deficit imposed during phase one of fruit growth, increased the proportion of cracked 'Cox's Orange Pippin' apples by two to three fold.

Sekse (1995a) reported significant differences in the occurrence of cuticular fractures on the fruit surface of sweet cherries, due to different irrigation regimes. Fruits from trees that were sheltered from rain and drip irrigation over a six week period and then supplied with abundant water just prior to harvest, showed significantly more fractures compared to fruits on trees

receiving drip irrigation regularly. Since fruit with cuticular fractures absorbed surface water more easily (Glenn and Poovaiah, 1989), these fruit were more vulnerable to fruit cracking. It was suggested that irregular water supply to the fruits causes the parenchymatous cells, and thereby the fruit itself, to grow irregularly. The wax-like cuticle is not correspondingly flexible and cuticular fractures develop. Maintaining a uniform turgor pressure build-up in the fruits by limited and regular water supply to the trees prior to harvest, would allow both normal fruit development and reduce cuticular fractioning on the fruit surface and thus fruit cracking (Sekse, 1995a).

According to Coit (1917), irregular water supply causing wide variations in the moisture content of the soil, produced a greater fluctuation in the growth of the interior than in the skin of the orange, resulting in split. He maintained, however, that this was only a contributing factor, because only a proportion of the fruit of any given tree would split.

For tomatoes, Peet (1992) found that high soil moisture tensions suddenly lowered by irrigation or rain, were the most frequent cause of fruit cracking. Low soil moisture tensions reduced the tensile strength of the skin. In addition, during rain or overhead irrigation, water penetrated into the fruit through minute cracks or through the suberised tissue around the stem scar. Maintenance of a limited and constant soil moisture content results in uniform and relatively slow fruit growth that would offer some protection against fruit cracking (Peet, 1992).

By contrast, Verner (1935) observed no increase in splitting when he caused sudden and definite fluctuations in soil moisture by inducing drought in 'Stayman Winesap' apple followed by flood irrigation. Watanabe *et al.* (1987) also found that moisture content had no pronounced effect on the incidence of splitting in 'Mutsu' apples. Kertesz and Nebel (1935) and Verner (1939) confirmed that the primary cause of cherry cracking was the absorption of water directly through the skin of the fruit and not through the root system. Verner and Blodgett (1931) attempted to induce cracking in three cherry cultivars by varying the frequency and duration of irrigation, but without success. Cracking occurred only with the presence of rain or if the fruits were immersed in water. The percentage of fruit cracked under natural conditions failed to show any correlation with soil moisture conditions at the time of cracking. Sawada (1931) concluded that extremes of soil moisture played no direct role on cracking of sweet cherries.

1.3.5.2 Relative humidity

An increase in water supply to the fruit tissues as a result of low rates of transpiration under conditions of high relative humidity, caused fruit cracking in several fruit (Verner, 1935 & 1938;

Meynhardt, 1956; Moreira, 1968; Teaotia and Singh, 1970). According to Meynhardt (1956), structural failure of the epidermis in table grapes occurred only at high atmospheric humidity.

Meynhardt (1964b) inspected full bearing 'Queen of the Vineyard' vines every morning during the maturation period up to harvest. No rain occurred during this period and the vines were not irrigated, as soil moisture content was well within the plant's available range. Splitting of the grape berries was associated with RH values above 50% during the day, followed by RH values above 95% during the night, either for a 24 or 48 hour period. Rixford (1918) reported the splitting of figs under conditions of high atmospheric humidity without rain or irrigation. Verner (1935) observed no apple cracking when rain was accompanied by humidity well below 90%, while lighter rain accompanied by relative humidity between 99 and 100% caused severe cracking. Tukey (1959) found that extended periods of high humidity, especially while the apples are small, may inhibit the potential formation or modify the composition of the cuticle sufficiently to cause it to lose its protective capacity.

Verner (1935) observed a definite relation between low rates of evaporation and the occurrence of apple splitting. Prolonged periods of low evaporation rates, even when there had been no rain for up to 6 days, resulted in severe splitting.

Bagging tomato fruits with polyethylene bags or covering the plants with a plastic tent enhanced cracking, even in fruits of a relatively crack-resistant cultivar (Singh and Young, 1970). This suggested that the temperature and humidity probably increased in the enclosed environment, which resulted in a reduction in transpiration and an increase in cell turgidity and so contributed to tomato fruit cracking. Cherry tomato plants grown under high humidity showed an increase in fruit cracking, compared to cherry tomatoes grown under low humidity (Ohta *et al.*, 1991). The highest penetration resistance of both the skin and flesh, and tensile strength of the skin, were found in fruits grown under low humidity.

1.3.5.3 Light exposure

In apple, prune, tomato and cherry fruit, the degree of sun exposure has been associated with cracking (Fisher, 1937a,b; Verner, 1936; Mrozek and Burkhardt, 1973; Peet, 1992). It appears as if apple cultivars differ regarding the influence of fruit exposure to sunlight on the incidence of fruit cracking. In 'York' apples, Reed and Crabill (1915), Fisher (1937a, b), Schrader and Haut (1938) and Shutak and Schrader (1948) found that skin cracking was limited almost entirely to the green (shaded) side of the fruit. Shutak and Schrader (1948) obtained a significant correlation between the amount of cracked 'York Imperial' apple fruits on a given tree and the

thickness of the cuticle. The sun-exposed red side of the fruit, which was less susceptible to skin cracking, possessed a thin cuticle and showed little distortion of the epidermal and subepidermal cell layers. On the shaded side of the fruit, which was more susceptible to skin cracking, the cuticle was thicker.

Tetley (1930) noted that in the apple cultivars 'James Grieve' and 'Beauty of Bath', most of the cracks developed on the sunny side of the apple. Tetley suggested that a cold period during fruit set produced a comparatively thick, non-elastic cuticle, especially on the exposed side of the apple, with the result that the epidermis was unable to resist the rapid swelling of the cells within, and consequently cracked. Verner (1938) found sound, densely shaded apples growing in the innermost parts of the tree with virtually no cracking. Apples in different parts of the tree and covered with brown paper bags for 3 to 4 weeks before harvest, showed 5% cracking compared to 41% in the control. Rootsi (1962) showed that apple cracking may increase with direct exposure of the fruit to sunlight. The sugar content was higher on the side of the fruit exposed to sunlight than on the shaded side. Rootsi (1962) attributed the lower incidence of cracking on the shaded side of fruit to a greater elasticity of the shaded tissues. In a study on the effect of insulation levels on stem-end fruit splitting of 'Gala' apples, Opara (1993) observed significantly higher levels of stem-end splitting in well-exposed fruit, compared to naturally shaded fruit. However, fruit quality of the well-exposed fruit were superior. Despite cultivar differences regarding the effect of sun-exposure on fruit cracking, cracking defects develop on the fruit side with a thicker, non-elastic cuticle (shaded or exposed) (Opara *et al.*, 1997).

In prunes, Mrozek and Burkhardt (1973) observed that the exposed side of the fruit experienced the highest temperature and the highest occurrence of side cracking. Peet (1992) suggested that high light intensity could play a role in increased cracking of tomato fruit apart from its association with high temperatures. Under high light conditions, fruit soluble solids and growth rates were higher, and these factors were sometimes associated with cracking.

1.3.5.4 Temperature

According to Bullock (1952), temperature is one of the most important factors contributing to cherry cracking and accounts for the great differences in susceptibility of the fruit to cracking in the field. In a laboratory trial where fruit were kept in chambers held at different temperatures, there was a linear increase in fruit cracking as the temperature increased from 1 to 40°C (Bullock, 1952). A Q_{10} of 1.55 was found for cherry cracking over the temperature range of 15 to 25°C, which is evidence of its importance. Due to the influence of temperature, the risk of cracking under orchard conditions is much greater during and just after rain on hot days than

cold days (Christensen, 1996).

At increasing temperatures (10, 30, 50°C), Lang and Düring (1990) observed a strong rise in internal pressure of grape berries and a significant decrease in skin stiffness and strength, which could result in grape splitting under certain circumstances. Similarly, with tomatoes, an increase in fruit temperature increased hydrostatic pressures of the pulp on the skin, resulting in immediate cracking in ripe fruits or delayed cracking in green fruits (Peet, 1992).

1.3.6 Cultural factors

1.3.6.1 Tree vigour, crop load and rootstock

Many factors that influence growth, also influence fruit cracking and splitting. Goodwin (1929) noted that the lower buds in debilitated apple trees had become so weakened that it was impossible for them to maintain sufficient vigour to produce fruit without cracks. Practically, all sound fruit were located near the top where the growth was stronger. Louw (1948) confirmed that vigorous growth tends to reduce cracking of apples. When neglected trees of 'Ohenimuri' apple were severely pruned, after not being pruned for several years, fruit cracking was almost entirely eliminated, due to vigorous growth that developed. Fisher (1937a,b), Schrader and Haut (1938) and Shutak and Schrader (1948) found that fruit on trees low in vigour and bearing a light crop, were susceptible to cracking. In cherries (Bullock, 1952), and stone fruit (Beattie *et al.*, 1989), heavily cropped trees tended to crack less than trees carrying a light crop.

Proctor and Lougheed (1980) reported more severe apple cracking in trees on dwarf rootstocks, which were also younger and bore a smaller crop load than the controls. However, Nilsson and Fernqvist (1956) found that vigorous rootstocks, such as M16, and seedlings were more susceptible to the development of fruit cracking in 'Ingrid Marie' apples. Studies by Watanabe *et al.* (1987) and Cobianchi *et al.* (1984) also noted rootstock effects on apple fruit cracking, but Comai and Widman (1981) could not find significant rootstock effects on fruit cracking in 13 'Stayman' clones. In sweet cherry cultivars, Cline *et al.* (1995a) observed differences in fruit cracking due to type of rootstock. These differences were partially ascribed to differences in water uptake by the roots (Cline *et al.*, 1995a). However, Granger and Frensham (1991) found no significant differences in rain-split fruit between three cultivars over five years.

1.3.6.2 Mineral nutrition

Differences in cracking susceptibility of fruit on different trees, or even on the same tree, can be attributed to the nutritional condition of the tree and fruit (Schrader and Haut, 1938). Deficiencies

in Ca and B have been suggested to account for the development of fruit cracks. Dube *et al.* (1969) attributed fruit cracking in 'Rymer' apples to B deficiency. Shear (1971) reported that high levels of N aggravated fruit splitting of 'York Imperial' apples. In 'Jonathan' apples, Tomana (1961) found that when seed development ceased and the fruit begin to enlarge, the N content of the flesh increased rapidly, resulting in cracking of the skin around the lenticels. Weissenborn and Gottwald (1965) reported a positive relationship between N manuring and the cracking of 'Holstein Cox' apples. 'Cox's Orange Pippin' apples showed worse cracking on clean cultivated plots, especially where N and K were applied, compared with trees that were in grass or which received K only (Montgomery, 1959). However, Stiles *et al.* (1959) found no influence of urea sprays on cracking of 'Stayman' apples. Fischer (1955) also found no evidence for apple fruit cracking due to nutrient deficiency.

1.3.6.3 Pesticides

Some authors (Schrader and Haut, 1938 and Asquith, 1957) observed that fruit cracking was aggravated by pesticide sprays, while others (Reed and Crabill, 1915; Fischer, 1955; Byers *et al.*, 1990) could find no difference between sprayed and unsprayed fruit regarding the incidence of this disorder.

Applications of Bordeaux mixture caused cracking of apple fruits (Moore, 1931). Cracking was also aggravated by late arsenate sprays (Schrader and Haut, 1938). In trials to control fruit pests in apple orchards, phosdrin caused severe cracking (Asquith, 1957), while high volume sprays of 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol at petal fall caused cracking and russeting of apple fruits (Anon, 1962).

Many researchers have found that surfactants, often applied with fungicides, herbicides or insecticides and known to increase water penetration through fruit cuticles, enhance apple fruit cracking (Noga and Bukovac, 1986; Byers *et al.*, 1990). Submerging apples in pesticide combinations or nutrient solutions generally did not affect fruit splitting, while several non-ionic and anionic surfactant-water solutions and a nutrient-surfactant combination increased fruit cracking (Byers *et al.*, 1990).

1.3.7 Fruit factors

Physiological and anatomical conditions of fruit have major effects on fruit cracking and splitting in apple (Tetley, 1930; Verner, 1935, 1938; Goldschmidt, 1962), cherry (Verner and Blodgett, 1931; Christensen, 1972c), grape (Meynhardt, 1964b; Considine, 1979), and tomato (Cortner *et al.*, 1969; Hankinson and Rao, 1979).

1.3.7.1 Fruit size and shape

In grapes, theoretical analyses of fruit shape and size suggested that these two parameters can influence the level and orientation of stresses in the dermal system that affect the incidence of rain-induced splitting (Considine, 1979; Considine and Brown, 1981). Considine (1981a) studied nine grape cultivars that represent a wide range of fruit size and shape found in *Vitis vinifera*. Fruit shape varied from round to elongate. No significant correlation could be detected between splitting susceptibility and fruit radius or shape.

In cherries, also no relationship could be found between cracking susceptibility and varietal determined fruit size (Christensen, 1975; Zielinski, 1964; Roser, 1996). However, a much stronger relationship existed between cracking susceptibility and fruit size within a variety than between varieties. Christensen (1975) found an increase in cracking from small to large fruits within the same variety. In 'Mutsu' apple fruit, Watanabe *et al.* (1987) also found that large fruit were more susceptible to splitting. Bullock (1952) found that fruit on cherry trees carrying a heavy crop tend to crack less than fruit of the same cultivar on trees with a light crop. The correlation between cracking susceptibility and cherry fruit size may account for these observations, as fruits from heavily cropping trees are normally smaller. Also in apples, factors that increase fruit size, tend to accentuate cracking (Nilsson and Bjurman, 1958).

In tomatoes, large fruit size was an anatomical characteristic of cracking susceptible cultivars (Peet, 1992). Also, tomato cultivars with few fruits per plant, were more susceptible to cracking.

1.3.7.2 Fruit firmness

Since cracking and splitting of fruit is caused by excessive absorption of water resulting in bursting of the skin, it may seem logical that firm-fleshed cultivars will have a greater tendency to crack and split than soft-fleshed ones. Uys (1996) developed a method to determine the contribution of turgor, berry flesh and skin to total firmness of a berry. The cultivars 'Dauphine' and 'Barlinka', which are relatively resistant to berry splitting, did not differ from 'Sultanina', which is susceptible to berry splitting, in any of the three firmness measurements. For cherries, neither Christensen (1975) nor Roser (1996) could find a significant correlation between fruit firmness and a tendency to crack. For tomato fruit, Zhenhua (1995) found that an increase in pectin content of the fruit was accompanied by an increase in fruit firmness and a decrease in the natural fruit cracking rate.

1.3.7.3 Soluble solids, osmotic potential and maturity

As water absorption into plant cells is predominantly an osmotic process and fruit cracking

seems to be related to water absorption (Verner and Blodgett, 1931), many investigators have attempted to associate differences in cracking or splitting with variations in the osmotic concentration of the fruit juice.

Several studies of cracking susceptibility in sweet cherries concluded that the osmotic concentration of the fruit soluble solids is a major factor controlling water absorption and cracking (Verner and Blodgett, 1931; Sawada, 1931; Bullock, 1952). Fruit of the cherry cultivar 'Bing' with a 14-16% sugar content showed 5% cracking after 2 hours immersion in water, while 21% of the fruit with a sugar content of 20-22% cracked during the same period (Verner and Blodgett, 1931). The rather simplistic conclusion was that cracking is directly affected by the osmotic concentration of the fruit juice. Sawada (1931) stated that cracking of the sweet cherries is due to an osmotic absorption of water that correlates with the degree of maturity. Fisher (1937a,b) also observed an increase in apple cracking as the fruit approached maturity. Anatomical characteristics of cracking-susceptible tomato cultivars were among others, low skin tensile strength and/or low extensibility associated with a certain stage of ripeness (Peet, 1992). According to Christensen (1972c), it is difficult to establish whether the increasing sugar content, or other fruit characteristics associated with advancing maturity are responsible for increased cracking susceptibility.

Meynhardt (1956) showed that grape splitting was related to the osmotic potential of the vacuolar solution within the berry. Split berries of 'Queen of the Vineyard' were halved, separating the basal from the apical part where splitting occurred (Meynhardt, 1964b). Higher osmotic and total soluble solid values were obtained for the apical parts of berries as opposed to basal parts. Meynhardt (1964b) also collected composite samples of split and sound berries from the same cluster and found that both the soluble solids and osmotic values of split berries were higher than those of sound berries. Comparable results were obtained by Verner (1935) for cracked 'Stayman Winesap' apples as compared to sound apples.

Verner (1936) observed that cherries frequently crack first at the apical end of the fruit. By dividing the fruit into sections from the apical to the stem end, Verner (1936) found, in accordance with Meynhardt's (1964b) results on grapes, an increasing sugar concentration gradient from the stem end to the apex. He argued that the higher osmotic concentration of juice at the apex accounted for a more rapid water absorption through the skin, resulting in an earlier formation of cracks in this region of the fruit. According to Christensen (1996), cracking in the apex can just as easily be explained by cuticular deformities in the apical region or the tendency for water drops to persist longer in this area before drying.

Although most of the water uptake through the fruit cuticle is predominantly osmotic, Tucker (1934), Kertesz and Nebel (1935), Zielinski (1964), Christensen (1972c) and Andersen and Richardson (1982) found no or very weak correlations between the sugar content or osmotic potential of sweet cherries and the tendency to crack. According to Christensen (1972c), the osmotic effect of the sugar content is considered to have a very slight influence on the varietal cracking susceptibility of cherries. Christensen (1972c) suggested that a high sugar content and an increasing cracking susceptibility are two independent characteristics, which both increase with advancing maturity. Although no significant correlation was found between osmotic concentration and the rate of water uptake (Christensen, 1972a), osmotic concentration may be more closely related to water uptake than to cracking, since cracking is a secondary phenomenon arising from water uptake (Wade, 1988). Tucker (1934) concluded that although sugar content can be of some importance, other varietal factors may influence cracking to a greater extent. Andersen and Richardson (1982) suggested that cuticular permeability, cuticular strength, cell wall strength or other structural factors may play a more important role in splitting.

1.3.7.4 Water-related properties of the fruit

Turgor develops when protoplasts that absorb water osmotically cease expanding in volume due to the relatively inflexible cell wall matrix (Wade, 1988). According to Considine and Kriedemann (1972), Wade (1988) and Sekse (1995b), turgor plays an important role in fruit cracking. Although Andersen and Richardson (1982) could find no simple relationship between turgor potential and cracking of cherries, since only 9% of variation in cracking was caused by turgor potential, turgor potential is responsible for cell expansion which leads to cracking (Wade, 1988).

A grape berry, placed in water or exposed to a humid atmosphere, behaves as a near-ideal osmometer, and the turgor pressure that develops will, if sufficiently high, rupture the epidermis (Considine and Kriedemann, 1972). The authors demonstrated that the epidermal tissue is less viscoelastic than the underlying pericarp tissue. Two uniform groups of Sultana berries, the one group intact and the other peeled, were immersed in distilled water. Within 30 minutes the intact fruit had ruptured. The peeled fruit on the other hand absorbed twice as much water as the intact fruit without any sign of splitting. Therefore, the epidermal tissue limits berry expansion and if the turgor pressure that develops in a grape berry is sufficiently high, rupture of the epidermis will occur. Predisposing factors that produce high turgidity in grape berries that will result in berry splitting, include abundant moisture supply and low evaporative demand on the vine as a whole. Cool and humid days with little wind, following an irrigation, towards the end of the growing season, are ideal conditions for the grape berries to attain maximum turgor. The

grapevine xylem has an exceptionally high conductivity, while the cuticular conductivity to water vapour loss from fruit is undoubtedly low. Under conditions of low evaporative demand, less moisture is diverted to foliar and fruit transpiration, so a build-up in turgor pressure in the berries develops that results in berry splitting. The vine's fruiting habit, foliage density, canopy architecture and even its row direction all influence evaporation/transpiration and thus the opportunity for build-up of turgor pressure.

Yamamoto (1973) found that sweet cherry fruits were liable to crack under the following circumstances : abundant moisture in the atmosphere, rapid fruit expansion, larger water potential gradients between the fruits and other parts of the tree, and increases in fruit water potential due to water absorption. Actual cracking was brought about by rapid water movement from other parts of the tree to the fruit due to reduced leaf transpiration rate caused by low wind velocity and low vapour pressure deficit, and also by water absorption through the fruit skin during rain.

Kertesz and Nebel (1935), after investigating the fruit pulp of a number of cherry varieties, concluded that the water-retaining capacity of the colloidal pulp is important in determining cracking susceptibility. The greater liquid retention by pulp of the cultivars that cracked severely, was due to the imbibitional properties of the greater amount of colloidal substance in these fruit. However, Christensen (1972c) could find no relationship between the water-holding capacity of the fruit pulp of 33 cherry varieties and their differences in cracking susceptibility, although he recorded vast differences between varieties.

The pericarp of sweet cherry fruit consists of parenchymatous tissue, of which vacuolar contents make up the major part of the fruit flesh (Sekse, 1995b). In mature fruits the vacuolar sap is high in soluble solids (Coombe, 1976) and its osmotic potential is therefore noticeably different from that of water. From water supplied through the vascular system, the parenchymatous cells build up turgor pressure that acts on the fruit skin from inside. The turgor pressure build-up is most probably due to swelling of vacuolar colloids as suggested by Kertesz and Nebel (1935), or by loading of water and solutes into cells (Sekse, 1995b). This build-up in turgor pressure is possibly the main force resulting in fruit cracking. Additional influx of surface water through the fruit cuticle, either osmotically driven through cuticular pores or directly through cuticular fractures, causes degradation of the dermal cell walls (Glenn and Poovaiah, 1989), and loss of the enclosing structure of the dermal layers. The turgor pressure acting from inside the fruit by means of water uptake through the vascular system of the fruit pedicel, then causes fruit cracking originating from the areas with destroyed epidermal cells (Sekse, 1995b).

The susceptibility of a sweet cherry cultivar to fruit cracking seems to be related to the rate and/or quantity of water absorption as well as the fruit's capacity to expand (Verner, 1937). Verner (1937) divided varieties into groups according to their susceptibility to fruit cracking. Severe-cracking varieties are characterized by rapid water absorption and low capacity for expansion. Moderate-cracking varieties either have a rapid absorption ability and a high capacity for expansion, or a slow absorption capability and a low capacity for expansion. Slight-cracking varieties are characterized by slow water absorption and high capacity for expansion. Although Christensen (1972a) and Wade (1988) showed that the rate of water absorption varied between cultivars, it only attributed partly to the fruit's susceptibility to cracking. In a study over three years with 26 cherry cultivars that differed regarding cracking susceptibility, Christensen (1972a) found that only 36% of the fruit cracking could be explained by rate of water uptake. The quantity of water absorption before cherry cracking occurred, or the threshold for water uptake, was also determined for the various cultivars. A multiple correlation between rate of water uptake, cracking index and cracking threshold for water uptake, resulted in a correlation coefficient of 0.684. Out of 11 major deviations from the linear correlation between rate of water uptake and cracking index, eight of them could be ascribed to their cracking threshold.

According to Cline *et al.* (1995a), cracking appeared to be related more to the rate of water absorption than to the quantity of water that accumulates. Cultivar differences were closely associated with indirect effects that skin permeability had on the rates of water absorption. For three subsequent years, Belmans and Keulemans (1996) examined cuticle thickness, the number of stomata and the nitrogen concentration in the fruit skin, as well as the rate and quantity of water absorption before cracking occurred in seven cherry cultivars that differed in cracking susceptibility. The cultivars more susceptible to cracking were characterised by a higher rate of water absorption. The rate of water absorption was influenced by the thickness of the cuticle and the number of stomata. The capacity of the fruit to absorb water was influenced by the nitrogen concentration of the skin and by cuticle thickness. However, these relationships were expressed only in some years. According to Belmans and Keulemans (1996), the possibility exists that cherry cultivars with thick cuticles or fewer stomata are less susceptible to cracking.

1.3.7.5 Morphological properties of the fruit skin

If one assumes that fruit cracking and splitting is at least partly associated with the amount or rate of water uptake through the fruit skin and the ability of the skin to adjust to this, then morphological characteristics of the fruit and especially the skin itself must play a major role in

these processes and the cracking/splitting response (Christensen, 1996). Skin anatomy, strength, elasticity/plasticity, cuticle integrity and stomatal function and density may all contribute to the quantity and rate of water uptake and cracking or splitting.

1.3.7.5.1 Cuticle

The cuticle, as the outermost structural element of the fruit skin, is the element to which the greatest amount of stress is applied. Commonly it's the site of initial failure which leads to skin cracking (Considine and Brown, 1981). The cuticle is a polymer membrane of heterogeneous composition. It consists mainly of cutin layers, which often contain varying proportions of cellulose, polyuronic acids, proteins, phenolic compounds and lipids (usually referred to as waxes) (Martin and Juniper, 1970; Schönherr, 1976). The waxes are either embedded in the cutin matrix or deposited superficially. The outermost cutin layer is highly hydrophobic, while the cutin layers beneath are hydrophilic. Schönherr (1976) demonstrated that the waxes are responsible for reducing the water permeability of cuticles. Extraction of cuticular waxes with lipid solvents from isolated membranes, increased their water permeability by a factor of 300 to 500.

Although the cuticle acts as an efficient water barrier (Sekse, 1995a), it is not impermeable to water. Direct absorption of water through the cherry cuticle is known to be an important mode of water penetration (Kertesz and Nebel, 1935; Verner, 1938; Westwood and Bjornstad, 1970). According to Anderson and Richardson (1982) and Glenn and Poovaiah (1985, 1989), cuticular penetration which occurs by diffusion or mass flow through cuticular cracks and other surface structures, may be important in determining susceptibility to fruit cracking.

According to Schönherr (1976) the cutin matrix contains polar pores which allows some diffusion of water, caused by a chemical potential gradient, through the cuticle. Cuticular water permeability depends on pore size, pore frequency and pore wall charge.

Cuticular penetration is also facilitated by stomata (Martin and Juniper, 1970), and Sawada (1931) showed that at least part of the water absorption into cherry fruit was through the stomata. Christensen (1972c) showed considerable differences between the varieties in number and size of fruit stomata. No correlation could be found between the density of stomata and the rate of water absorption or cracking susceptibility. However, the highly significant correlation that was evident between the size of the stomata and the rate of water absorption, seems to indicate that stomata may have some effect on cracking susceptibility. Glenn and Poovaiah (1989) studied the cherry cultivar "Bing" and found no water-damaged tissue at the

stomata, even if water damage occurred in areas adjacent to the stomatal region. On this evidence they contended that stomata were relatively unimportant in the development of fruit cracking, when compared to the stylar remnant and preharvest cuticular fractures.

An important property of the fruit cuticle influencing fruit cracking and splitting, is its rigidity due to its wax-like structure. Water injury in 'Bing' sweet cherry fruit was first detected as an increase in cell turgor followed by fracture formation in the cuticle that affected cuticular permeability and generally preceded fruit cracking (Glenn and Poovaiah, 1989). These fractures were minute and could only be detected with a microscope. Glenn and Poovaiah (1989) observed preharvest cuticular fractures on cherry fruit, which markedly increased the water absorption rate. The inconsistent occurrence of these fractures from year to year and from one area to another suggested that cultural or environmental factors are involved. Increased cuticular fracturing in sweet cherry fruit was demonstrated with irregular water supply to the tree (Sekse, 1995a). Similarly, in apple fruit, the development of preharvest cuticular fractures were reported, and are believed to be induced by environmental conditions that promote division and expansion of cells in the epidermal region (Faust and Shear, 1972). Considine (1982) described the occurrence of fine cuticular fractures in 'Sultana' grapes that develop due to water absorption, and can become very extensive prior to fruit cracking. Attempts to control the development of cuticular fractures through cultural practices may prove beneficial in reducing cracking susceptibility.

In certain fruits, other structural properties of the cuticle may have a definite influence on cracking and splitting. Tetley (1930) discovered that apple cultivars having cutin deposited only on the tangential walls of the epidermal cells, are less susceptible to cracking than cultivars having cutin deposits extending throughout the length of the radial walls or even completely surrounding the cells. Shutak and Schrader (1948) obtained a significant correlation between the amount of cracked 'York Imperial' apple fruits on a given tree and the thickness of the cuticle. The sun-exposed red side of the fruit, which was less susceptible to skin cracking, possessed a thin cuticle and showed little distortion of the epidermal and subepidermal cell layers. On the shaded side of the fruit, that was more susceptible to skin cracking, the cuticle was thicker. In a study of 13 sweet cherry varieties, Belmans *et al.* (1989) found a strong correlation between the thickness of the cuticle and cracking resistance. However, Bangerth (1968) could find no consistent correlation between the amount or composition of the cuticle and fruit cracking susceptibility. In a study of nine grape cultivars, cuticle thickness was not significantly correlated with resistance to splitting, and Considine (1981b) concluded that cuticle thickness is only one of several factors determining cracking or splitting susceptibility, and that it

is of secondary importance. Considine (1982) suggested that the strength of the cuticle plays an important role in the susceptibility of fruit to fracturing, cracking and/or splitting.

1.3.7.5.2 Dermal tissues

Various attempts have been made to correlate morphological parameters of the dermal tissues, with cracking or splitting susceptibility. For cherries, Kertesz and Nebel (1935) could find no relationship between the size of the epidermal cells and cracking susceptibility. However, a positive relationship could be detected between the thickness of the inner wall of the epidermal cells and cracking susceptibility.

Hankinson *et al.* (1977) studied histological properties of the grape skin such as the geometrical arrangement of cells and cell area. This information they compared with objective measurement of the texture of the grape skin, such as its tensile strengths and its resistance to puncture. The skins of two Muscadine grape varieties (*Vitis rotundifolia*), with very tough skins, were compared to the edible, thin skinned table grape variety, 'Thompson Seedless' (*Vitis vinifera*). Each of the grape cultivars has a single epidermal cell layer, with an outer layer of cutin. Underneath the epidermis is the hypodermis that is divided into two distinct layers. The first hypodermal layer consists of four or five rows of small, flattened collenchyma cells with thickened cell walls. This layer is followed by a second hypodermal layer of six to eight rows with larger, more rounded cells with thinner cell walls. The differences in average cell area for the Muscadine varieties and 'Thompson Seedless' are very small for the first three rows. However, after about the third row, the cells of the 'Thompson Seedless' begin to increase in size at a considerably faster rate than the two Muscadine varieties. The Muscadine varieties had significantly higher readings for failure stress and strain energy than 'Thompson Seedless'. Average cell area had an effect on the puncture resistance but not on the tensile strength of the skins. The skin thickness did not affect the tensile strength or the resistance of the skins to puncture. Considine (1981b) measured the number of layers of thickened cell walls and their thickness in the dermal tissue of nine grape cultivars. Cell wall thickness was significantly correlated with splitting susceptibility. Histological studies of the grape berry skin by Yamamura and Naito (1985) showed that cell wall thickness and size of cells in the subepidermal region were highly correlated with splitting. Tucker (1934) measured skin thickness of cherry fruit, but found no direct correlation between skin thickness and cracking susceptibility. However, he found a correlation between the calculated cubic centimetres of skin per gram of soluble solids in the fruit and cracking susceptibility.

According to Meynhardt (1964b), the anatomy of subepidermal tissues influences grape berry

resilience. Meynhardt (1964b) found a relationship between the length:width ratio of subepidermal cells, the number of subepidermal cell layers, and the susceptibility to berry splitting. In cultivars susceptible to berry splitting, the ratio between the longitudinal and radial subepidermal cell dimensions of the berry was comparatively small. A similar conclusion was drawn by Cortner *et al.* (1969) for tomatoes. In addition to the subepidermal cell dimension, the number of subepidermal cell layers may also possibly contribute towards a cultivars susceptibility or resistance to splitting (Meynhardt, 1964b). If the subepidermal tissue consists of a large number of cell layers, the resistance of the tissue to splitting is as a rule greater than in those tissues having fewer subepidermal cell layers. However, there was one exception to the rule. The grape cultivar 'Alphonse Lavallée', which is susceptible to splitting, possesses 10 subepidermal cell layers. Despite the relatively large number of cell layers, splitting in 'Alphonse Lavallée' berries may be due to the combined influence of a small subepidermal cell dimension ratio, and the small distance between the peripheral vascular strands and the berry skin. Compared with cell geometry, the number of subepidermal cell layers contributes less towards a cultivars susceptibility to splitting (Meynhardt, 1964b). Under circumstances which increased susceptibility to splitting, grape berries showed a thickened subepidermal layer, enhanced radial growth of the subepidermal cells and accumulation of pectic substances in the cell walls (Hiratsuka *et al.*, 1989).

Anatomical studies by Costa *et al.* (1983) showed that fruits of 'Stayman Winesap' apple were characterized by a lack of transition cells between the hypodermis and the fruit parenchyma. The hypodermic cells were small, thick-walled, tangentially orientated and depressed, while the parenchyma had large isodiametric cells with thin walls. Cell division in the hypodermic tissue ceased earlier than in the fruit parenchyma and the outer part of the fruit could not keep pace with that of its inner part. During periods of rapid growth, cracking of hypodermal cells could occur. These studies confirmed histological studies by Verner (1938) who suggested that the susceptibility of 'Stayman Winesap' apple to fruit cracking was due mainly to premature cessation or restriction of growth in the hypodermal layer. Weiser (1990) obtained similar results and hypothesized that the inability of the hypodermis to keep pace with the expansion of the fruit was due to a difference in cell wall composition and the consequent effect on wall extensibility.

Coombe (1960) found that cell division of the subepidermal tissue of grape berries of the cultivars 'Sultana' and 'Muscat of Alexandria' culminates about 30 days after anthesis. Thus, cell division of this tissue ceases some time before the berries become susceptible to splitting. Meynhardt (1956) found that the subepidermal cells of 'Queen of the Vineyard', a grape cultivar

susceptible to berry cracking, do not increase appreciably in length from about one month after anthesis until splitting begins. On the other hand, the subepidermal cells of 'Waltham Cross', a resistant cultivar, increases about 50% in length during the same time, while the radial dimension decreases.

In tomato fruit, Hankinson and Rao (1979) found that cultivars particularly resistant to concentric cracking possess flattened epidermal and hypodermal cells for their first few rows, while for cultivars resistant to radial cracking, the cutin penetrates into the third layer of cells. Peet (1992) noted that tomato cultivars with a thin skin, thin pericarp and shallow cutin penetration, are more susceptible to cracking. In cherry tomatoes, minute cuts on the surface of the fruit epidermis around the calyx, initiated fruit cracking (Ohta *et al.,* 1995). The cultivar in which the ratio of minute cut length to fruit circumference was the highest, showed the highest rate of cracked fruit at harvest. Thus, the occurrence of minute cuts ± 3 weeks after anthesis, was useful to determine susceptibility to fruit cracking among different cultivars of cherry tomato.

1.3.7.6 Mechanical properties of the fruit skin

The mechanical behaviour of the grape berry skin greatly influences the resistance of the berry to splitting (Lustig and Bernstein, 1985). In mature fruit, the skin is responsible for the ability of the fruit to withstand internal and external pressure changes and mechanical abuse under various climatic and environmental conditions. The buffering capacity of the skin develops during stage III of berry development which is characterized by rapid volume increase with cell expansion and also by an increase in deformability of the berry (Coombe, 1976; Coombe and Bishop, 1980).

The mature grape berry can be considered as an enclosed entity in which the internal pressure, or turgor pressure, is balanced by the skin stress (Nilsson *et al.*, 1958). Variations in the water content of the berry, which is dependant upon the soil, water and atmospheric condition, affects the turgor pressure in the berry as well as the skin stress of the berry. When the skin stress, caused by the turgor pressure, exceeds the rupture strength of the skin, the grape berry may split (Bernstein and Lustig, 1985).

Lustig and Bernstein (1985) measured the mechanical properties of the grape berry's skin in terms of skin stress and strain. Water intake increases the volume of the berry and consequently its skin area. The ratio of the increase in area to the initial area is termed strain. This strain increases the skin stress and the turgor pressure which balances this stress. The magnitude of

stress generated by a given increase in volume depends upon the mechanical properties of the skin. A grape cultivar resistant to splitting shows the behaviour of a berry with a soft, pliable skin which extends easily and therefore does not build up a high skin stress at a given water intake (Lustig and Bernstein, 1985). A cultivar less resistant to splitting depicts a tough skin which resists stretching and causes a higher rise of skin stress at the same water intake. At a level of skin strain which is to be expected during the diurnal variations of grape berry volume, the skin stress of the berry more resistant to splitting showed an increase of 57.5% above its initial stress. The skin stress of the less resistant berry at this point showed an increase of 113.6% above its initial stress. At the burst point of the less resistant berry, the skin stress of the more resistant berry only reached 77.6% of its burst stress (Lustig and Bernstein, 1985).

Lang and Düring (1990) developed techniques through which the stiffness, strength and extensibility of grape skin can be measured. These techniques involved the injection of known quantities of water into the fruit through fine hypodermic needles inserted through their stalks. Berries of nine cultivars were measured at a constant temperature. The cultivars differed in their elastic modulus, their bursting tension and their bursting strain. It was suggested that varietal differences in splitting resistance could be explained in terms of these mechanical properties of the skin. Niiuchi *et al.* (1960) found that tomato cultivars with highly elastic skins appeared to crack less.

1.3.8 Postharvest factors

Information on postharvest factors influencing postharvest berry split is virtually non-existent. The only publication which could be found was for 'Ribier' table grapes, packed in 8.2kg cases and held at room temperature (24°C) for 4, 8 or 12 hours, before storage at -0.5°C and 85-90% RH for 23 days (Leon and Lizana, 1990). A delay of 12 hours before cold storage caused berry cracking to increase to 11.24%, whereas it was only 6.4% with a 4 hour delay.

1.4 CONTROLLING OR REDUCING FRUIT CRACKING AND SPLITTING

Despite many research contributions to the phenomenon of fruit cracking and splitting, the search for reliable methods to adequately control the problem remains elusive (Opara *et al.*, 1997). Furthermore, success achieved in reducing cracking and splitting experimentally, has not been translated into the commercial fruit industry, due partially to difficulties in reproducing controlled conditions in the field. In certain instances, the cost of implementation would not be justified by the economic value of the crop (Bohlmann, 1962; Cline and Webster, 1994).

Many orchard management strategies have been applied to prevent or reduce preharvest fruit cracking and splitting, but their effectiveness varies greatly among fruits, cultivars, growing areas and conditions, and seasons (Opara *et al.*, 1997). Fruit producers have implemented a number of practices to minimize losses, such as reducing irrigation, installing temporary covers or shades to protect fruit from rain and applying spray materials to reduce water uptake by fruit. Because the severity of cracking or splitting normally increases as fruit approaches its peak of ripeness, growers of susceptible cultivars also tend to harvest earlier to prevent or reduce the amount of cracked fruit. This often results in the marketing of insufficiently coloured fruit, and fruit of inferior quality. In severe cases of fruit cracking and splitting, producers topwork susceptible cultivars, plant new cultivars resistant to cracking, or choose orchard sites where the probability of rainfall at the critical stage of the season is low.

1.4.1 Cultural methods

1.4.1.1 Mineral applications other than calcium

When cherry fruit were immersed in various chemical solutions, the cations were more effective in reducing cracking than the anions (Bullock,1952). Of the cations tested, calcium, copper, iron, aluminium, thorium and uranium reduced cracking. Cracking was reduced more as the valence of the cation increased, as long as the anion was monovalent. Preharvest spraying of cherry fruit and foliage with aluminium sulphate reduced the tendency of the fruit to crack at harvest. The spray left visible deposits on the fruit at harvest, but the residues could be removed with an acetic acid wash (Bullock, 1952).

Sprays of copper sulphate gave effective control of cracking (Powers and Bollen, 1947). A Bordeaux spray (a mixture of copper sulphate and calcium hydroxide) applied 5-6 weeks prior to harvest, reduced cracking in cherries from 80% in the controls to 4% in the treated trees (Foster, 1937). Verner (1939), Bohlmann (1962) and Powers and Bollen (1947) also found a reduction in fruit cracking with the use of the Bordeaux mixture. Verner (1939) and Bohlmann (1962) believed it was the calcium in the mixture which prevented fruit cracking, while Powers and Bollen (1947) concluded that it appears to be the copper, rather than the calcium, which gave the beneficial effects. A major drawback of the Bordeaux spray is that it causes spray damage on certain cultivars and leaves an unacceptable residue on the fruit. Copper hydroxide alone, at a low concentration or in combination with calcium hydroxide, resulted in a significant and consistent reduction of cracked fruit after moderate crack-inducing rainfall (Brown *et al*, 1995). The response to copper concentration was linear over the range tested, with the proportion of cracked fruit decreasing with increased copper concentrations in the sprays. Early applications

of copper-calcium sprays left minimal residues on the fruit at harvest and there was no obvious effect on other fruit quality factors.

In apples on boron-deficient soil, cracking has been reduced by boron applications (Bohlman, 1962). However, in 'Rymer' apples, soil application of boron was ineffective in reducing fruit cracking, while foliar sprays of 0.3% boric acid reduced fruit cracking considerably (Dube *et al.,* 1969). Powers and Bollen (1947) found a reduction in the rate of cracking by immersion of prunes in a 0.25% borax solution. Borax applied as a fertiliser reduced the cracking of cherries and prunes. Boron has been thought to reduce cracking through its influence on the elasticity and toughness of the cell walls of the fruit skin (Powers and Bollen, 1947). In another study (Bullock, 1952), boron inserted as a dry salt into holes drilled into the limbs of cherry trees, made no significant difference in the incidence of cracking of the cherry fruit. In tomato fruit, boron sprays reduced cracking significantly compared with control fruit (Gill and Nandpuri, 1970).

According to grower observations, overhead irrigation did not cause cherry cracking to the same extent as rain of equal duration (Ackley and Krueger, 1980). In laboratory tests, cherries were thoroughly wetted with either deionised water (which simulated rain), or with irrigation water which contained calcium and magnesium. The cracking of cherries wetted with irrigation water was consistently and significantly less than those wetted with deionised water (Ackley and Krueger, 1980). These results emphasise the marked effect of dissolved salts, particularly the divalent cations, on reducing cherry cracking.

1.4.1.2 Calcium

An estimated 60% of the total Ca^{2+} found in plant tissues is localised in the cell wall (Dey and Brinson, 1984). In the dermal tissue of flowering plants, the cells are enveloped by a cell wall composed of cellulose micro fibrils within a polysaccharide matrix that includes pectin gels ionically linked by Ca^{2+} bridges and proteins covalently cross linked (Carpita and Gibeaut, 1993). Ca^{2+} plays a critical role in maintaining or strengthening the structural integrity of the cell wall (Grignon and Sentenac, 1991). This may explain some of the beneficial effects of calcium applications in fruit splitting experiments. Applied calcium may interact with and strengthen cell walls in much the same manner as endogenous calcium (Callan, 1986). Regarding the mechanisms involved in cherry fruit cracking, histochemical studies indicated a breakdown of the cell wall structure in the epidermal region of water injured fruit (Glenn and Poovaiah, 1989). Calcium appeared to reduce cracking by delaying cell wall breakdown and thus protecting the epidermal region of the fruit from processes that lead to cracking. Autoradiographs of fruit

immersed in a solution containing $^{45}Ca^{2+}$, showed the epidermal region to be the site of Ca^{2+} action in altering fruit cracking (Glenn and Poovaiah, 1989). The use of chelators to extract Ca^{2+} from the cell wall, induced wall loosening, cell separation, cherry cracking and pectin solubilisation (Glenn and Poovaiah, 1989). The effect of the chelator could be completely negated by adding back an equal concentration of $CaCl_2$. Various formulations of foliar-applied fertilisers contain chelating materials that may increase cracking susceptibility.

The influence of Ca^{2+} may also be explained in terms of an effect on the cuticular pores (Sekse, 1995b) through which water and solutes penetrate (Schönherr, 1976). Application of chemicals with cations such as Ca^{2+} may influence the water permeability of cuticles by changing the chemical and physical environment within the pores (Sekse, 1995b). Calcium is known to decrease hydraulic permeability of cell membranes and to reduce the water absorption rate in sweet cherries (Verner, 1939).

A major drawback of preharvest calcium treatments is the ineffective uptake of the spray. The plant cuticle is the major barrier to spray penetration (Glenn and Poovaiah, 1985). Spray solutions penetrate the cuticle by diffusion and by mass flow through stomata, lenticels, polar pores and other surface breaks (Schönherr, 1976). *In vitro* studies, using isolated cuticles, were done by Glenn and Poovaiah (1985) to investigate the cuticular penetration of various calcium compounds, as well as the effect of temperature, viscosity and pH on the rate of calcium diffusion through isolated apple cuticles. Calcium chloride ($CaCl_2$) permeated the cuticle significantly faster than calcium acetate and calcium nitrate. Furthermore, the calcium penetration rate decreased with decreasing temperature. The decrease in the rate of diffusion at low temperatures seemed to be due to an increase in viscosity of the solution. Uptake of $CaCl_2$ tended to be higher at a pH of 3 than at a pH of 11. $CaCl_2$ molecules dissociated more under neutral or acid conditions than basic conditions. $CaCl_2$ not only dried slower than the other calcium compounds studied, but seemed less viscous which would enhance calcium movement further.

In other experiments, calcium acetate, applied 8 and 18 days before harvest reduced cracking, although the chemical deposits had to be removed with acetic acid (Bullock, 1952). Other researchers (Ono *et al.*, 1954) found that calcium caseinate sprays also greatly decreased cracking and did not leave residues on the fruit.

In laboratory trials, Callan (1986) evaluated combinations of calcium hydroxide ($Ca(OH)_2$), $CaCl_2$ and boron as preharvest sprays to reduce rain splitting of 'Lambert' sweet cherry. Three

sequential applications of $Ca(OH)_2$, with or without boron, reduced splitting more consistently than single applications of $Ca(OH)_2$ with boron. The addition of boron to the multiple $Ca(OH)_2$ applications did not increase effectiveness over $Ca(OH)_2$ alone. $Ca(OH)_2$ was more effective in reducing splitting than sprays of $CaCl_2$. Looney (1985) and Westwood and Bjornstad (1972) found $CaCl_2$ ineffective in reducing rain cracking. The reduction of fruit splitting by $Ca(OH)_2$ applications was also noted by Bullock (1952), Verner (1938) and Westwood and Bjornstad (1970,1972). According to Meheriuk *et al.* (1991), $CaCl_2$ and $Ca(OH)_2$ sprays generally reduced but did not eliminate rain splitting in 'Van' sweet cherries. Multiple sprays gave better protection than single sprays. Fruit shrivel and spray residue were observed with $Ca(OH)_2$ treatments. The residues were removed by water, but acid washes were recommended in studies by Verner (1938), Bullock (1952), and Westwood and Bjornstad (1970).

The incidence of split berries in the grape cultivar 'Alphonse Lavallée' was not significantly decreased by preharvest sprays with calcium nitrate, calcium nitrate plus Solubor® or calcium chloride plus Solubor® (Combrink *et al.*, 1982). Spraying 'Napoleon' cherry trees with 0.5% calcium nitrate significantly reduced fruit cracking compared with controls (Yamamoto *et al.*, 1992).

Organic complexes and chelates of calcium are available. Although these are safer and less corrosive to spray equipment, as well as less likely to result in chemical burn, there is evidence that less calcium is absorbed. Presumably the large molecules or the greater attachment of the calcium to the complex, results in lower intake (Tukey, 1983).

The poor ability of plants to take up calcium coincides with the large amount in most soils (Tukey, 1983). No relationship exists between the amount of calcium in the soil and the amount in the plant. The entire plant is usually not effected by calcium stress. This is usually confined to the fruiting part or growing point. Addition of calcium to the soil does not always correct calcium deficiencies, since the shortage of calcium may be beyond the plants absorption and mobilisation abilities to correct. Sprays of calcium directly to the surface of the fruit are more effective than soil applications. Foliar sprays are also applied, but there is little evidence that calcium entering the leaves moves to the fruit.

1.4.1.3 Fertilization, pruning and scoring

It is generally accepted that cultural practices resulting in the promotion of plant vigour, reduce the incidence of fruit cracking. Therefore, Campbell (1928) and Goodwin (1929) advocated practices such as heavy pruning, combined with manuring and cultivation, to prevent fruit

cracking and splitting. Rootsi (1962) suggested that the maintenance of adequate soil moisture and nitrogen supply can help in reducing the occurrence of apple cracking. According to Byers *et al.* (1990), two scores around the trunk of 'Stayman Winesap' apple trees inflicted with a carpet knife, reduced fruit cracking by 22%, without affecting fruit size, fruit colour or return bloom. In severe cases of apple cracking, Schmid (1960) recommended the top-working of affected trees to overcome the problem. To prevent grape splitting, Kelperis (1963) recommended pruning systems which allow good circulation of air and encourage transpiration. For tomatoes, fruit cracking could be reduced by as much as 60% by means of pruning (Brooks, 1961).

1.4.1.4 Moisture control

Maintaining an adequate water supply seems to lessen the amount of fruit cracking in cherries (Sekse, 1995a) and apples (Rootsi, 1962; Goode et al., 1975). Mezzetti (1959) suggested that apple cracking during cold storage could be prevented by maintaining a low RH during cold storage.

The exclusion of rain by shading apple trees during critical growth periods, resulted in fruit with less cracking (Jackson *et al.*, 1977). In sweet cherries, cracking has been prevented by covering fruit with paraffined paper (Sawada, 1931) or by excluding rain by means of waterproof tarpaulins (Verner and Blodgett, 1931). In contrast with these findings, many authors (Trought and Lang, 1991; Webster and Cline, 1994a and Meynhardt, 1956) observed cracked fruit in protected environments. Trought and Lang (1991) concluded that plastic covers that protect fruit from rain, may create small vapour pressure deficits that reduce the transpiration rate.

Levin *et al.* (1959) found that significant air circulation reduced cracking of wetted fruits under laboratory conditions. They suggested drying of the trees and the surrounding air by means of a helicopter or, alternatively, heating the air. Many producers shake their trees just after rain or spray high-velocity air with air-blast sprayers (Christensen, 1996). No evidence exists that confirms that these practices reduce fruit cracking. Drying orchards with blowers did not influence fruit cracking (Alani, 1980).

Sprays of hydrophobic compounds are often used to prevent or reduce transpiration of fruit. These anti-transpirants may retard the intake of external water by the fruit and may thereby reduce cracking (Davenport *et al.*, 1972). Single or multiple applications of Nutri-Save® (a polymeric coating), Pro-Stick® (a sticking agent) and Envy® did not affect rain splitting in 'Stella' cherries (Meheriuk *et al.*, 1991). Mobileaf® (a wax emulsion) and VaporGard® also failed to

reduce cracking of sweet cherries (Koffmann *et al.*, 1996). Spraying 0.001M 8-hydroxyquinoline sulphate twice daily on tomato plants did not affect cracking (Singh & Young, 1970). These results are in contrast to those of Davenport *et al.* (1972) and Intrieri (1974) in which anti transpirants reduced splitting.

Alani (1980) suggested that the action mode of wetters in washing out part of the hydrophobic surface waxes, could possibly increase water permeability of the fruit skin and transpiration. Field experiments with wetters on sweet cherries gave variable results with 0 to 80% fruit splitting (Alani, 1980). Koffmann *et al.* (1996) found that the wetting agents Citowett® and BS 100® failed to reduce splitting in sweet cherries. However, tests with four wetting agents, including Citowett®, applied 10 days before harvest on two cherry varieties, reduced splitting by 64-87% (Kampe, 1972). The flavour of the fruit was unaffected by the sprays.

1.4.2 Application of plant growth regulators

It has been suggested that fruit may split when skin stress, caused by a build-up in turgor pressure, exceeds the rupture strength of the fruit skin (Bernstein and Lustig, 1985). Due to the ability of plant growth regulators to modify cuticular and epidermal morphology such as to increase the plasticity of the fruit skin, researchers have investigated the use of several plant growth regulators to reduce the occurrence of fruit cracking and splitting (Opara *et al.*, 1997).

1.4.2.1 Daminozide (succinic acid 2,2-dimethylhydrazide)

Spray applications of daminozide two months before harvest, reduced fruit cracking in 'Stayman Winesap' apples by 93% (Kriedl, 1974). Byers *et al.* (1990) found that daminozide decreased fruit cracking in 'Stayman Winesap' apples only in certain years. Although Costa *et al.* (1983) noted a 66% reduction in 'Stayman Red' apple cracking following four spray applications of daminozide at 2000 ppm, there were adverse effects on fruit size, colour and yield in the year of application and on fruit size and shape the following year. Joosse (1982) also found a reduction in fruit splitting of 'Discovery' apples, but in contrast to the detrimental effects on fruit quality in 'Stayman Red' apple, daminozide also increased the yields. Despite the efficiency of daminozide in reducing fruit cracking, its application in agriculture, especially at high concentrations, is limited due to the adverse effects on fruit quality. Opara *et al.* (1997) suggested that the extent to which daminozide can reduce fruit cracking and its effect on fruit quality depends on various factors, including cultivar and the time, number and rate of applications.

1.4.2.2 Gibberellin

Apple cracking and splitting have been markedly reduced in various cultivars following gibberellic acid sprays (Joosse, 1982; Taylor and Knight, 1986; Byers *et al.*, 1990). According to Taylor and Knight (1986), the primary effect of gibberellic acid was considered to be the alleviation of stress within the fruit, which reduced the susceptibility to fruit splitting. Unrath (1991) studied the influence of concentration, spray interval, and the number of applications of gibberellic acid on the suppression of 'Stayman' fruit cracking. A concentration of 50 ppm and five applications at 3-week intervals were the optimum. Cracking was decreased by more than 80% compared to no gibberellin application. Byers *et al.* (1990) reported that combination treatments of gibberellic acid, daminozide, naphthaleneacetic acid (NAA), and VaporGard® (an anti-transpirant) reduced cracking from 93 to 22%. However, the reduction of fruit cracking by gibberellic acid alone and in various treatment combination was not consistent in other years. In citrus, Garcia-Luis *et al.* (1994) also observed this inconsistency. Fruit splitting in 'Nova' hybrid mandarin increased when gibberellic acid was applied at flowering, but the treatment reduced splitting when applied shortly after the end of fruit drop.

Sprays of gibberellic acid reduced cherry fruit cracking in the cultivar 'Brabanders' by 30-50%, compared with the untreated control (De Barsy *et al.*, 1988), while it failed to reduce cracking in the cherry cultivars 'Early Lyons', 'Merton Heart' and 'Ron's Seedling' (Koffmann *et al.*, 1996). According to Webster and Cline (1994b), the effectiveness of gibberellin on cherry splitting seems to be cultivar dependent. According to De Barsy *et al.* (1988), gibberellic acid appeared to thicken the cuticle and the radial walls of the epidermal cells, thereby reducing splitting. The attachment of gibberellin tapes on pear fruit (Maotani *et al.,* 1990) also reduced fruit cracking. In table grapes, gibberellic acid applications to increase fruit size, also increased the incidence of berry splitting in cultivars prone to this defect (Kononov, 1961; Jawanda *et al.*, 1974, Hiratsuka *et al.*, 1989; Laszlo and Saayman, 1991). Scanning electron microscopy showed that long cracks, invisible to the naked eye, developed most frequently during berry splitting in grapes sprayed with gibberellic acid (Hiratsuka *et al.*, 1989).

1.4.2.3 Paclobutrazol

Soil application of paclobutrazol significantly decreased fruit cracking in 'Seb' apples (Sankhla *et al.*, 1989) by minimizing excessive moisture and thermal stress. Similarly, soil application of paclobutrazol also reduced fruit cracking in the sweet cherry cultivar 'Hedelfinger R.' (Belmans, 1989). However, spray application of paclobutrazol at 250ppm increased the incidence of cracking in 'Niepling Stayman' apples (Visai *et al.*, 1989).

1.4.2.4 Promalin®

Applications of Promalin® were found ineffective to reduce fruit cracking in 'Stayman Red' (Costa *et al.*, 1983) and 'Niepling Stayman' apples (Visai *et al.*, 1989). Visai *et al.* (1989) attributed the ineffectiveness of Promalin® to unsuitable timing of the treatments, which were applied too early before the period of maximum susceptibility of the fruit to cracking.

1.4.2.5 Other plant growth regulators

Four spray applications of ethephon at 50ppm failed to reduce fruit cracking in 'Stayman Red' apples (Costa *et al.*, 1983), while ethephon at 100ppm decreased fruit cracking to less than 10% in a cherry tomato cultivar (Ohta *et al.*, 1992). Bullock (1952) found that sodium salt of α-naphthalene acetic acid (NAA), applied at 0.1-1ppm from 30-35 days before harvest, effectively reduced cracking in the sweet cherry cultivar 'Bing'. When applied closer to harvest, however, cracking was markedly increased. Westwood and Bjornstad (1972) found similar results. Yamamoto *et al.* (1992) found that spraying 'Napoleon' cherry trees with a combination of NAA and calcium nitrate reduced fruit cracking more effectively than either sprayed alone. The most effective combination was 1ppm NAA and 0.5% calcium nitrate, which reduced cracking significantly from 14.6 to 1.7%.

Although certain growth regulators reduced fruit cracking while others were ineffective, the results must be interpreted with caution because of considerable differences in region, cultivar, method and rate of application, and the time of application (Opara *et al.*, 1997). The effects of these factors and their interaction must be establish with a consideration of fruit quality attributes so that a more reliable spray strategy can be developed for the controlling of fruit cracking and splitting.

1.4.3 Metabolic inhibitors

The build-up in turgor pressure which leads to cracking, can develop only in cells with semi-permeable membranes, so turgor is linked to metabolism (Wade, 1988). It was therefore proposed that fruit cracking of sweet cherries is a metabolically active phenomenon, and the influence of several metabolic inhibitors on water uptake, respiration and cracking was examined.

Pre-treatment of sweet cherries with ether-saturated air for 1-2h before immersion in deionised water, inhibited cracking almost completely and reduced the respiration rate by almost half (Wade, 1988). Both the heavy metals silver nitrate and mercuric chloride inhibited water uptake and cracking in cherry fruit, but the silver nitrate was more effective. Potassium nitrate as

control treatment confirmed that silver was the active ion which inhibited cracking, and not the nitrate. Fluoride solutions caused inhibition of cracking, respiration and water uptake. Due to fluoride's high phytotoxicity, its potential use to control cracking is limited.

According to Pommier (1989), the physiological processes involved in fruit cracking are absorption of water into fruits, ion movements and respiration. Laboratory studies showed that neutralizing H^+ ions with a base (NaOH) reduced the electrochemical potential and hence water absorption by fruits. Respiration inhibitors, such as rotenone, were also effective in reducing water absorption.

1.4.4 Biotechnology

Genetic engineering was applied to improve the quality of tomatoes. The process involved the genetic transformation of tomatoes with polygalacturonase (PG) antisense constructs (Young, 1995). This resulted in reduced enzyme activities of PG and pectinesterase, which produced fruit more resistant to splitting and that ripened more slowly, thus delaying fruit softening.

1.5 CONCLUSIONS

It appears that no simple factor determines a grape cultivar's susceptibility to cracking or splitting, but that it is a combination of geometric, structural and physiological factors.

Various factors can initiate or aggravate berry split in cultivars susceptible to this defect. A vast amount of literature exists regarding pre-harvest factors influencing berry split, although there are some discrepancies concerning the influence of certain factors on berry split, e.g. fruit exposure to sunlight and the osmotic potential or total soluble solids of the fruit. However, very little information exists regarding the influence of postharvest factors on the incidence of berry split.

Despite the earliest efforts to control fruit splitting and cracking through various cultural measurements and the use of growth regulators, there is no guaranteed strategy to eliminate this disorder except by the breeding and selection of crack-resistant cultivars. It seems as if there are no detailed studies available, for table grapes specifically, which describe procedures for utilisation of such resistance in a breeding program. From a technical or scientific point of view, the development of biotechnology shows great potential to reduce or eliminate fruit cracking and splitting. However, the main impediments to the potential of biotechnology are issues of biosafety, ethics, the environment and public perceptions.

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2. LITERATURE REVIEW : FRUIT ABSCISSION

2.1. INTRODUCTION

Abscission is a process that culminates in the shedding of plant organs, such as leaves, flowers or fruits. For the purpose of this review, only fruit abscission will be discussed.

The majority of fruit on a plant may sometimes abscise almost simultaneously in response to environmental stresses, or, sometimes, portions of the total crop on the plant are shed throughout the period of fruit growth and development (Baird and Webster, 1979). Natural fruit abscission usually occurs during maturation and ripening, although in some species it also occurs soon after pollination and fruit set and during the period of differential growth of the young embryo. The percentage of the total plant body which is lost by premature abscission differs among species, within a species, and from season to season. Many species are characterised by alternate or biennial bearing, and the marked annual variations in the number of reproductive structures that abscise, are correlated to a considerable degree with the inherent patterns of periodicity of reproductive growth (Kozlowski, 1973). The dropping of immature fruits is a matter of widespread horticultural concern. Due to the premature abscission of 'Washington' navel orange over a six month period, Erickson and Brannaman (1960) found that the mature crop amounted to only 0.2% of its potential. The natural shedding of fruits can be both beneficial and deleterious (Baird and Webster, 1979). Natural abscission provides regeneration and extension of certain species by dissemination of both fruit and seed. Thinning of reproductive structures normally improves the quality of the remaining fruits. On the other hand, fruit abscission implicates a loss of useful plant products such as edible fruit, which is a loss in income for the producer of the fruit.

Modifications of the duration of fruit retention can be effected by removal of competing fruits and by the application of chemicals, including growth regulators, which induce or retard fruit abscission (Baird and Webster, 1979). In conjunction with mechanical harvesters, the application of chemical sprays to regulate fruit abscission has become increasingly common. Research on fruit abscission in citrus was initiated in 1950, when shortage of manpower and the high cost of harvesting were the main concern, especially in Florida (Wilson and Coppock, 1969). This encouraged citrus scientists to investigate chemical compounds to control the natural process of fruit fall and enable the introduction of mechanical harvesting as a common agrotechnical procedure in citrus orchards. In 1998, most citrus fruit were still harvested by hand, which resulted in reduced returns for citrus growers (Kazokas & Burns, 1998). The cost of

harvesting Florida citrus exceeded the cost of production (Muraro, 1996). Tree trunk shakers combined with abscission agents showed promise for automation and economics of harvesting (Whitney, 1995). However, the force needed to remove mature citrus fruit from the tree continues to be a major impediment to efficient and economical harvesting (Kazokas & Burns, 1998). A better understanding of the physiology and regulation of citrus fruit abscission is needed to develop effective automated harvesting management strategies based on abscission agents and mechanical harvesting units.

The most extensive studies on fruit abscission are those of plants in the families Rosaceae (apple, cherry, peach, plum, pear) and Rutaceae (citrus) (Baird and Webster, 1979). For grapes, research on the mechanism and control of berry abscission has been conducted in depth by various researchers world-wide since 1934. Despite all the research done on fruit abscission, its etiology and means of prevention are still not fully understood.

Nelson (1979) distinguished between "wet" and "dry" grape berry abscission. Sometimes rough handling may tear berries loose, leaving broken vascular strands as a wet brush still attached to the pedicel. This is a type of "wet" berry abscission. Another type of "wet" berry abscission occurs when a decay organism such as *Botrytis cinerea* Pers. infects the attachment area and causes the berry to slough off. This type of "wet" abscission has no wet brush adhering to the pedicel. Flexing of the stems from rough handling sometimes causes rupture at the base of the laterals and pedicels resulting in detached berries. This type of separation constitutes a form of "dry" berry abscission. A more serious form of "dry" berry abscission often occurs when the pedicel detaches effortlessly and neatly from the berry during postharvest handling. Many pedicels on the rachis occur without berries that have abscised in this manner.

Postharvest berry abscission with a "dry" wound is a major problem in table grape production. Since the first South African table grapes were exported to overseas markets, berry abscission has been one of the major quality defects occurring during cold storage and the shelf life period (T. Olivier, Pers. Comm.). In addition to a loss in marketable mass per grape bunch, berry abscission can lead to a discernible decline in consumer confidence and a corresponding drop in demand for, and price of the product. In highly competitive fresh fruit markets overseas, these repercussions are unaffordable for the South African table grape industry. In South Africa, the cultivars 'Waltham Cross', 'Thompson Seedless' and 'Dan-ben-Hannah' are to a greater or lesser extent susceptible to berry abscission, with annual rejections ranging between 1 and 5%. After four weeks in cold-storage, the occurrence of berry abscission can be as high as 25 to 33% (Smit, 1986). In an assessment of 'Thompson Seedless' grapes sampled from

retail stores in the UK, berry abscission was detected in 92% of the samples (Berry and Aked, 1996). An analysis was also carried out to determine the total weight of berries affected by the disorder as a percentage of the total weight sampled. Twenty four percent was lost due to berry abscission.

2.2. THE ABSCISSION PROCESS

The abscission process involves an orderly sequence of anatomical and physiological changes which culminate in cytolysis (Baird and Webster, 1979). Osborne (1968, 1989) divided the abscission process into three major stages (1) the *stimulus* stage, resulting from natural senescence or external factors (e.g. heat and drought stress, deficiency of minerals, mechanical injury, application of synthetic-exogenous plant growth substances); (2) the *signal* stage, marked by several internal parameters (e.g. increased ethylene production and a decrease in auxin level in the abscission zone, degradation of proteins and chlorophyll, etc.) and; (3) the *response* stage, characterised by synthesis of nucleic acids and specific proteins which results in the production of hydrolytic enzymes, e.g. cellulase and polygalacturonase, which are responsible for the degradation of the cell wall and the middle lamella. Two distinct types of structural changes occur during fruit abscission, *viz.* the detachment of the fruit from the plant at the abscission zone, and the protection of the exposed surface area on the plant (protective zone) after fruit abscission. The scar left after the fruit, pedicel or spur has abscised, is healed over by periderm formation.

2.2.1. Abscission zone

The abscission zone is a specialised cell layer which responds particularly to abscission inducing signals, so that abscission occurs at a genetically predetermined point (Brown, 1997). Fruit detachment may be preceded by differentiation of a discrete abscission zone through which abscission occurs, or the region of abscission may be structurally indistinguishable and only detectable histochemically (Baird and Webster, 1979). In fruit, two or three abscission zones develop. In particular species of cherry the first abscission zone develops at the fruit base, the second at the base of the pedicel, and the third at the base of the spur (Bukovac, 1971). Fabri and Betti (1982) distinguished between two abscission zones on the grape berry stem (pedicel) where abscission can occur. The one abscission zone is situated where the pedicel is attached to the bunch rachis and the other abscission zone is situated where the berry is attached to the pedicel. Citrus fruit has two abscission zones (Goren, 1981). During the first six to eight weeks after fruit set, young fruits abscise in the shoot-peduncle abscission zone.

Later, abscission is shifted to the peduncle-fruit abscission zone within the calyx area. However, there is a transition period of 2 to 3 weeks, during which abscission still occurs at both abscission zones (Goren, 1993). During this period, fruit first abscises at the peduncle-fruit abscission zone, and only then the remaining peduncle abscises at the shoot-peduncle abscission zone. After the transition period until maturity, the peduncle, if left after the abscission of fruit, will never abscise (Greenberg *et al.*, 1975).

Detachment of fruit is facilitated by developmental changes within the abscission zone (Baird and Webster, 1979). The degree of cellular differentiation within the abscission zone varies between species and reflects the relative ease with which fruit will separate naturally or are separated mechanically. Sour cherry (*Prunus cerasus* L.) fruits develop a distinct abscission zone and separate easily from the pedicel. However, fruits of sweet cherry (*Prunus avium* L.) do not develop a distinct abscission zone, and mature fruits are separated only with difficulty and often with injury (Stösser *et al.*, 1969).

The region of fruit abscission can often be determined externally by colour differences and by a narrow, constricted area at or close to the base of the abscising part (Baird and Webster, 1979).

The abscission region of most fruit is anatomically distinguishable from adjacent tissue (Baird and Webster, 1979; Goren, 1993). The abscission zone cells are typically thin-walled, densely packed and smaller than the adjacent cells. Certain structural components of the vascular tissue and its configuration normally differ from that in adjacent tissue. Xylem and phloem fibres are unusually small or absent. The abscission zone is usually considered as a region of weakness, although in some plants, this region is equally as strong as adjacent areas. In some fruit, such as mango and avocado, abscission is associated with tyloses formation in the vascular strands of the abscission zone (Barnell, 1939). It was suggested that the development of the abscission zone restricted water movement and that the restriction was probably due to the occlusion of xylem vessels by tyloses. In many plants, the presence of starch in the cells of the abscission zone is associated with abscission (Wilson and Hendershott, 1968, Kazokas and Burns, 1998; Bornman *et al.*, 1966). Crystals of calcium oxalate are also present in the parenchyma cells of the abscission zone of many fruits (Baird and Webster, 1979; Scott *et al.*, 1948). Lignin is typically absent or present in only very small amounts in the abscission zone and it is doubtful whether the parenchyma cells of the cortex of the abscission zone would undergo lignification or not (Baird and Webster, 1979). Furthermore, meristematic activity is not a conspicuous phenomenon in fruit abscission zones; only cell elongation without cell division was reported for citrus fruit (Wilson and Hendershott, 1968). However, regarding the abscission

of immature apple fruit, separation-related cell divisions occur at the junction of the pedicel and the spur (McCown, 1938).

2.2.2. Separation layer and separation process

Regarding the detachment of fruits, two separate sets of phenomena may be brought into operation (Baird and Webster, 1979). First is the formation of an anatomically distinct separation layer, through which abscission subsequently occurs. Secondly the actual separation of adjacent cells occurs as a result of chemical alterations in the cell walls. The separation layer typically encompasses one or more vertical rows of cells within the abscission zone. Initiation of layer formation in the pedicel-fruit abscission zone in the sour cherry (*Prunus cerasus* L. cv. Montmorency) occurs near the transition from Stage II to Stage III of fruit development (Wittenbach and Bukovac, 1974). Before this initiation, high fruit removal force (FRF) exists and ethylene does not significantly reduce the FRF during this period. After initiation of layer formation, there is a remarkable decrease in FRF and ethylene markedly reduces the detachment resistance. Stösser *et al.* (1969) investigated the site and histological changes associated with the separation layer formation in maturing sour cherry fruit. The layer consisted of 5-8 cell rows in the transition zone between the fruit and pedicel. The cells separated without rupturing of cell walls. The proximal and distal cells adjacent to the separation line were thin walled and most likely to separate. No abscission layer was formed through the vascular bundles and no cell division was observed during layer formation.

The separation layer can sometimes be detected prior to any anatomically or histochemically observable cell wall changes by a staining affinity of the parenchymatous cells which lie distal to the separation layer (Carns, 1966). When the abscission zone and adjacent tissue are treated with phloro-glucinol-hydrochloric acid, the walls of the distal parenchyma cells stain red. Separation eventually takes place through the unstained cells of the abscission zone (i.e. the separation layer) which adjoin the red-wall-stained cells. The significance of this staining reaction lies in the fact that it provides the first noticeable indication of the initiation of abscission.

Separation of cells during abscission can evidently commence in any tissue and region of the separation layer (Baird and Webster, 1979). Separation in apple fruits starts at the epidermis and then continues through collenchyma, vascular tissue, and sclerenchyma cells of the pith (McCown, 1943). Separation in muskmelon (*Cucumis)* originates internally, and is manifest by relatively simultaneous disconnection of a small number of adjacent parenchyma cells at several different places within the abscission zone (Webster, 1975). Disconnected cells consequently

collapse, resulting in several cavities within the zone. Continued cell separation, followed by cell breakdown, results in gradual broadening and eventual coalescence of cavities to form a single cavity extending through the abscission zone.

In fruit abscission the cell wall changes associated with the development of the separation layer involve : (1) hydrolysis or dissolution of pectin in the middle lamella, which results in loss of cementing effectiveness between contiguous cell walls; (2) dissolution of the lamella plus degradation of all or part of cell wall substances, particularly cellulose and hemicelluloses; and (3) mechanical breakage of non-living elements, which typically occurs through thick secondary walls of xylem elements (Baird and Webster, 1979). The separation of sour cherry fruit, for example, involves breakdown of pectic substances, non-cellulosic polysaccharides, cellulose and hemi-cellulose, and, eventually, fracturing of vascular tissue (Stösser *et al.*, 1969).

The histological changes associated with abscission layer formation result from the combined activities of several wall degrading enzymes (Sexton and Roberts, 1982). There are many different cell wall hydrolases, and only certain members of each family are involved in abscission (Brown, 1997). The enzymes most commonly associated with abscission include cellulases and pectinases, which hydrolyse cell wall constituents resulting in weakening of the cell wall, and several defense-related enzymes such as chitinase and β-1,3-glucanase (Sexton *et al.*, 1985). The hydrolytic enzymes produced in the cell organelles are termed endo-cellular enzymes (Abeles and Leather, 1971). These enzymes do not participate in the abscission process until they are secreted through the plasmalemma to the substrate in the cell wall, when they are termed exo-cellular enzymes. Abeles (1968) noted that the cell wall degrading enzymes are localized mainly in the separation layer, and their activity increases only after an induction period. Their activity is accelerated by ethylene and delayed by auxin and metabolic inhibitors. In addition to facilitating separation of primary-walled cortical cells of the abscission zone, hydrolytic enzymes (particularly cellulases) might also be involved in the rupture of non-lignified vascular elements (Osborne, 1968). Therefore, the action of cell wall hydrolases directly influences rupture at the abscission zone and ultimate detachment of the organ from the plant (Sexton and Roberts, 1982).

Cellulase has consistently been correlated with abscission (Horton and Osborne, 1967; Pollard and Biggs, 1970; Young, 1972; Rasmussen, 1973; Greenberg, *et al.*, 1975). Its activity increased in many different abscission zones as weakening of the abscission zone progressed (Sexton, 1994). Whether cellulase is involved in cell separation *per se* is unknown, nor is the identity of the linkages which are hydrolysed (Brown, 1997). Although the *in planta* substrate for

cellulase remains anonymous, the possible substrate may be xyloglucan polymers of hemicellulose (Brummel *et al.*, 1994). Several isoforms of cellulase exist, but not all are involved in abscission (Brown, 1997). Huberman and Goren (1979) observed nine isoenzymes of cellulase in the abscission zone of orange fruit. The activity of most isoenzymes and the abscission rate increased following ethylene treatment, whereas auxin delayed both the increase in activity of these isoenzymes and abscission. Two major isoenzymes of cellulase were characterized in bean: cellulase pI 4.5 participates in the elongation of cell walls, and pI 9.5 participates in the degradation of cell wall at the abscission zone (Linkins *et al.*, 1973). Cellulase pI 4.5 is enhanced by auxin, which affects cell elongation, and pI 9.5 is induced by ethylene which is involved in cell separation during abscission (Lewis and Varner, 1970; del Campillo *et al.*, 1990). Antibodies specific to pI 9.5 cellulase were used to demonstrate that this isoenzyme was undetectable prior to abscission, that its appearance correlated with a reduction in break-strength, and that it was localized in the abscission layer (del Campillo and Bennet, 1996; Sexton *et al.*, 1980). Although it is customary to characterise cellulase pI 9.5 as the abscission cellulase, del Campillo *et al.* (1990) and Reid *et al.* (1990) could sometimes not detect cellulase pI 9.5 during abscission, while it could be detected in tissues that did not participate in the abscission process.

Despite anatomical studies showing dissolution of the middle lamella (Addicott, 1982), and an increase in soluble pectin fractions during abscission (Morre, 1968), the activity of pectolytic enzymes in abscission zones has been more difficult to demonstrate than cellulase activity (Addiccott, 1982). Polygalacturonase (PG) activity correlated with the breakdown of pectin polymers that are present throughout the middle lamella of abscission zones (Sexton and Roberts, 1982). In abscission zones of citrus fruit, PG activity coincided with ethylene-induced fruit abscission, and its activity during abscission was similar and parallel to that of cellulase (Greenberg *et al.*, 1975). The PG protein involved in abscission in tomato was immunologically distinct from the PG isolated from fruit during ripening. Introduction of the antisense gene for fruit PG did not affect PG activity in the abscission zone (Taylor *et al.*, 1990). A specific PG gene expressed during tomato flower abscission was isolated and the amino acid sequence was only 42% identical to that of tomato fruit PG (Kalaitzis *et al.*, 1995). Expression of this gene showed the characteristics expected of an abscission-related hydrolase : Increase during ethylene-induced abscission, inhibition by auxin and silver thiosulfate (an inhibitor of ethylene action), and expression located specifically in the abscission zone. The correlation between expression of a PG mRNA and pod dehiscence also supports the involvement of a particular PG in abscission (Petersen *et al.*, 1996). Six other PG mRNAs were cloned from rape, but their expression did not correlate with dehiscence. Although there was a correlation between

ethylene production and pod dehiscence, ethylene had no effect on the dehiscence-related mRNA.

In the non-abscising shoot-peduncle abscission zone of mature citrus fruit no correlation exists between cellulase-and PG-activity and abscission (Goren *et al.*, 1973; Greenberg *et al.*, 1975). Although ethylene treatment increased their activity and cell wall degradation did occur at the abscission zone, cell separation was limited to "pockets" of cells located in the inner cortex within the abscission zone. The cells in these "pockets" underwent the entire separation process, but since the process did not occur in the whole abscission zone, the fruit did not abscise in this zone (Goren, 1981, 1993). This is the reason for the absence of abscission of fruit for approximately two months after fruit setting, despite the induction of hydrolytic enzymes.

Besides cellulases and pectinases, the enzymes peroxidase, uronic acid oxidase, chitinase and β-1,3-glucanase have been associated with abscission (Sexton *et al.*, 1985). These enzymes may not be directly involved in separation, but rather in wound responses and the protection of the exposed surface from pathogens. Pollard and Biggs (1970) could find no significant correlation between activity of pectin-methylesterase and abscission. Peroxidase, dehydrogenase and acid phosphatase are present in the abscission zones of sour and sweet cherry fruit during abscission layer formation (Poovaiah *et al.*, 1973). Their localized activity in this region through which separation takes place, indicates an association with abscission. Poovaiah *et al.* (1973) suggest that peroxidase may regulate endogenous levels of indoleacetic acid in fruit abscission. Localized dehydrogenase activity in the cherry abscission zone may simply indicate enhanced respiratory activity during the separation period (Baird and Webster, 1979). Acid phosphatase which is also associated with ripening and senescence, may influence permeability of membranes of abscission zone cells by impairing synthesis of RNA and protein (DeLeo and Sacher, 1970). However, Poovaiah *et al.* (1973) pointed out that since cherry fruit separation at the fruit-pedicel zone occurred only at fruit maturity (Bukovac, 1971), acid phosphatase may be only indirectly involved in the abscission process.

Delay of abscission with inhibitors of protein synthesis, suggest that *de novo* synthesis of RNA and protein is involved in fruit abscission (Stösser *et al.*, 1969). Based on protein inhibitor studies using bean explants, Abeles (1968) concluded that protein synthesis in the abscission zone was a prerequisite for synthesis of wall-degrading enzymes such as cellulase. Prior to cell separation in the calyx abscission zone of young lemon fruits, there was an increase in endoplasmatic reticulum and Golgi apparatus, which suggests the synthesis of protein and/or enzymes associated with degradation of primary cell walls (Iwahori and Van Steveninck, 1976).

Additional to pectin degradation in the abscission zone, cell walls in the abscission zone of sour cherry also lose calcium and magnesium during separation (Stösser *et al.*, 1969). Since pectins act as cementing substances between cells and are linked together by polyvalent cations such as calcium (Setterfield and Bailey, 1961), cell separation might reasonably be accountable for the loss in calcium and magnesium. Furthermore, the molecular chains of pectic acids are linked to hemicellulose and cellulose through calcium and magnesium bridges; thus the role of calcium and magnesium in maintaining cell wall integrity is well established (Tagawa and Bonner, 1957). However, Bukovac (1971) reported that the loss of calcium and the progressively deteriorating capacity of cherry abscission cells to bind ^{45}Ca as separation progresses, may be predominantly a reflection of general wall degradation.

Mechanical resistances (probably thick-walled vascular elements) influence the ultimate degree of separation in many fruits (Baird and Webster, 1979). Muskmelon fruits separate from the vine only after collapse of the vascular tissue (Webster, 1975). Separation of sour cherry fruit is ultimately effected by mechanical fracturing of the vascular strands, through which the differentiated abscission zone does not extend (Stösser *et al.*, 1969). Morré (1968) noted that in bean, the general solubilisation of pectin fractions associated with the break-strength decline alone was insufficient to assure actual separation. Only when the thick, cellulosic walls of the vascular strands were detached, was separation effected. In muskmelon fruit, this severing of vascular elements consistently coincided with a specific stage of fruit ripeness (Webster, 1975). Wittenbach and Bukovac (1972) noted that the most significant component of fruit abscission may be the ripening of fruit itself rather than some abscission process *per se*.

2.2.3. The protective zone

The protective zone develops on the pedicel or stem side of the abscission zone after fruit abscission has occurred and this zone is not structurally related to the abscission zone or to fruit separation (Baird and Webster, 1979). Meristematic activity in terms of cell divisions is associated with the formation of this protective zone. The cellular changes related to the elaboration of the protective zone are initiated prior to, or after fruit abscission. In mango fruit, a periderm, or protective region, forms after fruit abscission in tissue at the junction of the fruit stalk and the leafy twig (Barnell, 1939). Once withering and drying of the fruit stalk are well advanced, meristematic activity associated with phellogen formation commences. Some oranges show lignin deposition in the distal side of the separation layer in both the pith and cortical cells when abscission commences (Wilson and Hendershott, 1968). Lignification continues after the completion of separation, and contributes to the development of the

protective layer. In addition to lignin, cell walls of parenchyma cells in the protective zone characteristically contain suberin, and substances such as cutin and tannins may also be deposited (Barnell, 1939). Tyloses are abundant in the vascular tissue of the protective zone and the protoplasts of parenchyma cells gradually disappear.

2.3. PLANT HORMONES AND ABSCISSION

It is well established that plant hormones are directly involved in the control of abscission (Addicott, 1982). However, the role of endogenous growth substances such as auxins, abscisic acid (ABA), gibberellins (GA), cytokinins and ethylene in fruit abscission is complex and has not been clarified. There have been suggestions that fruit abscission is regulated by the interaction of various plant growth substances (Davies *et al.*, 1986). Auxin and cytokinins have been shown to delay abscission in excised organs or tissue while ABA and ethylene both accelerate abscission.

2.3.1. Auxin

Although it has been shown experimentally that auxins are very effective inhibitors of abscission, there is little indisputable evidence that auxin concentration regulates natural abscission (Sexton, 1997). Wareing and Phillips (1971) showed that periods of fruit abscission coincide with low levels of endogenous auxin. Therefore, Singh *et al.* (1985) suggested that exogenous application of auxins may supplement the endogenous level, thereby delaying abscission. The fact that precocious fruit abscission can often be reduced by application of auxin sprays, supports the theory that natural fruit abscission is related at least in part to low endogenous auxin levels in fruit. Studies by Luckwill (1948, 1953) regarding hormone production by apple seed in relation to fruit drop, support the view of auxin's involvement in fruit abscission. Preharvest abscission of apples was associated with low levels of auxin in the seed. The retardation of abscission by auxin and auxin-like growth regulators has been confirmed repeatedly with a variety of plants (Baird and Webster, 1979; Denney and Martin, 1994; Henderson and Osborne, 1994; Stern *et al*, 1995). This resulted in some important agricultural applications, including the prevention of preharvest abscission of apples and pears, delay of petal abscission in flowering cherry, and the reduction of young fruit abscission in tomatoes (Baird and Webster, 1979). In orange trees, leaves were shown to provide abscission-retarding material such as auxin to the fruit (Ben-Yehoshua and Eaks, 1970).

According to Gopalkrishna and Ekbote (1962), auxins prevent the breakdown of calcium pectate

in the middle lamella, thus preventing abscission. Convincing evidence indicates that auxin has a major controlling influence on both cellulase and PG. Goren (1981) found that auxin delayed an increase in cellulase and PG activity and abscission in citrus. If auxin is applied with ethylene, it prevents both abscission and the subsequent accumulation of both cellulase (Tucker *et al.*, 1988; del Campillo and Bennett, 1996) and PG mRNA (Kalaitzis *et al.*, 1995). Synthetic auxin applied to abscission zones treated with ethylene for 48 hours, reduced the levels of cellulase mRNA (Tucker *et al.*, 1988) and it inhibited further cellulase accumulation, even in the presence of ethylene (Osborne *et al.*, 1984).

It is well recognized that abscission proceeds in two stages : during the first stage auxin retards abscission, when ethylene is still unable to promote abscission (Addicott, 1970, 1982), while in the second stage, auxin accelerates abscission by promoting ethylene production (Abeles and Rubinstein, 1964; Addicott, 1982). In the former case, auxin inhibits the rise in the activity of the hydrolytic enzymes such as cellulase and PG (Greenberg *et al.*, 1975; Huberman and Goren, 1979); whereas in the latter case, auxin-induced fruit abscission results from enhanced ethylene production, which causes an increase in the synthesis and activity of the hydrolytic enzymes in the abscission zone (Goren, 1993).

2.3.2. Ethylene

A considerable amount of literature supports the involvement of ethylene in abscission of fruit (Brown, 1997). Strong evidence exists that ethylene accelerates fruit abscission (Burg, 1968; Brown, 1997), although, in many instances, it is not the only factor regulating abscission, and in some cases may not even be important (Brown, 1997). Although there is good evidence that ethylene is involved in accelerating abscission, there is no convincing evidence that its presence is an absolute requirement for abscission (Sexton, 1997). In many cases natural ethylene and/or 1-amino-cyclopropane-1-carboxylic acid (ACC) (a precursor of ethylene) levels are higher during periods of abscission in fruit, including early drop and during ripening (Burdon and Sexton, 1993). In such cases, the rise in ethylene production is probably responsible for promoting abscission. In general, abscission is a direct function of ethylene concentration in the tissue (Dedolph *et al.*, 1961; Burg, 1968; Abeles, 1972). When the ripening-related increase in ethylene production was blocked by antisense ACC oxidase in cantaloupe melon fruits, the fruit abscission zone was not activated and no fruit abscission occurred (Ayub *et al.*, 1996). Similarly, ethylene biosynthesis inhibitors, such as aminoethoxyvinylglycine (AVG), can delay the initial development of an abscission zone (Shellie, 1999; Ward *et al.*, 1999), or it can inhibit fruit abscission by suppressing ethylene evolution (Nito, 1985). Reports exist where silver thiosulphate (STS) completely terminated abscission for periods of one to several weeks

(Goszczynsla and Zieslin, 1993; Sexton *et al.*, 1995), implying that ethylene is essential for abscission to occur (Sexton, 1997).

When ethylene production does not correlate with abscission, ethylene responsiveness, which depends upon the developmental stage (Hoyer, 1996), may be an important factor (Brown, 1997). According to Sexton (1997), the extent of ethylene responses are dependent both on the concentration of ethylene and on the levels of ethylene receptors. It is suggested that changes in ethylene sensitivity are mediated by modulation of ethylene receptor levels during development (Payton *et al.* 1996). Mature 'Valencia' orange fruit undergo a period of non-responsiveness to abscission-inducing chemicals, during which less ethylene production is induced by the chemicals and the abscission zones are less responsive to exogenous ethylene (Wheaton *et al.*, 1977).

It is evident that in citrus, ethylene is the prime hormone which induces abscission, and ethylene is concurrently involved in the control of different processes which lead to abscission (Goren, 1981). Enhanced ethylene production has been associated with increased levels of cell wall degrading enzymes such as cellulase and pectinase in the fruit abscission zones (Rasmussen, 1973). For 'Valencia' orange and 'Tahiti' lime, Kazokas and Burns (1998) demonstrated that cellulase activity and gene expression are correlated with events in fruit abscission zones triggered by ethylene treatment. After six hours of ethylene treatment, cellulase mRNAs were expressed, followed by an increase in total cellulase activity. Light micrographs confirmed that abscission-related cell wall degradation commenced after 12h of continuous ethylene treatment of mature 'Valencia' orange fruit. The relatively short interval between ethylene exposure and the expression of cellulase mRNA and the increase in cellulase activity, suggests that the harvested citrus fruit contained low quantities of auxin-type compounds. In the abscission zone of mature 'Valencia' orange fruit, cellulase mRNA accumulation and cellulase activity continued during subsequent air storage that followed the ethylene treatment. In immature fruit, cellulase mRNA accumulation and cellulase activity was much less during the air storage period. The differences between mature and immature fruit regarding cellulase gene expression and cellulase activity measured after ethylene treatment and subsequent air storage, were probably attributable to differences in stage of fruit maturation. This may explain why mature fruit preferentially respond to abscission agents that cause rapid but transient ethylene production, while immature fruit remain attached to the tree.

In abscising bean leaves, ethylene treatment induced accumulation of a cellulase-specific mRNA followed by *de novo* synthesis of pI 9.5 cellulase (Tucker *et al.*, 1988). If ethylene is

withdrawn when abscission is in progress, the levels of cellulase mRNA decrease, indicating ethylene regulation of the expression of the abscission cellulase gene. Prior treatment with auxin prevented ethylene-induced accumulation of the pI 9.5 cellulase transcript. Burns *et al.* (1995) determined cellulase gene expression in ethylene-induced abscission zones of 'Valencia' citrus fruit. Two abscission-specific cellulase genes *Cel-al* and *Cel-bl* encoding cellulase proteins were isolated. Expression of *Cel-bl* was much greater in mature fruit during ethylene treatment. This suggests that each gene may encode a cellulase enzyme with a distinct biochemical function and/or compartmentation, or that it is active at distinct developmental stages (Kazokas and Burns, 1998).

The continuous presence of ethylene is required to sustain an accelerated rate of weakening of abscission zones (Brown, 1997). Ethylene-induced transcripts of genes encoding hydrolytic enzymes involved in abscission disappeared when bean leaf abscission zones were placed under hypobaric conditions to reduce the partial pressure of ethylene (Sexton *et al.*, 1985). This indicates that ethylene may be required for continued transcription of these genes. In addition, ethylene may control abscission at one or more post-transcriptional steps (Brown, 1997). When ethylene declines before separation has adequately progressed, the abscission process not only stops, but is reversed, and the break-strength increases (Biggs, 1971).

Berry and Aked (1996) and Yun and Lee (1996a), suggested that the presence of ethylene after harvest may stimulate berry abscission during storage. Ge *et al.* (1997) found that the level of ethylene released from 'Thompson Seedless' grapes was very low, but that the potential for ethylene release during cold storage was high. Because of the low permeability of the cuticle (Barmore and Briggs, 1972), the pedicel-fruit abscission zone may be the least resistant to ethylene diffusion from the fruit. Wu *et al.* (1992) and Ge *et al.* (1997) reported that for harvested grapes, the ethylene production rates of the cluster stalk, including most of the pedicel, were markedly higher than those of the fruits, and showed climacteric-like changes. The rate of berry abscission was significantly reduced when grapes were stored at 0°C in the presence of an ethylene scrubber (Yun and Lee, 1996a,b).

Auxin appears to be an important regulator of ethylene sensitivity and fruit abscission (Brown, 1997). According to Goren (1981) and Osborne (1989) the level of endogenous auxin in the abscission zone has to be reduced below a certain threshold before ethylene can induce the physiological processes which leads to abscission. It is known that ethylene reduces the level of endogenous auxin, and there is evidence that ethylene promotes the catabolism of auxin at the abscission zone (Ernest and Valdovinos, 1971). Although ethylene probably stimulates

some *in situ* auxin degradation by peroxidase, its main role in decreasing auxin levels is to reduce IAA transport to the abscission zones (Goren, 1981). It is apparent that the ethylene-induced formation of different conjugates is responsible for the inhibited transport of IAA from the leaf blade, lowering the level of IAA at the abscission zone. Okuda and Hirabayashi (1998) suggested that the reduction of IAA polar transport above the abscission zone is necessary to promote abscission of citrus fruit 'Kiyomi tangor' and that both IAA accumulation in the abscission zone and the IAA gradient between the peduncle and branch are involved in abscission. For apple fruits, Liu and Ma (1998) suggested that ethephon (an ethylene-releasing chemical) inhibits seed development and the export of IAA from fruits, and then activates polygalacturonase and cellulase biosynthesis in the abscission zone.

A modified auxin ethylene balance theory of abscission regulation seems to fit experimental facts best (Sexton, 1997). This envisages that the progress of abscission is determined by the inhibiting influence of auxin concentration counterbalanced by the accelerating effects of ethylene. The effectiveness of both these hormones is subject to modulation by factors such as receptor concentration, which influence the sensitivity of the tissue.

2.3.3. Abscisic acid

Davis and Addicott (1972) noted that large increases in abscisic acid were associated with young cotton fruit abscission. A single application of abscisic acid (ABA) effectively defoliated citrus trees and also induced extensive fruit abscission (Cooper and Henry, 1968). Cooper and Horanic (1973) demonstrated abscission of citrus fruit explants with ABA in the absence of ethylene, suggesting that ABA has a direct effect on abscission. According to Addicott (1983), fruit abscission is governed by the relative amounts of auxins and ABA. Although the levels of the auxin IAA is very low during Stage III of grape development, the ABA:IAA ratio, which is relatively high at this stage, can possibly be the reason for berry abscission (Wolf, 1991).

Wolf (1991) studied ABA levels during the developing and ripening periods of 'Waltham Cross' table grapes as well as after harvest during four weeks of refrigerated storage of the grapes. The ABA levels monitored during the berry development and ripening phases showed a significant peak between véraison and harvest as found by Scienza *et al.* (1978). Contrary to expectations, ABA levels rose significantly after harvest, during refrigeration. This rise could possibly be explained by the slow release and activation of the bound form of ABA to the physiologically active free form of ABA. Wolf (1991) exposed an experimental vineyard to possible berry abscission treatments, *viz.* water stress and gibberellic acid (GA) sprays for berry enlargement. Berry samples from the drought stressed vines had higher levels of ABA but a

lower incidence of berry abscission, compared with the non-stressed vines. Furthermore, in samples from the GA treatments, ABA levels were unchanged or slightly lower than the control, but the GA treatment resulted in the highest berry abscission. Thus, Wolf (1991) could find no clear correlation between changes in the levels of ABA and berry abscission for the cultivar 'Waltham Cross' under stress conditions. Rasmussen (1974) could find no significant changes in endogenous, bound or free, ABA in the abscission zones of young and mature grapefruits in the course of abscission.

The accelerating effect of externally applied ABA on abscission was observed in several plants (Addicott, 1982; Cooper *et al.*, 1968). In citrus fruit the role of ABA in abscission is not clear (Goren, 1981, 1993). In field experiments, ABA did not cause fruit abscission when applied to mature trees (Cooper *et al.*, 1968). However, in laboratory trials with fruit explants, ABA induced abscission (Rasmussen, 1974). Interrelationships were found between ABA and tissue injury, which may explain why ABA does not induce abscission when applied in the field to intact citrus trees, but promotes abscission in fruit explant systems where injury is involved (Goren, 1993). The fact that ABA had positive effects on abscission only at relatively high concentrations, indicates that the effect of ABA in citrus may be indirect. It was shown that when ABA induces the activity of hydrolytic enzymes and abscission, it is via the induction of ethylene formation which is the primary hormone affecting the physiological processes that control abscission (Sagee *et al.*, 1980; Goren, 1993).

2.3.4. Gibberellin and cytokinin

In most plants, gibberellin and cytokinin are not considered as primary hormones in the control of abscission (Goren, 1993). However, in table grapes, the application of gibberellic acid (GA_3) reduced berry abscission in certain instances (Rizk *et al.*, 1974), and aggravated the problem in other cases (Steenkamp and Uys, 1982; Smit, 1986; Cooper *et al.*,1993). Also, grape bunches treated with kinetin, reduced berry abscission (Dhillon *et al.*, 1985).

2.4. FACTORS INFLUENCING ABSCISSION

2.4.1. Application of growth regulators and other chemicals

The use of growth regulators and other chemicals to control abscission has four objectives : (a) to provoke thinning of fruitlets in heavy bearing trees; (b) to prevent fruitlet drop in low bearing trees; (c) to induce fruit loosening for mechanical harvesting; and (d) to prevent preharvest fruit abscission (Goren, 1981). All four of these objectives are reliant on the same physiological and

biochemical processes, and an understanding of these processes is necessary for the development of agrotechnical procedures using growth regulators and other substances for the promotion or prevention of fruit abscission.

Application of gibberellic acid (GA_3) two weeks after fruit set, reduced berry abscission in 'Thompson Seedless' grapes (Rizk *et al.*, 1974), while GA_3 sprays one week after 'Waltham Cross' berries had reached pea size aggravated the problem (Steenkamp and Uys, 1982). Cooper *et al.* (1993) reported that increasing GA_3 concentrations (0 - 30ppm at flowering and 0-100ppm after fruit-set) had a significant effect on berry length and diameter of 'Thompson Seedless' grapes, while berry abscission after cold storage increased progressively. Promalin® (GA_{4+7} + benzyladenine) had a similar effect on berry growth, but reduced berry abscission (Cooper *et al.*, 1993). Retamales and Cooper (1993) measured the fruit removal force (FRF) as well as pedicel and rachis flexibility in 'Thompson Seedless' grapes treated with different GA_3 concentrations. Contrary to what other authors had postulated, the FRF increased, indicating greater detachment resistance, as the GA_3 concentration increased. However, pedicel and rachis flexibility were reduced with increasing GA_3 dosage. It is suggested that this loss of flexibility was directly responsible for the higher occurrence of berry abscission noted under these conditions. Vines, treated once after flowering with GA_3 at 10, 15 or 20ppm increased berry resistance to detachment (Celestre, 1964). For navel oranges, treatments with 25 and 100ppm GA_3, followed by 5 ppm of 2,4,5-trichlorophenoxyacetic acid (2,4,5-TP) a month later, resulted in significant increases in the drop of mature fruit (Henz and Leyden, 1961).

Ben-Tal (1990) found that the time of GA_3 application has a greater effect on the rate of berry abscission than the number of GA_3 applications given, or the concentration of GA_3 at each application. Late GA_3 applications (more than ±15 days after fruit set) contributed the most to berry abscission. The use of gibberellic acid can aggravate the incidence of berry abscission to a level of 30 - 40% above that of the untreated grapes (Smit, 1986).

Synthetic auxins are used commercially as both a fruit thinning-agent and to prevent or reduce fruit abscission, thereby displaying opposite effects on fruit abscission at different stages of development (Brown, 1997). These auxins are thought to increase abscission of young fruit by increasing ethylene production (Walsh *et al.*, 1979), while in mature fruit reduce abscission by reducing the sensitivity of the abscission zone to ethylene (Brown, 1997). The auxins alpha-naphthalene acetic acid (alpha-NAA), parachlorophenoxy acetic acid (PCPA), 4-chlorophenoxy acetic acid (4-CPA) and Planofix® (a commercially available form of alpha-NAA) have been reported to reduce postharvest berry abscission in various grape cultivars (Weaver, 1953;

Lavee, 1959; Narasimham *et al.*, 1967; Rao *et al.*, 1968; Daulta *et al.*, 1981; Singh *et al.*, 1985). Preharvest sprays of NAA significantly reduced postharvest berry abscission in 'Cheema Sahebi' grapes (Dass *et al.*, 1974). A mixture of NAA and PCPA also reduced postharvest berry abscission. The synthetic auxin 2,4,5-TP (3ppm) applied at the pea-size stage reduced loose berries, but increased the incidence of berry crack and heat spot significantly (Wagener, 1985). 2,4,5-TP(3ppm) on its own and in combination with 5ppm PCPA, applied as dip treatments one week after the berries had reached pea size, reduced the incidence of loose berries significantly (Steenkamp and Uys, 1982). However, serious scald injury on the grapes was observed with 2,4,5-TP above 5ppm. Results obtained with only PCPA varied considerably (Wagener, 1985). In cherries, fruit drop has been controlled successfully with the amide of NAA (Dirigol-N®), which does not damage leaves and shoots like NAA itself (Schumacher, 1961). Spraying bunches of newly set litchi fruit with concentrations of 2,4,5-TP and NAA ranging from 10-100ppm, progressively reduced the fruit drop (Prasad and Jauhari, 1963). For the litchi cultivar 'Mauritius', fruitlet drop was reduced and consequently yield significantly increased by spraying with 2,4,5-TP (Stern and Gazit, 1997). However, 2,4,5-TP is considered a potential health hazard and causes foliage injury. The synthetic auxin 3,5,6-trichloro-2-pyridyl-oxyacetic acid (3,5,6-TPA) was found to be as effective as 2,4,5-TP in reducing fruitlet abscission, without causing foliage damage. In 'Redchief Delicious' apples, abscission was induced by cutting the fruits in half transversely through the seed cavity (Ward *et al.*, 1999). NAA applied two or four days after cutting, delayed fruit abscission. In citrus, auxins and particularly the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), are used to delay preharvest abscission and to keep the fruit on the tree to delay harvest (Goren, 1993). Early treatment with 2,4-D delayed frost-induced fruit abscission as a result of a significant delay in the rise of cellulase activity at the fruit abscission zone (Zur and Goren, 1977). On the other hand, the synthetic auxin NAA sprayed on mandarin trees after flowering, promoted the abscission of fruitlets by enhancing ethylene production, and thus, cellulase activity at the abscission zone of fruitlets (Iwahori and Oohata, 1976).

The application of exogenous ethylene promotes abscission of a variety of mature and immature fleshy fruit, a response which is exploited in horticulture to manipulate fruit load and to assist mechanical harvest (Kahn *et al.*, 1997; Goren, 1993; Kollàr and Bukovac, 1996). The minimal concentrations of ethylene necessary are normally between 0.1 and 1.0μL^{-1} (Sexton, 1997). The extent of response seems dependent on an interaction between concentration, duration and developmental stage and may vary in different organs on the same plant (Hoyer, 1996). Mechanical harvest of paprika pepper (*Capsicum annuum* L.) produces a mixture of marketable and unmarketable fruit (Kahn *et al.*, 1997). A single application of ethephon (2-chloroethyl

phosphonic acid), an ethylene-releasing chemical, as a controlled abscission agent, removed late-developing flower buds, blooms and green fruit, and consequently, increased the percentage of marketable red fruit in a once-over harvest. To facilitate mechanical harvesting in citrus, the chemicals cycloheximide (CHI) and ethephon are used to promote abscission (Goren, 1993). Abscission agents operate by releasing ethylene either directly by breakdown of the agent, through metabolism of the agent, or by tissue injury (Davies *et al.*, 1976). Unlike ethephon, which releases ethylene immediately on entering the tissue, CHI weakens the peduncle by injuring the fruit peel, causing increased ethylene production (Wilson *et al.*, 1982). Once this ethylene is released, it enhances the synthesis and the activity of hydrolytic enzymes in the abscission zone and reduces FRF (Rasmussen, 1980).

Since leaf abscission zone cells are controlled by a mechanism similar to that of fruit abscission zone cells, any treatment with an ethylene-releasing chemical designed to promote fruit abscission, may cause an undesirable drop of leaves (Goren, 1993). Ethrel at 1000ppm induced mature fruit drop and severe leaf drop in Washington Navel oranges (El-Zeftawi, 1970). After 12 days, 98% of the fruit and 90% of the mature leaves had dropped. Naphthalene acetic acid (NAA) is a chemical that promotes citrus fruit abscission by inducing ethylene evolution, but the concentrations recommended do not induce leaf abscission (Goren, 1993). Ethrel, sprayed at 200-300 ppm, facilitated mechanical harvesting of sour cherry fruit by significantly reducing the fruit removal force (Kollàr and Bukovac, 1996). A shorter shaking time was required during mechanical harvesting, which resulted in a higher quality of machine-harvested fruit and less machine damage to the trees. Ethrel sprayed at these concentrations caused neither significant leaf abscission nor necrosis that would have a negative effect on the production or life span of the trees.

Ethephon has various effects on grapes. In some cultivars it improves fruit color (Blommaert *et al.*, 1975) and enhances ripening (Blommaert *et al.*, 1974). Ethephon is also used to enhance the formation of the berry abscission layer, thus reducing the FRF, thereby improving the efficiency of mechanical grape harvesting (Szyjewicz *et al.*, 1984). Hedberg and Goodwin (1980) found a berry loosening effect seven days after ethephon application, with an increase in loose berries with increasing ethephon concentrations. Complete abscission of grape berries was possible with high concentrations of ethephon. Slight absorption of ethephon took place through the berry cuticle, and the rachis and pedicels seem to be the active site for ethephon absorption (Weaver *et al.*, 1972). Various factors, including cultivar, concentration, timing, method of application, pH, adjuvants, temperature and vine water status influence grapevine responses to ethephon (Szyjewicz *et al.*, 1984).

The herbicides sulfonylurea and imidazolinone, which are widely used to control broadleaf weeds, also promote abscission of citrus fruit (Wilcox and Taylor, 1997). When sprayed on fruiting branches, these chemicals induced fruit abscission of mature oranges but did not affect leaves (Burns *et al.*, 1999). In general, application rates for fruit abscission were at least an order of magnitude lower than those recommended for herbicidal activity. Abscission agents used for citrus fruit removal caused a burst of ethylene evolution from fruit followed by variable fruit drop within 2 to 4 days (Holm and Wilson, 1977). The sulfonylurea metsulfuron-methyl increased internal ethylene production in fruit 2 to 5 days after treatment (Burns *et al.*, 1999). Orange fruit abscission commenced as ethylene production peaked and was 72% 10 days after treatment. High temperatures (average 33°C) markedly suppressed ethylene production and fruit abscission of metsulfuron-methyl-treated fruit. Application of sulfonylureas to stimulate fruit abscission should therefore be avoided during periods of high temperature. Sulfonylurea and imidazolinone compounds, when sprayed for citrus fruit abscission, caused variable peel pitting, although no detrimental effect on internal quality was evident. The greatest potential for these compounds exists for fruit destined for the processing market.

Spraying grape bunches during the flowering period with aminoethoxyvinylglycine (AVG) inhibited flower and berry abscission in 'Kyoho' grapes by suppressing ethylene evolution (Nito, 1985). Kim *et al.* (1999) found that prebloom application of ABA and AVG was effective in the inhibition of berry shattering of 'Kyoho' grapes. In apple fruits where abscission was induced by cutting the fruits in half transversely through the seed cavity, AVG applied two or four days after cutting delayed abscission (Ward *et al.*, 1999). In muskmelon, applications of 260mg.L^{-1} AVG 18 or 12 days prior to harvest, delayed the initial development of an abscission zone (Shellie, 1999).

For many plants silver thiosulphate (STS) has been shown to delay or prevent the negative effect of endo-and exogenous ethylene on fruit quality (Veen, 1983). STS considerably reduced fruit abscission caused by ethylene in *Capsicum annuum* 'Janne'. (Hoyer, 1998). Because STS contains silver, which is considered as a potent environmental pollutant, its commercial use has some environmental restrictions (Serek and Reid, 1993). Alternative strategies have therefore been investigated, including the use of inhibitors of ethylene biosynthesis, and inhibitors of ethylene binding (Serek *et al.*, 1994a), for preventing the undesirable effects of ethylene. A gaseous compound, 1-methylcyclopropene (1-MCP), which inhibits ethylene perception, inhibited a range of plant responses to ethylene, including ethylene-induced ripening and flower abscission (Serek *et al.*, 1994b).

Berry abscission was reduced in grape bunches treated with kinetin (Dhillon *et al.*, 1985). According to Ben-Arie *et al.* (1997), forchlorfenuron (CPPU) is currently the only compound with cytokinin-like activity that can be used on edible crops, due to its low mammalian toxicity. A single post-bloom application to grapes may increase berry size to the same extent as with several applications of GA_3. As with GA_3, fruit ripening and maturation are delayed following CPPU application. Differences between the physiological effects of these two growth regulators are apparent during the grapes postharvest life. Whereas GA_3 increases rachis desiccation and berry abscission, CPPU reduces desiccation and berry abscission on certain grape cultivars due to its thickening effect on the stems and pedicels. Wolf *et al.* (1994) confirmed that CPPU reduces berry abscission, but due to its inhibition effect on colour development in black grape cultivars, it is not recommended for use on cultivars such as 'Dan-ben-Hannah'. A postharvest spray of the synthetic cytokinin, benzyladenine, in combination with urea, reduced berry abscission in 'Waltham Cross' (Smit, 1986).

Stösser and Dinh (1973) found that sprays with calcium chloride reduced abscission in sweet and sour cherry fruit. Abscission was progressively inhibited with increasing concentrations of calcium chloride (Stösser, 1975). Calcium chloride and sodium benzoate reduced post-harvest berry abscission in 'Himrod' table grapes considerably (Singh *et al.*, 1985). Baker *et al.* (1977) reported that sodium benzoate inhibited ethylene production. Calcium nitrate (1%) applied 10 days before harvest, reduced the incidence of berry abscission (Singh *et al.*, 1989). Magnesium chloride was less effective than calcium chloride or calcium nitrate in reducing fruit abscission, and potassium chloride had no effect (Stösser, 1975). The rest-breaking material calcium cyanamide sprayed on 'Waltham Cross' vines two weeks before bud break, reduced berry abscission significantly, but delayed maturation considerably (Wagener, 1985). The fluoride compound NH_4F induced a complete abscission layer in sweet cherry fruit (Stösser and Dinh, 1973).

Smit (1986) reported that Cycocel or CCC, with chlormequat chloride as active ingredient, can reduce abscission during fruitset when sprayed at a pre-flowering stage, as well as berry abscission during cold storage, when sprayed at the pea-size stage. However, CCC sprayed at certain concentrations, suppresses growth excessively, reduces lignification of summer canes and delays ripening in 'Waltham Cross' table grapes. Other detrimental side effects of CCC are berry cracking as well as small and flaccid berries.

2.4.2. Surfactants

Surfactants, often added to a spraying solution primarily to improve wetting and to enhance

penetration of the active ingredient into the plant, may impose distinct stress symptoms in plants (Noga and Bukovac, 1986). Ethylene production was used as a quantitative indicator for surfactant-induced stress. Increased concentrations of Citowett® (an alkylaryl-polyoxyglycolether) and Tween 20® (a polyoxyethylene sorbitan monolaurate) enhanced ethylene production in leaves and fruit of 'Golden Delicious' apples. The application of these surfactants also induced fruit abscission, and a positive correlation was established between surfactant concentration and the extent of fruit drop.

2.4.3. Biotechnology

At present, the prospects of the chemical industry producing new selective synthetic chemicals that will promote citrus fruit abscission without causing peel damage and with minimal leaf drop, are small, since the cost of screening of new chemicals and their registration is very high (Goren, 1993). In addition, due to the current tendency to avoid chemicals in agriculture due to health and environmental reasons, the chemical industry has lost interest in abscission-inducing chemicals. However, an increasing interest is evident to explain the abscission process from the molecular biological point of view. Sexton *et al.* (1980) attempted to define and characterise the cellulase involved in the separation process of 'Red Kidney' beans at molecular level by means of specific antibodies. Using this method, *de novo* synthesis of the enzyme at the abscission zone was shown (del Campillo *et al.*, 1990). The main objective of the molecular study of abscission must be the control of genes of the hydrolytic enzymes, and the development of genetic engineering techniques to help create differences in the sensitivity, and thus in the expression of genes responsible for ethylene-induced synthesis of these enzymes (Goren, 1993). If the sensitivity of the leaf abscission zone to ethylene is reduced, the concentration that promotes *in vivo* biosynthesis of the hydrolytic enzymes in the abscission zone of the fruit may fall below the threshold required for leaf abscission, or vice versa. Consequently, only one of the organs will abscise in response to ethylene, with the other organ remaining unaffected. Another possibility could be to produce transgenic cellulase and PG antisense gene repression plants in which the leaf abscission zone will not respond to ethylene-releasing treatments. This method has been successfully utilized to suppress polygalacturonase gene expression in tomato fruit in order to delay ripening (Smith *et al.*, 1988; Giovannoni *et al.*, 1992).

2.4.4. Fruit characteristics

Hedberg and Goodwin (1980) found that the natural ease of grape berry removal differs between grape cultivars. This may be explained by the ratio of berry weight to berry/pedicel contact area. Cultivars with a high berry removal force have relatively low berry weight to

berry/pedicel contact area ratios. Microscopic examination of the tissues at the junction of the pedicels and berries showed differences in anatomy between 'Dulcet', a non-abscising variety, and 'Thomas', an abscising variety (Sherman and Nevins, 1963). Differentiation of abscission cells in the vascular tissue connecting the pedicel and berry was found in 'Thomas' but not in 'Dulcet'. Gersch *et al.* (1998) examined eight genotypes of cayenne pepper (*Capsicum annuum*) and identified two genotypes that differed significantly in the ease of fruit detachment. Mature fruits of the genotype that did not separate exhibited a distinct region of sclerified cells, which extend from the periphery of the fruit into the receptable for 25-30 cell layers. By contrast, mature fruits of the more readily detachable genotype had 10-15 layers of sclerified cells at the region of detachment. It was concluded that the presence of more sclerified cells and increased lignification in the less detachable genotype probably contributed to the differences in ease of detachment.

Fruits with more seeds, which are sources of auxins, are less likely to abscise (Edgarton and Greenhalgh, 1969). Grape berries with three or more seeds do not abscise as easily as those with less seeds (Beyers, 1936).

A cluster-dipping experiment with the abscission-promoting compounds ethrel, abscisic acid and a morphactin was done on 'Thompson Seedless' at four developmental stages, *viz* prebloom, bloom, fruit-set and about two weeks after fruit-set (Weaver and Pool, 1969). The compounds were most effective in promoting abscission when applied during the prebloom, bloom and fruit-set stages. However, with post fruit-set application abscission did not usually occur.

Hedberg and Goodwin (1980) found that natural berry abscission varied with maturity. The incidence of loose berries was measured weekly at about 8:00 am, over an eight week period near harvest. For the cultivars 'Shiraz' and 'Cabernet Sauvignon', the incidence of berry abscission generally increased during the first four weeks of measurement, where after it declined. The reduction in natural abscission occurred after maximum berry weight was reached.

In a study done by Wolf (1991), the level of berry ripeness was a major factor in stimulating berry abscission. 'Waltham Cross' berries with ripeness levels below 17°Brix were found to be more prone to berry abscission than berries with higher ripeness levels. By contrast, Nelson (1979) found that berry abscission is aggravated by advanced maturity.

2.4.5. Pre-harvest factors

The premature fall of navel oranges was attributed to abnormal water relations within the plant, resulting in cell wall changes in the abscission zone, and finally in complete separation of cells (Coit and Hodgson, 1918). Although Barnell (1939) could not correlate changes in water relations and separation in avocado and mango fruits, he noted that the nature of the abscission zones in these fruits might be exceptional in the insignificant development of tyloses in the xylem tissue. He concluded that the presence of tyloses might limit water supply and therefore affect separation.

Berry abscission is aggravated by any factor giving rise to moisture stress in vines during berry development (Uys, 1980). In general, vineyards that suffer from water stress due to drought or incorrect irrigation, are more prone to both pre- and post harvest berry abscission. Uys (1980) reported an increase of up to 50% in berry abscission when vines experience severe water stress. However, bunches from drought stressed vines showed a significantly higher degree of ripeness than bunches from non-stressed vines (Wolf, 1991). Due to the higher ripeness levels, bunches from the drought stressed vines had a lower occurrence of berry abscission than those from the non-stressed vines. Thus, the level of ripeness appeared to be the dominant factor influencing berry abscission.

Water stress in vines can also be induced by over-cropping and excess vigour, due to the excessive demand for assimilates and water (Uys, 1980). Thus, bunches harvested from over-cropped vines and/or vines with excessive vegetative growth, are very susceptible to both pre- and post harvest berry abscission. Warm weather and hot, dry winds can also cause insufficient moisture to supply all the vine's evaporation needs. This can result in a certain measure of water stress that can aggravates berry abscission (Wolf, 1991).

Hegazi and El-Barkoki (1994) found that 'Flame Seedless' grapes grown in sandy soil, on newly reclaimed desert land, had lower rates of berry abscission than grapes of the same cultivar grown in a clay soil on Delta old land. The suitable environmental conditions of climate and soil in the desert land produced grapes of better overall quality after storage.

To test the relationship between berry abscission and ambient temperature, bunches of 'Semillon' grapes at a ripeness level of 22°Brix were given a standard shake test at different times during a day (Hedberg and Goodwin,1980). A negative correlation between berry abscission and temperature was evident. Plant water potential follows a diurnal cycle causing variations in berry turgidity. Hedberg and Goodwin (1980) suggest that grape berries may be

more prone to berry abscission during the cooler hours of the day because the berries are more turgid than at higher temperatures.

Fruit abscission increases when plants are subjected to heat and water stress, conditions which increase ethylene production and reduce auxin transport (Ofir *et al.*, 1993). The bulk of fruit on a plant sometimes abscise almost simultaneously in response to change in daylength or temperature (Baird and Webster, 1979).

Both early girdling at flowering and later girdling at about 4 weeks after flowering control berry abscission (Smit, 1986). For the cultivar 'Waltham Cross', late girdling is more beneficial, because it suppresses growth less, and it advances maturation. In more vigorous cultivars susceptible to berry abscission, such as 'Dan-ben-Hannah' and 'Thompson Seedless', girdling closer to flowering may have greater advantages. Wolf *et al.* (1991) found that girdling, applied when 50% of the berries were 4-5mm in diameter, reduced the incidence of berry abscission in 'Thompson Seedless' table grapes.

2.4.6. Post-harvest factors

Extremely high temperatures (30°C and higher) during harvest, increases the amount of postharvest berry abscission (Wolf,1991). Beyers (1936) found that grapes harvested at a low temperature early in the morning, developed less berry abscission than grapes harvested at higher temperatures in the afternoon. Berry abscission also occurs when grapes are harvested under warm, dry conditions and delayed for 12 hours or longer before cold storage (Wagener, 1985).

Water stress can induce increases in ethylene production in plant tissues (Berry and Aked, 1996). Therefore, it was suggested that a reduction in dehydration during the storage of grapes by using modified atmosphere packaging (MAP), can possibly reduce ethylene production and thus berry abscission. MAP reduced weight loss, and MAP with the ethylene oxidant $KMnO_4$ reduced berry abscission (Yun and Lee, 1996a). Ge *et al.* (1997) found that the SO_2 treatment of table grapes reduced berry abscission by reducing the abscisic acid content and the release of ethylene from the grapes, and by increasing the levels of IAA and GA. MAP combined with a SO_2 treatment, or MAP combined with $KMnO_4$ and a SO_2 treatment, only reduced berry abscission slightly (Yun and Lee, 1996a).

To determine post-storage quality, 'Perlette' grapes were packed in polyethylene bags with 0.56, 0.84, 1.12, 1.40 and 1.68% perforations (Sandhu *et al.*, 1990). With the lowest perforation

(0.56%), minimum berry abscission was observed, while maximum berry abscission was recorded with the highest perforation (1.68%). An increase of 1% in the intensity of perforation resulted in an increase of about 0.48% in berry abscission. Contradicting this, grapes stored in non-perforated polyethylene liners had more berry abscission than when perforated liners were used (Landania and Dhillon, 1987).

Cooper *et al.* (1993) reported that berry abscission did not increase with the extension of the cold storage period at 0°C up to 45 days. By prolonging the storage period at ambient temperature (shelf-life period), additional berry abscission is induced (Hegazi and El-Barkoki, 1994; Berry and Aked, 1996). Storage of grape bunches at 4°C greatly reduced berry abscission, compared with storage at 25°C (Wu *et al.*, 1992) When grapes were stored at 0°C in the presence of an ethylene scrubber, the rate of fruit abscission was reduced significantly (Yun *et al.*, 1995).

Fruit abscission in sour cherry explants was influenced by temperature, light, O_2 and CO_2 (Wittenbach and Bukovac, 1973). An increase in temperature from 15 to 35°C caused a dramatic decrease (50%) in FRF. Abscission was delayed by light. After an 80 hr treatment, the FRF of explants held in light was 50% higher than for those kept in dark. A low O_2 as well as a high CO_2 environment significantly delayed abscission. CO_2 probably acted as a competitive inhibitor of ethylene action (Burg and Burg, 1967). 'Waltham Cross' grapes stored under controlled atmospheric (CA) conditions of 21%O_2 and 5%CO_2 developed a significantly higher percentage berry abscission, compared with CA conditions of 1%O_2 and 0%CO_2 or 2%O_2 and 1%CO_2 (Làszlò, 1985).

Post-harvest irradiation of grape bunches with x-rays, gamma rays or microwaves could not reduce berry abscission significantly (Wagener, 1985). Gamma rays at a dosage of 10-20 kilorad could reduce berry abscission, induced by gibberellic acid, in cold-stored 'Waltham Cross' grapes by 50% (Smit, 1986). Low intensity microwaves could also reduce the occurrence of berry abscission. It is surmised that irradiation directly or indirectly inactivates specific enzymes that are involved in the separation layer, and thus, in berry abscission, e.g. enzymes which dissolve the intercellular compounds of pectins, cellulose or hemicellulose.

2.5. CONCLUSION

The most significant component of fruit abscission may be the ripening stage of the fruit itself, which may influence the modulation of receptor levels of the different plant hormones, and thereby the sensitivity of abscission zones to these hormones. Morphological and biochemical

studies as well as biotechnology have greatly increased our knowledge on the physiological events that precede the separation process. It is likely that, by application of molecular techniques, even more will be revealed regarding the enzymes involved in abscission, their localization, and their regulation by plant hormones. Further knowledge will also be gained about the regulation of ethylene and auxin synthesis, and sensitivity in abscission zones.

Primarily, the problem of berry abscission is genetic of nature. It is induced or aggravated not by a single factor, but is the result of interactions between various factors. Therefore, the problem can not be solved by rectifying a single factor and disregarding the other contributory factors. A vast amount of literature is available regarding the abscission process and the enzymes and plant hormones involved in the separation of fruit, although very little work has been done on table grapes specifically. Furthermore, only a small amount of information exists regarding the influence of postharvest factors on the incidence of berry abscission.

Despite the earliest efforts to control berry abscission through cultural practices and the use of growth regulators, there is no guaranteed strategy to eliminate this disorder, except by the breeding and selection of cultivars resistant to this problem. It seems as if there are no detailed studies available, for table grapes specifically, which describe procedures for utilisation of such resistance in a breeding program. From a technical or scientific point of view, the development of biotechnology shows great potential. Mutant and transgenic plants with altered synthesis of, and responses to, plant hormones, may possibly enable reduction or elimination of berry abscission. However, the main obstruction to the potential of biotechnology are issues of bio-safety, ethics, the environment and public perceptions.

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PAPER I - The influence of harvest maturity, harvest temperature and perforated liners on the quality of cold stored 'Thompson Seedless' table grapes, with special reference to berry split and berry abscission.

THE INFLUENCE OF HARVEST MATURITY, HARVEST TEMPERATURE AND PERFORATED LINERS ON THE QUALITY OF COLD STORED 'THOMPSON SEEDLESS' TABLE GRAPES, WITH SPECIAL REFERENCE TO BERRY SPLIT AND BERRY ABSCISSION.

ABSTRACT

The effect of harvest maturity, harvest temperature and type of liner on the quality of 'Thompson Seedless' table grapes after storage at -0.5°C for 5 weeks and a subsequent shelf-life of one week at 10°C, was investigated. Berry split decreased significantly for grapes harvested at more advanced maturities. However, grapes harvested too mature showed an increase in stem desiccation and the development of Botrytis *decay. The incidence of berry abscission also appeared to increase with increased harvest maturity. The harvesting and packing of grapes at high pulp temperatures aggravated berry split significantly, especially for grapes packed in non-perforated bags. A reduction in berry firmness and an increase in moisture loss was also evident for grapes harvested at high temperatures, compared to grapes harvested at lower temperatures. Perforated bags significantly reduced berry split by between 80 to 90 percent, compared to grapes packed in non-perforated bags. The levels of SO_2 damage were also reduced significantly by perforated bags. The amount of moisture loss from grapes in perforated bags was significantly higher than from grapes in non-perforated bags. During the 1999 season, this resulted in a significant reduction in berry firmness and a significant increase in stem desiccation. Grapes harvested at low temperatures and packed in perforated bags showed significantly higher levels of* Botrytis *decay during the 1999 season. The influence of harvest temperature and liner type on berry abscission was only evident during one season, and is therefore not conclusive. The commercial use of perforated bags to reduce berry split shows immense potential. Optimum perforation size and density needs to be determined to reduce berry split and SO_2 damage, but without increasing the levels of* Botrytis *decay and moisture loss, which would lead to more flaccid berries and increased stem desiccation.*

KEYWORDS : 'Thompson Seedless', berry split, berry abscission, harvest maturity, harvest temperature, type of liner, grape

INTRODUCTION

Vitis vinifera L. cv. 'Sultanina' (Thompson Seedless) is one of the most important table grape

varieties in South Africa. It comprised approximately 20% of the total volume of 38.9 million cartons exported from South Africa by various exporters during the 1999 season. Postharvest cracking or splitting and abscission of 'Sultanina' berries are serious quality disorders and are responsible for considerable financial losses. Of the 28 South African table grape cultivars exported by Capespan (Pty) Ltd. during the 1999 season, 'Thompson Seedless' showed the highest incidence of berry split and the second highest incidence of berry abscission.

In most of the table grape production areas of South Africa, excessively high temperatures prevail during the harvesting period. This results in grape berries with pulp temperatures of 30°C and higher, especially when harvested later in the day. Some producers harvest throughout the day, but make use of pre-coolers to reduce the field heat of grape berries from ambient to approximately 18°C before packing. Other producers, with or without pre-cooler facilities, harvest only during the early morning hours while the pulp temperatures are lower than approximately 25°C. There are also table grape producers, especially in the Western Cape, who harvest throughout the day but have no facilities to reduce excessively high field heat temperatures of the grapes before packing. In this trial, the quality of grapes harvested in the morning at low temperatures, was compared to grapes harvested in the afternoon at high temperatures, without field heat removal prior to packing in both instances. Quality was assessed after cold storage, with special reference to berry split and berry abscission.

The type of packaging material used plays a vital role in the maintenance of the postharvest quality of perishable products. Typically, export grapes are packed in 4.5kg closed-top corrugated fibre-board cartons, with individual bunches in plastic or polycote carrybags, and the entire carton content enclosed in a non-perforated 20µm polyethylene liner. An SO_2 generator to control decay is also included inside the liner on top of the grapes. The use of non-perforated liners has many advantages (Combrink *et al.*, 1978). For example, moisture loss is minimised by using non-perforated bags, and subsequently, stem desiccation and berry flaccidity is prevented. In addition, *Botrytis* decay can be controlled effectively because sufficient levels of SO_2 are contained within the bag (László *et al.*, 1981). Quality problems associated with the use of non-perforated bags include berry split and SO_2 damage. Furthermore, the impermeable bag prevents cold air from coming into direct contact with the grapes and therefore the forced-air cooling process is slow, which reduces cold room throughput.

In an attempt to alleviate quality disorders which occur when non-perforated bags are used for table grapes, and simultaneously improve temperature management, it was decided to evaluate the use of perforated bags for improving the quality of 'Sultanina' table grapes. According to

Mitchell (1978), perforations in polyethylene liners influence the vapour pressure differences between the inside of the berry and the surrounding atmosphere, and hence, affect moisture loss from the berries. The number and size of perforations in the liners also influence gas concentrations of various gases such as carbon dioxide, oxygen and, to a lesser extent, ethylene which are either produced or consumed by the grapes (Rooney, 1995). The type of liner also influences how sulphur dioxide is released from the generator which in turn impacts on decay control efficacy and the damage potential. In this study, the effect of the type of liner employed was assessed by conducting comprehensive examinations after a five week cold storage period, since this is the duration typically used for produce exported from South Africa.

In table grape research, contradictory literature exists regarding the influence of harvest maturity on the incidence of berry split and berry abscission. Nelson (1979) found that berry abscission is aggravated by advanced maturity. By contrast, 'Waltham Cross' berries with ripeness levels below 17°Brix were found to be more prone to berry abscission than berries with higher ripeness levels (Wolf, 1991). Several studies on berry splitting susceptibility concluded that the osmotic concentration of the fruit soluble solids, and therefore fruit maturity, is a major factor controlling water absorption through the berry skin and, hence, berry split (De Villiers, 1926; Verner and Blodgett, 1931; Meynhardt, 1964). By contrast, Tucker (1934), Kertesz and Nebel (1935) and Andersen and Richardson (1982) found no or very weak correlations between the sugar content or osmotic potential of fruit and its tendency to split. To clarify discrepancies concerning the effect of harvest maturity on berry split and berry abscission in 'Thompson Seedless' table grapes, this study also examined the effect of harvest maturity on overall quality.

MATERIALS AND METHODS

Experiment 1 : Harvest temperature and type of liner

Trials were conducted in 1998 and 1999 in a 'Thompson Seedless' vineyard in the Wellington area in the Western Cape, South Africa. The trial block was planted in 1986, on Ramsey rootstock. The vines were trained onto a slanting trellis system with a 1.8m in-row and a 3.6m between-row planting distance and irrigated by means of a drip irrigation system. The recommended gibberellic acid (GA_3) sprays for 'Thompson Seedless' were applied (Wolf *et al.*, 1991). This comprised three sprays of 5ppm during flowering and three sprays of 20ppm when the berries were between 4 and 8mm in diameter. The vines were girdled when the berries reached the pea-size stage. Normal cultural practices were followed as far as irrigation,

fertilisation, cluster preparation, pest and disease control and foliage management were concerned.

In 1998, the 'Thompson Seedless' grapes were harvested with total soluble solids (TSS) of 16.7°Brix and titratable acid level (TA) of 0.65%. Differences in berry temperature were obtained by sampling in the morning and in the afternoon. The ambient temperature in the morning was 25.6°C, the relative humidity (RH) was 61% and the pulp temperature of the grape berries was 24.5°C. In the afternoon, the ambient temperature was 28.4°C, with a RH of 61% and a pulp temperature of 29.4°C.

In 1999, the grapes sampled had a TSS of 14.7°Brix and a TA of 0.86%. Grapes were sampled in the morning at an ambient temperature of 19.0°C, an RH of 72% and berry pulp temperatures of 16.9°C. The afternoon harvest was conducted at an ambient temperature of 33.5°C, a RH of 24.5% and berry pulp temperatures of 30.0°C.

For measurement of TSS and TA, 50 randomly selected berries from five randomly selected grape bunches were used. The berries were juiced in a liquidiser and filtered. The percentage soluble solids of the filtrate, expressed as degree-Brix, was measured using a bench top Atago Palette PR100 digital refractometer with automated temperature compensation. Because the acid fraction of the total soluble solids is usually very small compared with the sugar fraction, the soluble solids were considered as the sugar content. Titratable acidity, expressed as percentage tartaric acid, was determined by titrating a 10g aliquot of juice with 0.1N NaOH to a pH end-point of 7, using an auto Metrohm 665 Dosimat titrator. Pulp and ambient temperatures were recorded with a probe connected to a Kane-May 22 digital thermometer, while a whirling hygrometer was used to determine the wet and dry bulb temperatures. Relative humidity was then read from a psychrometric chart.

Thirty-two cartons of grapes were sampled at each harvest stage. Of these, half were packed in macro perforated high density polyethylene liners, 20µm thick, with a perforation density of 0.39% made up of randomly distributed holes of 5mm diameter. The other half of the grapes were packed in non-perforated low density polyethylene liners with a thickness of 20µm. The grapes inside the liners were packed in 4.5kg closed-top cartons. A 'Uvasys' sulphur dioxide (SO_2) generator sheet (supplied by Grapetek, South Africa) was enclosed in each of the polyliners, positioned on top of the grapes. Polyethylene liners containing the grapes and the 'Uvasys' SO_2 sheet were folded, closed with gum tape and placed under passive cooling within 3 hours of packing. The grape cartons were packed four layers high on a pallet and stored at

-0.5°C and a RH of 85-90% for five weeks, simulating transport by ship and accumulation overseas prior to sale. Fruit quality was assessed at ambient temperature (±24°C) immediately after this five week cold storage period, and again after a simulated shelf life of 7 days at 10°C. Polyliners were kept closed during shelf life. Grapes were examined for the quality defects berry split, berry abscission, *Botrytis* decay and SO_2 damage. All of the grapes in each carton were examined. The incidence of each defect was expressed as a percentage of the total grape mass per carton. Moisture loss was also determined per replicate, by labelling and weighing one grape bunch from each carton at harvest. In 1998, the labelled bunches were weighed after the five week cold storage period as well as after an additional week at 10°C. In 1999, the bunches were only weighed at the end of the simulated shelf life. It was assumed that the loss in fresh mass during storage was mainly due to moisture loss during transpiration. Moisture loss was expressed as a percentage of the initial fresh mass measured at harvest. All the grape bunches per carton were rated for stem condition on a five-point scale, where 1 = fresh, green stems and 5 = dry, brown stems. On this scale, a rating above 3.5 is deemed unacceptable.

Berry skin strength (only measured during 1999 season) and berry firmness were also assessed at both examination stages. The berry firmness and skin strength were measured on 20 berries randomly selected per carton. To obtain an accurate, repeatable reading for fruit firmness that corresponds with the force applied when pressing a grape berry with the fingers, the TA-XT2 texture analyzer, manufactured by Stable Micro Systems Ltd, England, was used. The TA-XT2 was pre-set to apply a compression force on the berry with a cylinder probe 2mm in diameter. The force, in Newton's, necessary to indent the grape berry a distance of 1mm was measured. It was assumed that this distance simulates the average distance that a berry will be compressed by the fingers of a consumer. The force required to penetrate the skin of the berry was also determined, and was considered to be an indication of the berry's skin strength. The entire measuring process was computer controlled to ensure a constant movement of the probe and improve the repeatability of the readings.

The experimental design was completely randomised, and the treatment design was a 2 x 2 factorial with harvest temperature and polyliner type as factors. Each treatment was replicated eight times, with one carton constituting a single replicate. Therefore, for each treatment combination, eight replicates were examined at the end of cold storage, and another eight were examined after shelf life. Data were subjected to an analysis of variance using the General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

Experiment 2 : Harvest maturity

The experiments were carried out in two 'Thompson Seedless' vineyards, namely, in the Wellington area in 1999, and in the Saron area in 2000. The Wellington trial block was planted in 1986, on Ramsey rootstock, at a spacing of 1.8 x 3.6m, and trained onto a slanting trellis system. The Saron trial block was planted in 1989, on Richter 99 rootstock, with a 2 x 3m spacing onto a double gable system. Both vineyards were irrigated by means of a drip irrigation system. The commercially recommended gibberellic acid (GA_3) sprays were applied (Wolf *et al.*, 1991), and the vines were girdled when the berries were between 5 and 6mm in diameter. Standard cultural practices were followed regarding irrigation, fertilisation, cluster preparation, pest and disease control and foliage management.

Grapes were harvested at four different maturities after horticultural maturity, as defined by Watada *et al.* (1984), was reached, with approximately weekly intervals between sampling dates. Samples of 100 berries per replicate were randomly collected at each harvest maturity for measurements of TSS, TA and berry skin colour, the latter which was only measured in the 1999 season. The TSS and TA was determined as described for experiment 1. Berry skin colour was measured using a Nippon NR3000 colorimeter. For each replicate, measurements were taken of five berries and the mean value was determined. Colour was expressed in terms of hue angle (h°), L*-value and chroma (C*). The colour space coordinates a* and b* were used to calculate the h° and C* (colour intensity) values. Hue angle refers to the angle formed by the line from the origin to the intercept of the a* (x-axis) and b* (y-axis) co-ordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992). L* is a measurement of the lightness of the fruit and its value may range from black = 0 to white = 100.

The grapes sampled on each harvest date were packed in non-perforated low density polyethylene liners 20µm thick inside 4.5kg closed-top cartons. The 'Uvasys' SO_2 sheet was enclosed in each of the polyliners, on top of the grapes. Polyethylene bags containing grapes and the 'Uvasys' SO_2 sheet were folded, closed with gum tape and placed under cold storage within 3 hours after packing. The grapes were cold stored at -0.5°C and 85-90% RH for 5 weeks. Fruit quality was assessed at ambient temperature (±24°C) after this storage period (only in the 1999 season), and after a further simulated shelf life period of 7 days at 10°C. The polyliners were kept closed during shelf life. Grape quality was assessed in terms of berry split, berry abscission, stem condition and *Botrytis* decay, while moisture loss and berry firmness were also quantified. All the grape bunches per carton were examined. The incidence of berry split and berry abscission was expressed as a percentage of the cartons total grape mass.

Stem condition was rated on a five-point scale, where 1 = fresh, green stems and 5 = dry, brown stems.

The experimental design was a randomised complete block, with each block replicated six times. Each individual plot consisted of four vines. Experimental vines were selected on the basis of vigour, uniformity of vegetative growth and cluster development. One way ANOVA was used to analyse the data with the General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990). Six individual carton replicates were assessed after five weeks of cold storage (only in the 1999 season), and another six after the shelf life period for each harvest maturity.

RESULTS AND DISCUSSION

Experiment 1 : Harvest temperature and type of liner

In 1998, the interaction between harvest temperature and type of liner was not significant ($P>0.05$) for any of the quality parameters measured after storage (Table 1). Therefore, data were pooled across harvest temperatures and liner types. After five weeks storage at -0.5°C, moisture loss and berry firmness were significantly influenced by temperature at harvest. Grapes harvested at high pulp temperatures in the afternoon showed a significant increase in moisture loss and a significant decrease in berry firmness, compared to grapes harvested at lower temperatures in the morning. By contrast, berry split, berry abscission, stem condition, SO_2 damage and *Botrytis* decay were not significantly ($P>0.05$) influenced by temperature at harvest. Where perforated polyliners were used, moisture loss was significantly greater than in the case of non-perforated bags, but levels of berry split were reduced significantly, from 2.12% in non-perforated bags to 0.44% in perforated bags. Although the incidence of berry split was relatively low compared to commercial experience where the occurrence of berry split can exceed 30% of the total grape mass per carton, a reduction of 79.2% in berry split by using a perforated bag can be of great value for vineyards with a higher susceptibility to berry split. Despite higher moisture loss in perforated bags, berry firmness and stem condition were comparable to grapes packed in non-perforated bags. Berry abscission and levels of SO_2 damage and *Botrytis* decay were not influenced significantly ($P>0.05$) by the type of liner.

The significantly higher level of moisture loss from grapes harvested in the afternoon, with berry pulp temperatures of 29.4°C, as opposed to 24.5°C for grapes harvested in the morning (Table

1), can be ascribed to higher transpiration rates at higher temperatures. Transpiration refers to internal water loss through stomata, cuticle or lenticels (Salisbury and Ross, 1985). The driving force for transpiration is the difference in water vapour pressure within the fruit and in the atmosphere beyond the boundary layer that surrounds the fruit (Mitchell, 1978). Vapour pressure of water increases rapidly with temperature, so that at 30°C in saturated air, the vapour pressure of water is 4.3kPa, and at 20°C the vapour pressure is less than 2.4kPa. Due to higher pulp temperatures of grapes harvested in the afternoon, these grapes would have exhibited a higher vapour pressure, compared to grapes harvested in the morning at lower pulp temperatures. Therefore, at the onset of cold storage, a higher vapour-pressure difference existed between grape bunches harvested in the afternoon and the air that enclosed the grape bunches beyond the boundary layer inside the liners, compared to grapes harvested in the morning with a lower vapour pressure. This would result in more moisture loss of grapes harvested at high temperatures. These grapes were also significantly less firm, compared to grapes harvested in the morning. Turgor contributes to berry firmness, thus changes in the water status of the berry can change berry firmness (Uys, 1997). According to Nelson (1979), any decrease in firmness of grape tissue is due to flaccidity from water loss, as there is slight, if any, hydrolysis of the intercellular pectic compounds during storage. Although differences in berry firmness could have been evident already at harvest, it is suggested that the significantly higher moisture loss of grapes harvested in the afternoon resulted in a further reduction in berry firmness.

The higher amount of moisture loss from produce in perforated liners compared to non-perforated liners, can be attributed to extended periods of a water vapour gradient between the grapes and the atmosphere that surrounds the berries, before an equilibrium in relative humidity is reached. At the onset of cooling, grapes lost moisture by means of transpiration, as previously discussed. In the perforated liner, moist air could diffuse through the perforations to the drier air outside the liner, due to vapour pressure differences between the inside and outside of the liner. Therefore, more moisture could evaporate from the grapes to the surrounding air beyond the boundary layer before an equilibrium relative humidity would have been reached. Although no significant difference in stem desiccation or berry firmness could be detected between grapes in perforated bags and grapes in non-perforated bags, water loss equates to loss of saleable weight, and thus constitutes a direct loss in income.

Perforated bags reduced berry split significantly probably because the stage of reaching dewpoint was postponed or prevented. During the cooling process, the moist air that surrounds the grapes beyond the boundary layer is cooled, and dew point is reached when the air

becomes saturated with water vapour, i.e. RH = 100%. With further cooling, water starts to condense on the inner surface of the liner which is in contact with the grape bunches, therefore free moisture becomes present on the fruit surface. Due to solutes (i.e. sugars) present in the grape berries and because of moisture loss at the onset of cooling, the grape berries exhibit a lower water potential than the water on the fruit surface. Thus, water diffuses into the berries in response to this water potential gradient, from the boundary layer, through the cuticle and cell membranes into the epidermal cells. An increased turgor pressure develops. According to Considine and Kriedemann (1972), Wade (1988) and Sekse (1995), turgor pressure plays an important role in fruit splitting. Osmotic entry of excessive water by the epidermal cells causes rupture of the cells. In the case of perforated liners, moist air could diffuse through the perforations so that the stage of reaching dewpoint could be postponed or prevented. Thus, no or less water presumably condensed inside the liner that could wet the fruit surface. Consequently, less water was available for excessive osmotic absorption by the fruit skin which could result in berry split.

After five weeks storage at -0.5°C followed by a week at 10°C, grapes harvested at a high temperature were significantly less firm and showed significantly higher berry split and significantly lower berry abscission than grapes harvested in the morning at a lower temperature (Table 1). Although moisture loss was influenced significantly by harvest temperature during the five weeks storage at -0.5°C, no influence was evident after the simulated shelf life. Stem condition, SO_2 damage and *Botrytis* decay were not influenced by temperature at harvest. Where perforated polyliners were used, moisture loss was significantly higher than in the case of non-perforated bags, but levels of berry split and berry abscission were reduced significantly in perforated bags, compared to non-perforated bags. Despite the higher moisture loss from grapes in perforated bags, berry firmness and stem condition were comparable to grapes packed in non-perforated bags. The levels of SO_2 damage and *Botrytis* decay were not influenced significantly ($P>0.05$) by the type of liner.

The significant difference in berry firmness between grapes harvested in the morning and grapes harvested in the afternoon, that was evident after the simulated shelf life period, can possibly be attributed to moisture loss, although a reduction in firmness is not only a function of moisture loss. Although it was only evident after five weeks storage at -0.5°C, grapes harvested at a high temperature showed a significantly higher amount of moisture loss than grapes harvested at a low temperature. According to Nelson (1979), a decrease in firmness of grape tissue is due to flaccidity from water loss. The aggravation of berry split for grapes with high temperatures at harvest, can also be elucidated in terms of the quantity of moisture loss. Due to

higher levels of moisture loss from grapes harvested in the afternoon, more moisture would be available to condense during the cooling process, as the atmosphere which surrounds the grapes becomes saturated with moist air. Also, along with moisture loss, the osmotic potential in plant cells becomes more negative, thus lowering the water potential (Wills *et al.*, 1998). Thus, for grapes harvested in the afternoon the water potential would have decreased to lower values, and the water potential gradient between the grapes and the condensed water on the fruit surface would have been higher than for grapes harvested in the morning with less moisture loss. Therefore, a higher rate of water absorption could have occurred for grapes harvested in the afternoon, which could be responsible for the higher incidence of berry split. The significantly higher incidence of berry abscission that was evident after the shelf life period for grapes harvested in the morning at low temperatures, is difficult to explain. It is in contrast with results obtained by Beyers (1936) where grapes harvested at a low temperature in the morning developed less berry abscission than grapes harvested at higher temperatures in the afternoon.

The effect of perforated liners on moisture loss and berry split, that manifested after the shelf life period, has previously been explained. The significant reduction in berry abscission by the perforated bag can possibly be explained in terms of ethylene concentration inside the polyethylene bag. Ethylene production of harvested grapes exists, and the production rate of the cluster stalk, including most of the pedicel, can even show climacteric-like changes (Wu *et al.*, 1992). Strong evidence also exists that ethylene accelerates fruit abscission (Burg, 1968; Berry and Aked, 1996). Therefore, during the shelf life period, a higher concentration of ethylene could possibly have been present in the non-perforated liner that stimulated berry abscission. By contrast, in perforated bags, the ethylene produced would have been lost to the atmosphere with less effect on the product. In addition, perforated bags resulted in significantly less berry split, therefore less injury which could result in a higher production of ethylene. Landania and Dhillon (1987) also found more berry abscission when non-perforated liners were used, compared to perforated liners. However, Sandhu *et al.* (1990) found that an increase of 1% in the intensity of perforation resulted in an increase of about 0.48% in berry abscission. Therefore, it seems as if the intensity and size of the perforations are critical.

Irrespective of the treatment, grapes examined after storage at -0.5°C, showed a higher incidence of berry abscission compared to grapes examined after shelf life (Table 1). The change in temperature from -0.5°C to approximately 24°C inside the examination room was rapid in the case of the examination at the end of cold storage. For the examination at the end of shelf life, there was a less drastic change in temperature, namely from -0.5°C to 10°C at the

beginning of shelf life, with a further increase to 24°C during the examination at the end of shelf life. It is possible that the rapid temperature increase was responsible for more berry abscission than the more gradual increase in temperature. Irrespective of the treatment, *Botrytis* decay increased during the simulated shelf-life period. For grapes harvested in the afternoon or grapes packed in non-perforated bags, the incidence of berry split more than doubled during the simulated shelf-life period. By contrast, for grapes harvested in the morning or grapes packed in perforated bags, berry split only increased slightly during the seven days at 10°C.

In 1999, after the grapes had been stored for five weeks at -0.5°C, a significant interaction between harvest temperature and type of liner occurred regarding the incidence of berry split (Table 2). In general, the incidence of berry split was relatively low, compared to the commercial experience in certain vineyards. However, grapes harvested in the afternoon and packed in non-perforated liners had significantly higher levels of berry split than any of the other treatments. Harvest temperature had no significant influence on any of the other quality parameters measured after five weeks cold storage. Perforated liners resulted in significantly more stem desiccation and significantly less SO_2 damage. Despite the statistical differences between liner types in SO_2 damage, the values were only 0.64% and 0.02%, which are lower than what is experienced commercially. Such a small difference, even if statistically significant, would not influence the decision on the use of perforated versus non-perforated liners. Furthermore, the type of liner did not influence berry firmness, berry skin strength, berry abscission or *Botrytis* decay significantly.

The aggravation of berry split by high harvest temperatures and by packing grapes in non-perforated liners was consistent with the 1998 findings. Therefore, in 1999, it was to be expected that grapes harvested at high temperatures and packed in non-perforated bags would exhibit the highest incidence of berry split, compared to the other treatments. Perforated liners reduced the incidence of berry split significantly for grapes harvested at high temperatures, when compared to non-perforated liners. Thus, the negative effect of high temperatures on berry split was minimised by the use of perforated liners, while non-perforated liners performed satisfactorily on grapes harvested at low temperatures. As was evident during the 1998 season, grapes in perforated liners showed higher levels of moisture loss than grapes in non-perforated liners (Table 2). This explains why grapes in perforated liners had significantly more stem desiccation after five weeks storage at -0.5°C. The lower levels of SO_2 damage on grapes packed in perforated liners can be ascribed to the lower SO_2 concentration inside the polyethylene liner. In the perforated bag, SO_2 gas would have diffused from a high

concentration inside the bag, through the perforations, to a lower concentration outside the bag. Therefore, concentrations of SO_2 inside the perforated bag would be lower than in the non-perforated bag, which would result in lower levels of SO_2 damage in the former. The amount of SO_2 damage is also influenced by moisture levels in the bags (Nelson, 1983; Peiser and Yang, 1985). Increased vapour pressure of water causes accelerated breakdown of $NaHSO_3$, and this gas can cause excessive bleaching of the grapes. Therefore, as previously explained, non-perforated liners could lead to a higher build up of moisture, which could increase the levels of SO_2 damage.

After the grapes had been stored for five weeks at -0.5°C followed by an additional week at 10°C, significant interaction occurred between harvest temperature and type of liner for moisture loss and the incidence of *Botrytis* decay (Table 2). Grapes harvested in the afternoon and packed in perforated liners had significantly higher levels of moisture loss than any of the other treatments. In general, grapes packed in perforated liners lost significantly more moisture than grapes packed in non-perforated liners, irrespective of the harvest temperature. The incidence of *Botrytis* decay was significantly higher for grapes harvested in the morning and packed in a perforated liner, compared to the other treatments. Grapes harvested in the morning were significantly firmer, the berries skin strength was significantly higher and the grapes showed significantly less berry split than grapes harvested in the afternoon. Harvest temperature had no significant ($P>0.05$) influence on berry abscission, stem desiccation or SO_2 damage, as measured after the shelf life period. The use of perforated liners resulted in a significant reduction of berry firmness, berry split and SO_2 damage. The type of liner did not influence berry skin strength, berry abscission or stem condition significantly.

Similar to 1998 results, moisture loss was exacerbated by harvesting at high temperatures and by packing in perforated liners. The influence of harvest temperature and type of liner on the levels of *Botrytis* decay can be explained in terms of the SO_2 concentration available to control *Botrytis cinerea*. SO_2 gas released by the 'Uvasys' dual release SO_2 sheet kills the conidia of *Botrytis cinerea* on the surface of the berry (Harvey, 1955). Couey and Uota (1961) showed that the toxicity of SO_2 increases about 1.5 times for each 10°C rise in temperature between 0°C and 30°C. Also, high humidity inside the polyethylene bag triggers the release of SO_2 from the sodium metabisulphite impregnated SO_2 sheets (Làszlò *et al.*, 1981). The higher the humidity, the more effective SO_2 is as a fungicide (Ginsburg *et al.*, 1973). Thus, grapes harvested at a higher temperature would develop a high humidity more rapidly inside the polyethylene bag. This would trigger a rapid release of SO_2 gas, and with the higher temperature, would increase the toxicity of the SO_2, thereby resulting in more effective control of *Botrytis* decay. Non-

perforated liners result in a higher humidity and a higher concentration of SO_2 gas inside the liner than inside a perforated liner. Therefore, it is possible that grapes harvested in the morning at low temperatures and packed in perforated liners, would have a higher decay potential. The higher berry skin strength values obtained for grapes harvested at low temperatures, is in accordance with research done by Lang and Düring (1990), who observed a significant decrease in berry skin stiffness and strength with increasing temperatures. Additional to the previous explanation on why grapes with higher temperatures are more prone to berry splitting, these grapes also had a lower skin strength which could increase their susceptibility to berry splitting. According to Lustig and Bernstein (1985) and Lang and Düring (1990), the mechanical behaviour of the grape berry skin greatly influences the berry's resistance to splitting. When the skin stress, caused by the turgor pressure, exceeds the rupture strength of the skin, the grape berry may split (Bernstein and Lustig, 1985). The significant reduction in berry firmness of grapes packed in perforated liners can be ascribed to a significant increase in moisture loss. The reduction in berry split and SO_2 damage by using perforated liners has previously been explained.

Experiment 2 : Harvest maturity

Grapes harvested at different maturity stages showed significant differences in TSS and TA in 1999 (Table 3) and 2000 (Table 4). TSS increased and TA decreased with later sampling, thereby indicating an advance in maturity. No significant difference in colour, as measured with the Nippon NR3000 colorimeter, could be detected between the grapes sampled at different harvest maturities during the 1999 season.

In 1999, after the grapes had been stored for five weeks at -0.5°C, no significant difference in berry split could be detected between grapes harvested at different maturities (Table 5). Grapes sampled at harvest 3 with a TSS of 18.4°Brix and a TA of 0.53% developed significantly more berry abscission. The stem condition of grapes harvested at the two more advanced stages of maturity, was significantly inferior due to desiccation, compared to grapes harvested less mature. No *Botrytis* decay was detected on grapes from any treatment.

After an additional week's storage at 10°C, no significant difference occurred between harvest maturities for berry split or stem desiccation (Table 5). However, grapes harvested at the two most advanced maturities showed a significantly higher incidence of berry abscission than grapes harvested less mature.

After the storage period at -0.5°C, berry abscission showed no definite trend that could be attributed to harvest maturity. However, after the additional 7 days at 10°C, the incidence of berry abscission increased with advanced harvest maturity, with sugars higher than 18.4°Brix and TA's lower than 0.53%. This is consistent with the findings of Nelson (1979), who also reported that abscission is aggravated by advanced maturity. According to him, as a result of continuous maturation of the vascular tissue at the pedicel attachment, an abscission zone may develop. A cork cambium develops from parenchyma cells in the abscission zone and, as a result, intercellular bonding weakens, with concomitant abscission taking place.

The increase in stem desiccation with increased maturity, which was evident after five weeks storage at -0.5°C, is probably also due to a more developed abscission layer in riper fruit. Since the stems have a much larger surface area to mass ratio than the grape berries (Ginsburg and Combrink, 1972), and because they have a high concentration of stomata and lenticels (Nelson, 1979), the stems lose moisture more readily than the berries. As a result, water diffuses from the berries to the stems in response to a water potential gradient. As the berries ripen, the abscission layer develops, and this can possibly create an obstruction for water movement from the berries to the stems. Therefore, grapes at advanced ripeness levels are probably more susceptible to stem desiccation. The elevation of the storage temperature during the simulated shelf life, would stimulate moisture loss due to creation of a larger difference in vapour pressure between the grapes and the surrounding air space. This influence on moisture loss, and consequently, stem desiccation, could probably surpass the influence of harvest maturity on stem desiccation. Therefore, no significant difference in stem desiccation occurred after the shelf life period.

In 2000, after the grapes had been stored for five weeks at -0.5°C followed by a week at 10°C, grapes harvested at different stages of maturity showed significant ($P<0.01$) differences in berry split, stem desiccation and *Botrytis* decay (Table 6). No significant difference in berry abscission occurred between grapes harvested at the different maturities. Since stem desiccation increased with advancing harvest maturity, similar to the 1999 findings, the explanation would be the same as previously presented.

The incidence of berry split decreased significantly for grapes harvested at the last two maturities (Table 6). By harvesting 'Thompson Seedless' grapes at a TSS of 20°Brix and a TA of 0.4%, instead of at a TSS of 17.3°Brix and lower, and a TA of 0.5% and higher, the incidence of post-storage berry split was reduced by approximately 87%. Two maturity related fruit factors can possibly influence the grape berry's susceptibility to split, *viz.* the osmotic concentration of

the fruit soluble solids, and the properties of the berry skin.

Since water absorption into plant cells is predominantly an osmotically driven process and post-harvest fruit splitting seems to be related to water absorption (Verner and Blodgett, 1931), many researchers have attempted to correlate differences in splitting with variations in the osmotic concentration of the fruit juice. Several studies on cracking susceptibility in sweet cherries concluded that the osmotic concentration of the fruit soluble solids is a major factor controlling water absorption and cracking (Verner and Blodgett, 1931; Sawada, 1931; Bullock, 1952). Sawada (1931) stated that cracking of sweet cherries is due to an osmotic absorption of water that correlates with the degree of maturity. Similarly, with apples and prunes, splitting potential increased with advanced maturity (Fisher, 1937a,b; Mrozek and Burkhardt, 1973). By contrast, Tucker (1934), Kertesz and Nebel (1935), Zielinski (1964), Christensen (1972) and Andersen and Richardson (1982) found no, or very weak correlations, between the sugar content or osmotic potential of sweet cherries and the tendency to crack. Tucker (1934) concluded that while sugar content could be of some importance, other factors influenced split potential to a greater extent. Andersen and Richardson (1982) suggested that cuticular properties or other structural factors may play a more important role in splitting.

If one assumes that fruit splitting is at least partly associated with the amount or rate of water uptake through the fruit skin and the ability of the skin to adjust to this, then morphological characteristics of the fruit skin must play a major role in these processes and the splitting response (Christensen, 1996). The cuticle, as the outermost structural element of the fruit skin, is the site of initial failure which leads to skin cracking (Considine and Brown, 1981). The waxlike cutin layers of the cuticle of grape berries consists mainly of oleanolic acid (79%) and long chain alcohols with traces of esters, fatty acids, aldehydes and paraffins (Radler, 1965a, b). Schönherr (1976) demonstrated that the waxes are responsible for reducing the water permeability of cuticles. Extraction of cuticular waxes with lipid solvents from isolated membranes, increased their water permeability by a factor of 300 to 500. Various properties of the cuticle may influence fruit splitting, such as the permeability of the cuticle (Anderson and Richardson, 1982; Glenn and Poovaiah, 1985, 1989), the thickness of the cuticle (Shutak and Schrader, 1948; Belmans *et al.,* 1989) and the strength of the cuticle (Considine, 1982). De Villiers (1962) reported that, other conditions being equal, immature fruit loses water more readily than well-matured fruit. The author attributed this difference to the increase in cutinisation of the skin of the berries as they mature. This supports the experience of many growers that 'Emperor' grapes should not be harvested for storage until well matured (Winkler *et al.*, 1974). Therefore, the increase in cutinisation of the skin as the grape berries mature, can

possibly decrease the water permeability of the cuticle and increase the thickness and strength of the cuticle. Consequently, these changes can increase the resistance of the grape berries to split. This, in part, could explain why 'Thompson Seedless' grapes that were harvested more mature developed less berry split in the 2000 season.

Grapes harvested at the most advanced maturity, developed significantly higher levels of *Botrytis* decay than grapes harvested less mature (Table 6). Resveratrol is a grapevine phytoalexin related to *Botrytis cinerea* (grey mold) resistance that is synthesized in the skin cells of grape berries of *Vitis vinifera* L. and *Vitis labrusca* L. (Jeandet *et al.*, 1991). Resveratrol production decreases during maturation, and hence, there could be a decrease in natural resistance to *Botrytis cinerea* infection as grape maturity increases. This would explain the significant increase in *Botrytis* decay for grapes harvested at the most advanced maturity.

Contrary to the influence of harvest maturity on berry abscission during the 1999 season, harvest maturity had no significant influence on berry abscission during the 2000 season. Also, 'Thompson Seedless' grapes derived from the Wellington trial block were less susceptible to berry split than 'Thompson Seedless' grapes derived from the Saron trial block. This probably explains why harvest maturity showed no significant influence on berry split during the 1999 season, at such low incidences.

Since table grapes are non-climacteric fruit, they do not ripen after harvest (Kanellis and Roubelakis-Angelakis, 1993). Table grapes should therefore only be harvested after they reach the optimum stage of acceptability in appearance, flavour and texture. Grapes harvested immature, are more susceptible to berry split and have poor eating quality. However, grapes harvested over mature, are prone to stem desiccation and the development of *Botrytis* decay. Therefore, to ensure optimum quality of cold stored 'Thompson Seedless', it is imperative to harvest as soon as possible after horticultural maturity has been reached, and to remain within the optimum maturity window. It is suggested that a TSS of 18°Brix be used as an indication of horticultural maturity for 'Thompson Seedless' table grapes.

CONCLUSIONS

Berry split is aggravated by packing 'Thompson Seedless' grapes at high pulp temperatures, especially if the grapes are packed in non-perforated bags. The incidence of berry split can be reduced by between 80 and 90% by packing grapes in perforated instead of non-perforated

liners. Perforated bags also reduce levels of SO_2 damage. However, due to significantly more moisture loss from grapes in perforated bags, compared to non-perforated bags, the risk of higher fruit and stem desiccation and softer berries exists. Grapes packed in a perforated liner are cooled much more rapidly in a forced-air cooling system (as is used commercially) than grapes packed in a non-perforated liner, and pulp temperature is the most critical factor in determining the vapour pressure of the berry. Therefore, the faster rate of cooling that is accomplished by using a perforated liner, may compensate to some extent for the higher vapour pressure difference existing later once the fruit is on temperature. This can alleviate the problem of moisture loss. The control of *Botrytis* decay in perforated liners can be less effective. This suggests that storage duration in perforated bags will be less than with non-perforated bags, from a decay control point of view. Optimum size and density of perforations needs to be determined to reduce berry split without excessive loss of moisture from the grapes, and SO_2 gas from the air space surrounding the product. The influence of harvest temperature and liner type on berry abscission was only evident during the 1998 season, and is therefore not conclusive. Advanced maturity increased grape resistance to berry split. However, grapes harvested too mature are prone to stem desiccation and the development of *Botrytis* decay. The occurrence of berry abscission also appeared to increase with increased harvest maturity. Consequently, to ensure optimal quality, 'Thompson Seedless' grapes should be harvested as soon as horticultural maturity has been reached, which appears to be at approximately 18°Brix. Taste requirements will also influence optimum harvest maturity, and a balance between taste and the other quality parameters discussed in this communication will be essential to facilitate export market requirements.

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Table 1 : Effect of harvest temperature and type of liner on the overall quality of 'Thompson Seedless' table grapes after five weeks storage at -0.5°C, and after five weeks cold storage and a shelf life of one week at 10°C (1998 season).

Parameters	Harvest temp (A)[1]		Liner type (B)[2]		Prob > F[3]		
	Low	High	Non-perf	Perf	A	B	A x B
After 5 weeks storage at -0.5°C							
Moisture loss (%)	1.37	1.91	0.85	2.44	0.008	0.001	0.340
Firmness (N)	1.12	0.90	1.00	1.01	0.001	0.831	0.973
Berry split (%)	1.24	1.32	2.12	0.44	0.283	0.001	0.284
Berry abscission (%)	2.16	1.73	2.06	1.83	0.261	0.551	0.234
Stem condition[4]	2.13	2.38	2.38	2.13	0.308	0.308	1.000
SO_2 damage (%)	1.30	0.97	1.32	0.95	0.990	0.917	0.089
Botrytis (%)	0.69	1.73	1.40	1.02	0.096	0.635	0.062
After 5 weeks storage at -0.5°C + 1 week at 10°C							
Moisture loss (%)	1.69	1.65	0.85	2.48	0.802	0.001	0.253
Firmness (N)	1.14	0.95	1.05	1.04	0.001	0.674	0.438
Berry split (%)	1.53	3.68	4.53	0.67	0.049	0.001	0.623
Berry abscission (%)	1.34	0.68	1.25	0.77	0.004	0.028	0.778
Stem condition	1.94	2.09	2.01	2.03	0.421	0.825	0.573
SO_2 damage (%)	1.23	2.80	2.59	1.44	0.176	0.194	0.688
Botrytis (%)	5.51	6.54	4.68	7.37	0.848	0.331	0.861

1 Data pooled across 'Liner type'. Low = berry pulp temperature of 24.5°C, high = berry pulp temperature of 29.4°C

2 Data pooled across 'Harvest temperature'.

3 Two-way ANOVA table with complete randomised factorial design for factor A (harvest temperature) and factor B (liner type).

4 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

Table 2 : Effect of harvest temperature and type of liner on the overall quality of 'Thompson Seedless' table grapes after five weeks storage at -0.5°C, and after five weeks cold storage and a shelf life of one week at 10°C (1999 season).

Parameters	Liner Type (B)	Harvest temp (A)[1]		Liner type (B)[2]		Prob > F[3]		
		Low	High	Non-perf	Perf	A	B	A x B
<u>After 5 weeks storage at -0.5°C</u>								
Firmness (N)		1.12	1.02	1.05	1.09	0.094	0.461	0.314
Skin strength (N)		2.92	3.01	2.84	3.09	0.616	0.151	0.442
Berry split (%)[4]	Non-perf	0.16[a]	1.98[b]			0.007	0.003	0.004
	Perf	0.11[a]	0.05[a]					
Berry abscission (%)		0.62	0.32	0.46	0.48	0.094	0.835	0.702
Stem condition[5]		2.22	2.16	1.97	2.41	0.746	0.030	0.519
SO_2 damage (%)		0.26	0.40	0.64	0.02	0.492	0.004	0.383
Botrytis (%)		0.54	0.31	0.31	0.54	0.318	0.344	0.371
<u>After 5 weeks storage at -0.5°C + 1 week at 10°C</u>								
Moisture loss (%)[4,6]	Non-perf	0.43[a]	0.28[a]			0.004	0.001	0.001
	Perf	1.14[b]	1.90[c]					
Firmness (N)		1.08	0.94	1.06	0.96	0.004	0.030	0.350
Skin strength (N)		3.20	2.77	2.93	3.04	0.004	0.424	0.082
Berry split (%)		0.73	1.53	2.04	0.22	0.048	0.001	0.949
Berry abscission (%)		0.63	0.68	0.58	0.73	0.804	0.458	0.422
Stem condition		2.59	2.50	2.50	2.59	0.653	0.653	0.881
SO_2 damage (%)		1.17	1.21	1.96	0.41	0.933	0.002	0.978
Botrytis (%)[4]	Non-perf	1.26[a]	1.68[a]			0.013	0.016	0.001
	Perf	3.84[b]	1.23[a]					

1 Data pooled across 'Liner type'. Low = berry pulp temp. of 16.9°C, high = berry pulp temp. of 30.0°C

2 Data pooled across 'Harvest temperature'.

3 Two-way ANOVA table with complete randomised factorial design for factor A (harvest temperature) and factor B (liner type).

4 Interaction between factor A and B. Data not pooled.

5 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

6 Moisture loss was determined only after shelf life. Interaction between factor A and B. Data not pooled.

Table 3 : Maturity of 'Thompson Seedless' table grapes determined immediately after harvesting at four stages from a vineyard in Wellington in the 1999 season, with approximately weekly intervals between stages.

Parameters	Harvest maturity				Prob > F[1]
	1	2	3	4	
TSS (°Brix)	15.2[a]	17.8[b]	18.4[b]	21.0[c]	0.001
TA (%)	0.84[a]	0.68[b]	0.53[c]	0.47[d]	0.001
Colour (L-value)	45.4	45.9	45.7	45.7	0.921
Colour (hue)	117.9	118.5	120.9	109.4	0.431
Colour (chroma)	13.4	13.1	12.8	13.0	0.969

1 One-way ANOVA table with randomised block design.

Table 4 : Maturity of 'Thompson Seedless' table grapes determined immediately after harvesting at four stages from a vineyard in Saron in the 2000 season, with approximately weekly intervals between stages.

Parameters	Harvest maturity				Prob>F[1]
	1	2	3	4	
TSS (°Brix)	16.8[a]	17.3[a]	20.4[b]	20.4[b]	0.001
TA (%)	0.57[a]	0.50[b]	0.45[c]	0.42[c]	0.001

1 One-way ANOVA table with randomised block design.

Table 5 : Effect of harvest maturity on the overall quality of 'Thompson Seedless' table grapes sampled from Wellington in 1999, after five weeks storage at -0.5°C, and after five weeks cold storage and a shelf life of one week at 10°C.

Parameters	Harvest maturity				Prob > F[1]
	1	2	3	4	
After 5 weeks storage at -0.5°C					
Berry split (%)	0.4	1.5	1.0	1.0	0.339
Berry abscission (%)	0.1[a]	0.8[ab]	1.4[b]	0.6[ab]	0.035
Stem condition[2]	1.83[a]	2.33[a]	3.08[b]	3.08[b]	0.002
After 5 weeks storage at -0.5°C + 1 week at 10°C					
Berry split (%)	1.0	0.9	1.5	3.2	0.225
Berry abscission (%)	0.2[a]	0.3[a]	1.1[b]	1.1[b]	0.003
Stem condition[2]	2.2	2.5	2.0	2.1	0.340

1 One-way ANOVA table with randomised block design.

2 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

Table 6 Effect of harvest maturity on the overall quality of 'Thompson Seedless' table grapes sampled from Saron in 2000, after five weeks storage at -0.5°C and a shelf life of one week at 10°C.

Parameters	Harvest maturity				Prob>F[1]
	1	2	3	4	
Berry split (%)	22.4[b]	25.5[b]	1.5[a]	4.6[a]	0.001
Berry abscission (%)	1.2	0.9	2.1	1.3	0.244
Stem condition[2]	1.0[a]	1.3[a]	2.2[b]	1.9[b]	0.001
Decay (%)	0.2[a]	0.1[a]	0.1[a]	1.2[b]	0.009

1 One-way ANOVA table with randomised block design.

2 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

PAPER II - Effect of field heat removal prior to packing, and delay periods before and after packing on the quality of 'Thompson Seedless' table grapes, with special reference to berry split and berry abscission.

EFFECT OF FIELD HEAT REMOVAL PRIOR TO PACKING, AND DELAY PERIODS BEFORE AND AFTER PACKING ON THE QUALITY OF 'THOMPSON SEEDLESS' TABLE GRAPES, WITH SPECIAL REFERENCE TO BERRY SPLIT AND BERRY ABSCISSION.

ABSTRACT

In 1998, cooling of 'Thompson Seedless' grapes harvested at an average pulp temperature of 23.7°C was delayed for 3, 8 and 12 hours at an ambient temperature of 25.8°C and a relative humidity (RH) of 57%, before packing in non-perforated polyethylene liners commenced. Forced-air cooling (FAC) commenced immediately after packing. In 1999, grapes were harvested at an average pulp temperature of 27.7°C and then either subjected to field heat removal (FHR) for 1.5 hours or kept in the shade for the same duration. After FHR, the average berry temperature was 18.9°C, while at the end of the period in the shade the berry temperature was 27.7°C. Grapes lost 0.17% moisture during FHR, while grapes kept in the shade lost 0.25%. After packing in non-perforated polyethylene liners, cooling of grapes was delayed for 6, 12 or 18 hours at an average temperature of 23.4°C, before FAC commenced. In trials conducted in both 1998 and 1999, after FAC, the grapes were stored for five weeks at -0.5°C and an additional week at 10°C. Fruit quality was assessed directly after the delay periods, after the five week cold storage period, and again after an additional simulated shelf life of seven days at 10°C. FHR prior to packing had no beneficial or adverse effect on berry split and berry abscission. Although FHR resulted in grapes with lower levels of Botrytis *decay and stem desiccation after five week's cold storage at -0.5°C, no beneficial effect of FHR regarding these defects was evident after the simulated shelf life period. Delay periods prior to packing aggravated berry abscission, but did not influenced berry split significantly. Packaging delays of 12 hours resulted in a significant increase in berry abscission, compared to grapes delayed for only 3 or 8 hours. In addition, the incidence of* Botrytis *decay increased significantly for grapes delayed for 12 hours before packing. Grapes delayed for 18 hours before FAC commenced, had significantly higher levels of berry split directly after the delay period, compared to grapes delayed for 6 or 12 hours. At the end of the simulated shelf life period, berry split levels were distinctly higher than at the beginning of shelf life, indicating that breaks in the cold chain promote split. No significant difference in berry abscission occurred between grapes delayed for different periods. Although grapes delayed for 6 hours exhibited significantly more* Botrytis *decay after five weeks storage at -0.5°C, no significant difference in* Botrytis *decay could be detected between the different treatments after the shelf life period.*

KEYWORDS : 'Thompson Seedless', berry split, berry abscission, field heat removal, delay periods, grape

INTRODUCTION

'Sultanina' is one of the most important export table grape cultivars in South Africa, and is predominantly grown in hot inland regions where high field heat temperatures in excess of 38°C can occur. Certain metabolic processes in table grapes, like for all other perishable commodities, continue during postharvest handling and marketing (Nelson, 1979), and are regulated by the catalytic action of enzymes (Salisbury and Ross, 1985a). Enzyme activity is temperature sensitive, and therefore temperature is a great determinant of fresh produce deterioration rate, and, consequently, of potential storage life. With highly perishable commodities, a few hours delay before start of cooling can cause damage which cannot be overcome by subsequent good handling practices (Nelson, 1979).

Temperatures favourable for maximum growth and development of rot organisms often coincide with field temperatures encountered during fruit harvest (Nelson, 1956). Without rapid cooling, rot organisms may quickly consume the produce. The inhibiting effect of low temperatures on these organisms varies, dependant on the organism involved. The most problematic postharvest decay organism in table grapes is *Botrytis cinerea* Pers. It is especially dangerous because it grows vigorously at vineyard temperatures of 10 to 25°C, which are often the norm. Even in storage at a temperature of -1°C, *Botrytis* will continue to grow, albeit slowly.

Probably the most compelling reason why table grapes should be cooled promptly after harvest, is to reduce the rate of water loss (Nelson, 1979). This phenomenon is strictly a physical factor related to the evaporative potential of the surrounding air. Due to relatively high fruit temperatures that prevail from the time the grapes are harvested until cooling is well under way, the duration of this phase has a profound effect on the rate of deterioration.

In practice, delays before and after packing, prior to the onset of FAC, are sometimes inevitable. Co-operative cooling facilities which may be some distance away from the pack-house, are often responsible for delays before the commencement of cooling. A rule of thumb law often quoted is that every hour saved between harvesting and cooling adds a day to the shelf life of grapes (Ginsburg *et al.*, 1973). Evidence, based on South African experience and some experimentation, suggests that a delay of 6 to 12 hours between harvesting and the onset of FAC results in acceptable quality maintenance of cold stored grapes. The influence of delay

periods at high temperatures on physiological processes such as respiration, moisture loss, and the development of *Botrytis* decay, have been studied extensively (Ryall and Harvey, 1959; Combrink *et al.*, 1975; Nelson, 1979). However, the influence of delay periods before and after packing on the incidence of berry split and berry abscission following five weeks cold storage at -0.5°C, is uncertain.

To reduce the field heat of grape berries as soon as possible after harvest, some producers use pre-coolers set at approximately 18°C to partially cool the product prior to packing. To date, no conclusive evidence has been published to indicate whether or not FHR, using pre-cooling prior to packing, has a causative or preventative effect on the development of berry split and berry abscission during cold storage.

The aim of this study was to investigate the effect of field heat removal, as well as delay periods before and after packing, on the quality of cold stored 'Thompson Seedless' table grapes.

MATERIALS AND METHODS

Experiment 1 : Delays before packing

The study was conducted in 1998 on 12 year old 'Thompson Seedless' vines grafted on Ramsey rootstock. The vineyard in the Wellington area in the Western Cape, South Africa, was drip irrigated. The vines were supported on a slanting trellis system with a vine and row spacing of 1.8 and 3.6m, respectively. The recommended gibberellic acid (GA_3) sprays to promote berry enlargement, which comprise three sprays of 5ppm during flowering and three sprays of 20ppm when the berries are between 4 and 8mm in diameter, were applied (Wolf *et al.*, 1991). The vines were girdled when the berries reached the pea-size stage. Normal cultural practices were followed as far as irrigation, fertilisation, cluster preparation, pest and disease control and foliage management were concerned.

Grapes were harvested with total soluble solids (TSS) of 16.9°Brix and titratable acid levels (TA) of 0.62%, and at an average pulp temperature of 23.7°C. After harvest, the grapes were delayed without pre-cooling for 3, 8 and 12 hours before packing. The average ambient temperature and relative humidity (RH) during the delay periods was 25.8°C and 57%, respectively. The grapes were packed in 4.5kg closed top cartons, with individual bunches in plastic carrybags, and the entire carton content enclosed in a non-perforated 20µm polyethylene liner. A 'Uvasys' SO_2 generator to control decay was included inside the liner, on top of the

grapes. The grapes were subjected to FAC approximately 50 min after packing. Approximately 48 hours were required to reduce the temperature from an average of 25.6°C to -0.5°C.

Directly after forced-air cooling, the grapes were stored at -0.5°C and 85-90% RH for five weeks, simulating transport by ship and accumulation overseas prior to sale. Fruit quality was assessed at ambient temperature (±24°C) directly after this five week cold storage period, and again after a simulated shelf life of seven days at 10°C. Polyliners were kept closed during shelf life.

For measurement of TSS and TA at harvest, 50 randomly selected berries from five randomly selected grape bunches were used. The berries were juiced in a liquidiser and filtered. The percentage soluble solids of the filtrate, expressed as degree-Brix, was measured using a bench top Atago Palette PR100 digital refractometer with automated temperature compensation. Because the acid fraction of the total soluble solids is usually very small compared to the sugar fraction, the soluble solids were considered as the sugar content. TA was determined by titrating a 10g aliquot of juice with 0.1N NaOH to a pH end-point of 7, using an auto Metrohm 665 Dosimat titrator. Pulp and ambient temperatures were recorded with a probe connected to a Kane-May 22 digital thermometer, while RH was measured using a whirling hygrometer to determine the wet and dry bulb temperatures. RH was then read from a psychrometric chart.

After five weeks of cold storage and the subsequent shelf-life period, grapes were examined for the quality defects moisture loss, berry split, berry abscission and *Botrytis* decay. All of the grapes in each carton were examined. The incidence of each defect was expressed as a percentage of the total grape mass per carton. Berry split was recorded as such when any split, whether restricted to the epidermis, or deeper into the mesocarp, was visibly evident. Artificial berry split due to external stresses from manual or mechanical sources, e.g. berry split caused by excessive pressure on the fruit during lidding, was not taken into account. All berries with "dry" scars, found loose in the carry bags after removal of the bunch, were recorded for berry abscission. Berries that were torn loose due to improper handling or berry split around the pedicle end, were not recorded. All of the grape bunches per carton were rated for stem condition on a five-point scale, where 1 = fresh, green stems and 5 = dry, brown stems.

Moisture loss was determined by labelling and weighing one grape bunch per carton at harvest, after the delay period, after the five week cold storage period, and again at the end of the shelf-life period. It was assumed that the reduction in fresh mass during storage was mainly due to

moisture loss as a consequence of transpiration. Moisture loss was calculated as a percentage of the initial fresh mass measured at harvest.

The experimental design was completely randomised. Each treatment was replicated eight times, with one carton constituting a single replicate. Eight replicates were examined at the end of cold storage, and another eight were examined after shelf life. Data were subjected to an analysis of variance using the General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

Experiment 2 : Field heat removal and delays after packing

The trial was conducted in the 1999 season on 'Thompson Seedless' grapes from the Saron area in the Western Cape, South Africa. The trial block was planted in 1989, on Richter 99 rootstock. The vines were trained onto a double gable system with a 2m in-row and a 3m between-row planting distance, and irrigated by means of a drip irrigation system. The commercially recommended gibberellic acid (GA_3) sprays were applied (Wolf *et al.*, 1991), and vines were also girdled when the berries were between 5 and 6mm in diameter. Standard cultural practices were followed regarding irrigation, fertilisation, cluster preparation, pest and disease control and foliage management.

Grapes with a TSS of 18.9°Brix and TA of 0.63% were harvested at an average pulp temperature of 27.7°C. The grapes were either subjected to FHR for 1.5 hours or kept in the shade for the same duration. The average dry bulb temperature and RH in the FHR chamber was 19°C and 82%, respectively. An average dry bulb temperature of 27.3°C and a RH of 52% was measured in the shade. After FHR, the average berry temperature was 18.9°C, while at the end of the period in the shade the berry temperature was 27.7°C. The experimental grapes were packed as described for experiment 1. After packing, the grapes were delayed for 6, 12 or 18 hours at an average temperature of 23.4°C, before FAC was initiated. After FAC, the grapes were stored at -0.5°C and 85-90% RH for five weeks. Fruit quality was assessed at ambient temperature (±24°C) after this cold storage period, and again after a simulated shelf life of 7 days at 10°C. The polyliners were kept closed during shelf life. Grapes were examined for the quality defects berry split, berry abscission, moisture loss, and *Botrytis* decay. All of the grapes in each carton were examined. The incidence of each defect was expressed as a percentage of the total grape mass per carton. All the grape bunches per carton were also rated for stem condition on a five-point scale, where 1 = fresh, green stems and 5 = dry, brown stems.

For measurement of TSS, TA, temperatures, relative humidity and moisture loss, the same methods as described in experiment 1 were used. Moisture loss over storage time was determined by comparison of bunch mass measured at harvest against the mass at the end of the simulated shelf life.

The experimental design was completely randomised, and the treatment design was a 2 x 3 factorial with FHR and delay periods as factors. Each treatment was replicated eight times, with one carton constituting a single replicate. For each treatment combination, eight replicates were examined at the end of cold storage, and another eight were examined after shelf life. Data were subjected to an analysis of variance using the General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Experiment 1 : Delays before packing

Directly after the delay periods, the grapes were examined for any visual defects. The stems showed no visible desiccation and no berry split could be detected (data not shown). Grapes delayed for 8 or 12 hours, showed significantly more moisture loss than grapes only delayed for 3 hours before packing commenced (Table 1). This can be ascribed to prolonged transpiration at a high rate. Transpiration refers to internal water loss through stomata, cuticle or lenticels (Salisbury and Ross, 1985b). The driving force for transpiration is the difference in water vapour pressure within the fruit and in the atmosphere beyond the boundary layer that surrounds the fruit (Mitchell, 1978). This difference between the vapour pressure of the grapes, which is a function of temperature and RH, and that of the surrounding air, which is also a function of temperature and RH, is called the vapour pressure deficit (Salisbury and Ross, 1985b). According to the psychrometric chart, grapes harvested at an average pulp temperature of 23.7°C had a vapour pressure of approximately 3.0kPa, if it is assumed that the RH of the internal atmosphere within the fruit is 100%. The vapour pressure of the surrounding air with an average temperature of 25.8°C and a RH of 57%, as was evident during the delay periods, was approximately 1.8kPa. With a vapour pressure deficit of approximately 1.2kPa, a relatively high rate of transpiration would have occurred. During the delay periods, the grapes were kept in open lug boxes in a large room with temperature regulation. Thus, the ambient temperature was kept constant, and the pulp temperatures of the grapes did not change dramatically. Therefore, the difference in RH between the intercellular spaces inside the fruit and the air

surrounding the fruit, would have been the main reason for prolonged transpiration at a high rate during the delay periods. However, once the packed grapes were closed inside the non-perforated polyethylene liner and FAC commenced, the temperature and RH dynamics would have changed completely. At the onset of FAC, a high rate of transpiration would have occurred due to vast differences in temperature and RH between the grapes and the surrounding air. However, within a few hours the temperature of the grapes would have decreased rapidly, and because non-perforated polyethylene liners are excellent water vapour barriers, the RH of the air surrounding the grapes would have increased due to moisture loss. Consequently, the water vapour pressure in the atmosphere that surrounds the fruit inside the bag, increases more rapidly than in the atmosphere that surrounds the grapes in the open lug boxes. Therefore, the transpiration rate decreases inside the non-perforated bag and consequently less moisture loss occurs. Thus, the longer the duration of the delay period before the grapes are packed and closed inside the polyethylene liner, the longer transpiration at a high rate occurs.

Berry abscission increased significantly when grapes were delayed for 12 hours before packing, compared to grapes delayed for shorter periods (Table 1). This effect was most evident immediately after the delay periods, but also after five weeks storage at -0.5°C. Even a delay of only 3 hours before packing resulted in a high level of berry abscission, namely 1.95%. In general, the incidence of berry abscission during storage was relatively low, compared to commercial experience for 'Thompson Seedless' table grapes. Commercially, berry abscission that occurs before packing is not recorded and is generally underestimated.

Two separate phenomena may be involved in fruit abscission (Baird and Webster, 1979). Firstly, an anatomically distinct separation layer between the pedicel and the berry, or between the pedicel and the rachis is formed, through which abscission subsequently takes place. For table grapes, this layer in the berry attachment area is formed at a very early stage of berry development (Wagener, 1985). Secondly, the actual separation of cells adjacent to the abscission layer takes place as a result of chemical alterations in the cell walls (Baird and Webster, 1979). The cell wall changes leading to separation of cells involve hydrolysis of the middle lamella, breakdown of all or part of the cellulosic cell wall, and mechanical breakage of non living elements, such as the vascular elements. It is possible that the breakdown of cell wall constituents is caused by the catalytic action of enzymes such as pectinmethylesterase, polygalacturonase and cellulase. Wagener (1985) hypothesised that the separation process may be activated by harvesting. A hormone then probably acts as a "messenger" to secrete hydrolytic enzymes in the abscission zone which initiates the separation process. Enzyme activity is temperature sensitive and increases two to four times for each 7.7°C temperature rise

(Salisbury and Ross, 1985). It is assumed that the delay temperature of approximately 25.8°C for prolonged periods was favourable for enzyme activity involved in the separation process. According to Wagener (1985), berry abscission is aggravated by any factor giving rise to moisture stress in the plant up to and including harvesting time. It is further assumed that postharvest factors which result in excessive moisture loss, also aggravate berry abscission. The significantly higher moisture loss from grapes delayed for 12 hours (0.92%), compared to the moisture loss from grapes delayed for only 3 hours (0.38%), could have been the stimulus for the significantly higher incidence of berry abscission for grapes delayed for 12 hours. That grapes delayed for 8 hours also showed significantly higher moisture loss than grapes delayed for 3 hours, without an associated significant increase in berry abscission, is difficult to explain. However, it is possible that the time at the higher temperature plays a more dominant role than moisture loss itself, and that a time period of longer than 8 hours was required to degrade cell walls to the extent that it caused abscission.

After five weeks storage at -0.5°C, no significant ($P>0.05$) difference in moisture loss, berry split and stem condition could be detected between the different treatments (Table 1). Grapes delayed for 12 hours before packing showed a significant increase in berry abscission and *Botrytis* decay, compared to grapes delayed for shorter periods. Commercially, the occurrence of 1.93% *Botrytis* decay is unacceptably high. Relative to the level of berry abscission that was evident directly after the delay period, less berry abscission occurred during cold storage. The low temperature of -0.5°C during storage most probably decreased or inhibited the activities of hydrolytic enzymes involved in berry abscission.

After five weeks cold storage and an additional week at 10°C, grapes delayed for different periods before packing showed no significant ($P>0.05$) difference regarding moisture loss, berry split, berry abscission and stem condition (Table 1). The levels of *Botrytis* decay were significantly higher for grapes delayed for 12 hours, compared to grapes delayed for only 3 or 8 hours before packing commenced. In general, the incidence of *Botrytis* decay was unacceptably high after five weeks cold storage and an additional week at 10°C.

Decay is an ever-present hazard in the postharvest handling of table grapes (Nelson, 1956). Several fungi can incite postharvest decay, of which *Botrytis cinerea* Pers. is the most important. Surface contamination and even latent infections by this fungus can result in high decay levels during storage. *B. cinerea* grows vigorously at vineyard temperatures of 10 to 25°C, but growth is slowed as the temperature is lowered. The extended delay period of twelve hours at an average temperature of 25.8°C and a RH of 57% probably enhanced germination of *B. cinerea*

spores on the berry surface, as well as the further development of latent infections. As was the case in this study, this could result in significantly higher levels of *Botrytis* decay for grapes delayed for 12 hours before packing, compared to grapes delayed for shorter periods. The stimulatory influence of delay periods on *Botrytis* decay was evident after five week's storage at -0.5°C, but was further promoted by raising the temperature to 10°C during shelf life. Additional to more vigorous growth of *B. cinerea* at higher temperatures, the elevation of storage temperature would also result in condensation on the grapes. This condensation would promote further *Botrytis* decay through spore germination.

Irrespective of the treatment, grapes examined directly after the delay periods showed a higher incidence of berry abscission than grapes examined after five or six weeks storage (Table 1). Stem desiccation increased slightly during the simulated shelf-life period, while the incidence of berry split and *Botrytis* decay more than doubled during shelf-life, irrespective of the treatment.

The enormous increase in berry split when the storage temperature was raised to 10°C, can be explained in terms of the physical processes, namely condensation and osmosis. In thermodynamic terms, the grape bunches are separated from the surroundings by a boundary layer that envelopes each grape berry (Wills *et al.*, 1998). In this layer, the water vapour pressure is approximately in equilibrium with that of the produce itself. When the storage temperature is raised from -0.5°C to 10°C, the temperature of the moist air that surrounds the grape bunches increases more rapidly than the temperature of the grape berries. The warmer air closest to the grape berries in the boundary layer is therefore cooled down by the colder grape berries. As the moist air cools down, dew point is reached at a certain temperature and condensation is initiated. Due to solutes (i.e. sugars) present in the grape berries and because of moisture loss at the onset of cooling, the grape berries exhibit a lower water potential than the water that condenses on the fruit surface. Therefore, water diffuses into the berries in response to the water potential gradient, from the boundary layer, through the cuticle and cell membranes into the epidermal cells. An increased turgor pressure develops. According to Considine and Kriedemann (1972), Wade (1988) and Sekse (1995), turgor pressure plays an important role in fruit splitting. It is suggested that osmotic entry of excessive water into the epidermal cells causes rupture of the cells, resulting in berry split.

Experiment 2 : **Field heat removal and delays after packing**

Interaction between the factors FHR and delay periods was not significant for any of the quality parameters measured (Table 2). Therefore, data were pooled across FHR treatments and delay

periods.

The removal of field heat before packing did not significantly (P>0.05) influence moisture loss, berry split or berry abscission, as determined directly after the delay periods (Table 2). Grapes delayed for 18 hours after packing and before the onset of FAC exhibited significantly more moisture loss and berry split than grapes delayed for 6 or 12 hours. The delay periods had no significant (P>0.05) influence on the incidence of berry abscission, as determined directly after the delay periods.

After five weeks storage at -0.5°C, FHR had no significant (P>0.05) influence on berry split or berry abscission (Table 2). However, grapes subjected to FHR showed significantly less stem desiccation and *Botrytis* decay, compared to grapes kept in the shade. After five week's cold storage, the influence of delay periods on berry split was still evident, with higher split levels associated with longer delays. Grapes delayed for 18 hours showed significantly higher berry split, compared to grapes delayed for only 6 hours. The delay periods had no significant (P>0.05) influence on berry abscission or the stem condition. Grapes delayed for only 6 hours before the onset of FAC had significantly higher levels of *Botrytis* decay than grapes delayed for longer durations.

After five weeks storage at -0.5°C, followed by a week at 10°C, no significant (P>0.05) influence of FHR on any of the parameters was evident (Table 2). Grapes delayed for 12 hours showed significantly less berry split, compared to the other delay periods. The simulated shelf life period resulted in increased levels of berry split. Berry split increased during the shelf-life period. No significant (P>0.05) difference in berry abscission, stem condition or *Botrytis* decay was evident between delay periods.

The removal of field heat from grapes by reducing pulp temperatures from 27.7°C to 18.9°C prior to packing, had no significant influence on the incidence of berry split or berry abscission, irrespective of the examination stage. The benefit of FHR in this study lies in the reduction of stem desiccation and *Botrytis* decay, as was evident after five weeks storage at -0.5°C. FHR would likely have had a more prominent positive effect on overall quality if the grapes were harvested at temperatures higher than 30°C. Due to the reduction of fruit temperature and a high RH of 82% inside the FHR-chamber, moisture loss from grapes subjected to FHR was confined to 0.17%, compared to a moisture loss of 0.25% from grapes kept in the shade for the same duration of time, at an ambient temperature and RH of 27.3°C and 52%, respectively (data not shown). Desiccation of the grape stem is considered as a secondary symptom of

water loss (Nelson, 1979). Therefore, the reduction in moisture loss from grapes subjected to FHR could have resulted in less stem desiccation. The fact that higher levels of *Botrytis* decay occurred in the product at 27.3°C in the shade, compared to 19°C in the FHR-chamber, suggested that the higher temperature was more favourable for decay development from previous infection.

Although FHR resulted in grapes with lower levels of *Botrytis* decay and stem desiccation after five week's cold storage at -0.5°C, no beneficial effect was evident after the simulated shelf life period, which is indicative of the quality received by the consumer. Therefore, while the retailer would benefit from the use of FHR, it is doubtful whether or not the quality advantage would be evident to the consumer, if only the parameters measured in this trial are considered. There may be other parameters that were not evaluated but that may conceivably have been affected. For producers who do not want the expense of setting up a FHR chamber, these results suggest that it would benefit quality by harvesting 'Sultanina' during the coolest part of the day. According to Làszlò and Saayman (1992), the best and most economical method of eliminating field heat in hot regions is to harvest 'Sultanina' after midnight to just before sunrise. Given the volumes and infrastructure constraints the South African table grape industry are currently facing, such a practice will be most feasible.

The significant increase in moisture loss from grapes delayed for 18 hours before the onset of FAC, as opposed to grapes delayed for 6 or 12 hours, can be ascribed to an extended period of transpiration at a high temperature. Irrespective of whether field heat was removed or not, more transpiration would occur the longer the grapes stood at 23.4°C, with lower RH levels in the atmosphere that surrounded the fruit than within the intercellular spaces in the grape berries.

A higher incidence of berry split for grapes delayed for 18 hours was evident after the delay period as well as after five week's cold storage at -0.5°C. Berry split that occurred during the delay periods at 23.4°C was approximately 85% less (based on the average value of data pooled across 'delay periods') than the amount of split measured after five week's storage at -0.5°C. During the delay periods, the atmospheric humidity inside the non-perforated bags would increase rapidly due to moisture loss from warm grapes. According to De Villiers (1962) and Meynhardt (1956), berry split can occur at high atmospheric humidity. Therefore, it is to be expected that berry split would already be evident after the 6 hour delay period. The more flaccid berries, due to excessive moisture loss, would only have been exposed to the moisture that they themselves had transpired. This could lead to split or merely the re-absorption of transpired moisture. However, the amount of berry split that was evident, is possibly related to

the rate of moisture absorption rather than the amount of absorbed moisture itself. According to Cline *et al.* (1995), cracking of cherries appeared to be related more to the rate of water absorption than to the quantity of water that accumulates. The significant increase in berry split for grapes delayed for 18 hours could be due to prolonged exposure to high atmospheric humidities. The significant differences in berry split which were evident after five weeks cold storage at -0.5°C can be explained in terms of moisture loss. Along with moisture loss, the osmotic potential in the plant cells becomes more negative, thus lowering the water potential (Wills *et al.*, 1998). Therefore, for grapes delayed for 18 hours and where moisture loss was probably greater, the water potential in the berries possibly decreased to lower values than for grapes delayed for shorter durations with less moisture loss. Hence, a higher rate of water absorption would have occurred, since the water potential gradient from high on the surface to low in the berry would have been greater for grapes delayed for 18 hours, than with shorter delays. Water absorption by the grape berries results in an increase in turgor pressure, and according to Considine and Kriedemann (1972), Wade (1988) and Sekse (1995), turgor pressure plays an important role in fruit splitting. Therefore, it is suggested that the greater inward movement of condensate into grape berries exposed to longer delay periods, resulted in the higher levels of split.

During the simulated shelf life period, berry split further increased, and grapes delayed for 12 hours before cooling showed significantly less berry split than grapes delayed for 6 or 18 hours. This is difficult to explain. The increase in berry split when the storage temperature is raised to 10°C, has been explained in experiment 1.

Grapes delayed for 6 hours before the onset of FAC showed significantly higher levels of *Botrytis* decay after five week's storage at -0.5°C, compared to grapes delayed for extended periods. This can be explained in terms of the amount of SO_2 which is released from the SO_2 generator at high temperatures (J.F. Fourie, Pers. Comm.). SO_2 gas released by SO_2 sheets kills the conidia of *Botrytis cinerea* Pers. on the surface of the berry (Harvey, 1955). Couey and Uota (1961) showed that the toxicity of SO_2 increases about 1.5 times for each 10°C rise in temperature between 0°C and 30°C. Thus, delay periods of 12 and 18 hours at 23.4°C could have increased the toxicity of the SO_2, which would result in more effective control of *Botrytis* decay, compared to a delay period of only 6 hours. After the shelf life period, no significant ($P>0.05$) difference regarding *Botrytis* decay could be detected between the different treatments.

CONCLUSION

FHR for 1.5 hours at 19°C prior to packing had no beneficial or adverse effect on berry split and berry abscission. Although FHR resulted in grapes with lower levels of *Botrytis* decay and stem desiccation after five weeks cold storage at -0.5°C, no beneficial effect was evident after the simulated shelf life period, which is indicative of quality received by the consumer. Therefore, while the retailer would benefit from the use of FHR, it is doubtful whether or not the quality advantage would be evident to the consumer. For producers who do not want the expenses of setting up a FHR chamber, these results suggest that it would benefit quality by harvesting 'Sultanina' during the coolest part of the day or during the night (Làszlò and Saayman, 1992).

Delay periods prior to packing aggravated berry abscission, but did not influence berry split significantly. Grapes delayed for 12 hours showed a significant increase in berry abscission and *Botrytis* decay, compared to grapes delayed for only 3 or 8 hours. Considering that the absence of fungal decay is the most important quality prerequisite in table grapes (Ginsburg and Combrink, 1972), it is of vital importance to pack grapes with as short a delay period as possible.

Grapes packed in non-perforated liners and delayed for different durations after packing, before the onset of FAC, showed significant differences regarding the incidence of berry split. Grapes delayed for 18 hours before FAC commenced, had significantly higher levels of berry split directly after the delay period, compared to grapes delayed for 6 or 12 hours. After the simulated shelf life period, levels of berry split were approximately 114% higher than at the beginning of shelf life, which indicated that breaks in the cold chain stimulated splitting. No significant difference in berry abscission occurred between grapes delayed for different periods. Although grapes delayed for 6 hours showed significantly more *Botrytis* decay after five week's storage at -0.5°C, no significant ($P>0.05$) difference in *Botrytis* decay could be detected between the different treatments after the shelf life period. To minimise the amount of berry split, FAC should be applied as rapidly as possible after the packing of grapes in non-perforated liners.

Finally, for the consistent supply of high-quality table grapes that will ensure success in a highly competitive market, inputs do not stop at harvest. To ensure that the quality is maintained until the product is consumed, it is imperative to commence cooling of the grapes to -0.5°C as soon as possible. Delays before or after packing must be avoided. After FAC, good cold storage management without temperature fluctuations is important. The possible beneficial effects of FHR will have to be tested using grapes harvested at temperatures above 30°C.

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Table 1 : Effect of a 3, 8 and 12 hour delay period at 25.8°C before packing, on the overall quality of 'Thompson Seedless' table grapes, as examined directly after the delay periods, after five weeks storage at -0.5°C, and after five weeks cold storage and a shelf life of one week at 10°C.

Parameters	Delay periods (h)			Prob > F[1]
	3	8	12	
Directly after delay period				
Moisture loss (%)	0.38[a]	0.89[b]	0.92[b]	0.001
Berry abscission (%)	1.95[a]	1.62[a]	3.47[b]	0.003
After 5 weeks storage at -0.5°C				
Moisture loss (%)	0.70	0.76	0.43	0.330
Berry split (%)	0.93	1.02	0.72	0.776
Berry abscission (%)	0.71[a]	0.68[a]	1.15[b]	0.020
Stem condition[2]	1.75	1.88	1.50	0.493
Botrytis (%)	0.58[a]	0.13[a]	1.93[b]	0.001
After 5 weeks storage at -0.5°C + 1 week at 10°C				
Moisture loss (%)	0.74	0.73	1.05	0.535
Berry split (%)	3.47	4.38	3.81	0.870
Berry abscission (%)	0.40	0.41	0.27	0.711
Stem condition[2]	2.25	2.44	2.88	0.262
Botrytis (%)	3.40[a]	1.31[a]	7.04[b]	0.005

1 One-way ANOVA table with completely randomised design.
2 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

Table 2 : Effect of field heat removal at 19°C and delay periods of 6, 12 and 18 hours at 23.4°C after packing on the overall quality of 'Thompson Seedless' table grapes, as examined directly after the delay periods, after five weeks storage at -0.5°C, and after five weeks cold storage and a shelf life of one week at 10°C.

Parameters	Field heat removal (A)[1]		Delay periods (B)[2]			Prob > F[3]		
	No	Yes	6h	12h	18h	A	B	A x B
Directly after the delay period								
Moisture loss (%)[4]	0.35	0.30	0.25[a]	0.27[a]	0.46[b]	0.273	0.001	0.123
Berry split (%)	0.24	0.80	0.26[a]	0.08[a]	1.23[b]	0.091	0.013	0.195
Berry abscission (%)	2.16	2.40	2.11	2.74	2.00	0.378	0.058	0.603
After 5 weeks storage at -0.5°C								
Berry split (%)	4.51	3.24	2.23[a]	4.21[ab]	5.18[b]	0.140	0.022	0.732
Berry abscission (%)	1.95	1.94	1.80	2.22	1.83	0.974	0.456	0.166
Stem condition[5]	2.38[b]	1.98[a]	2.18	2.21	2.14	0.021	0.941	0.380
Botrytis (%)	2.75[b]	1.96[a]	3.14[b]	1.82[a]	2.10[a]	0.042	0.017	0.573
After 5 weeks storage at -0.5°C + 1 week at 10°C								
Berry split (%)	6.55	8.33	8.44[b]	5.08[a]	8.81[b]	0.051	0.002	0.115
Berry abscission (%)	1.61	1.66	1.90	1.69	1.31	0.863	0.269	0.355
Stem condition[5]	2.31	2.38	2.14	2.39	2.50	0.686	0.245	0.359
Botrytis (%)	2.31	2.19	2.21	1.79	2.75	0.830	0.324	0.939

1 Data pooled across 'Delay periods'.

2 Data pooled across 'Field heat removal'.

3 Two-way ANOVA table with complete randomised factorial design for factor A (field heat removal) and factor B delay periods).

4 Moisture loss was only determined directly after the delay period.

5 Stem condition was ranked from 1 – 5, where 1 = green stems and 5 = dry, brown stems.

PAPER III - The influence of storage duration and elevation of storage temperature on the development of berry split and berry abscission in 'Thompson Seedless' table grapes.

THE INFLUENCE OF STORAGE DURATION AND ELEVATION OF STORAGE TEMPERATURE ON THE DEVELOPMENT OF BERRY SPLIT AND BERRY ABSCISSION IN 'THOMPSON SEEDLESS' TABLE GRAPES.

ABSTRACT

'Thompson Seedless' grapes, packed in non-perforated polyethylene bags, were cold stored at -0.5°C and 83% RH for 0, 1, 2, 4 and 8 weeks. After storage at -0.5°C for these periods, the grapes were either held at -0.5°C for another two or five days, or grapes were transferred to 10°C and 88% RH for two or five days. The polyliners were kept closed during the different storage periods. Fruit quality examinations were conducted after the specified periods at -0.5°C, and after each additional two and five days at both temperatures. Berry split increased with prolonged storage at -0.5°C. A linear function (y = 0.58x - 1.14) with a R^2-value of 0.97 described this increase in berry split over 61 days storage at -0.5°C. Transferring the grapes from -0.5°C to 10°C resulted in a further increase in berry split. Grapes stored for 0, 1, 2 and 4 weeks at -0.5°C followed by 5 days at 10°C, showed a significant increase in berry split, compared to grapes stored only at -0.5°C for the same period. Although significant differences in berry abscission occurred, no definite trend was observed that could be ascribed to storage period at -0.5°C or to an elevation in storage temperature from -0.5°C to 10°C.

KEYWORDS : 'Thompson Seedless', storage temperature, storage duration, berry split, berry abscission

INTRODUCTION

Grapes are highly perishable commodities, and their market life is a function of time and temperature, with the degree of deterioration related directly to the length of exposure to higher temperatures, regardless of when exposure occurs (Nelson, 1979).

The period between harvest and marketing of table grapes can be divided into different phases, and each phase can play a vital role in maintaining fruit quality. The time period and temperature of the phase between harvest and packing, as well as between packing and the onset of forced-air cooling (FAC), can determine the difference between top-quality and unacceptable quality fruit (Combrink *et al.*, 1975; László and Saayman, 1992). The duration of the FAC process can also have a significant influence on the market quality of the grapes (Ginsburg and Combrink, 1972). In South Africa, grapes packed in non-perforated polyethylene

bags are subjected to FAC which reduces grape temperature to -0.5°C within a maximum of 72 hours. The grapes are mainly cooled by means of conduction, since the cold air forced through the cartons on pallets, is prevented from direct contact with the grapes by the bags (Ginsburg *et al.*, 1978). After FAC has lowered the product temperature to -0.5°C, the cold storage phase during transport and the accumulation period is also of vital importance in determining post-storage quality. This is mainly due to the length of the period, and the risk of temperature fluctuations which may occur.

Following FAC, fruit can be accumulated for 3 to 12 days at -0.5°C before being loaded onto ships. Sea export of South African table grapes to Middle East markets takes approximately 14 days, to European markets it takes more or less 16 days, and to the Far East and USA the shipping period can last 22 days. Additional to this, depending on market conditions, South African grapes can be held for a further 7 to 18 days overseas before being sold to the retailer. The extended periods of sea freight, and the potential requirement to roll stocks until there is sufficient demand to ensure profitable returns, has resulted in a pressing need to increase the storage life of table grapes under refrigerated conditions, so that fruit can be marketed for longer periods.

Table grape cultivars have different cold storage potentials, which can vary from 5 to 10 weeks (Ginsburg, 1965). A cultivar's storage potential is determined by its susceptibility to quality defects during cold storage, such as physiological disorders, fruit rot and desiccation. Decay excluded, berry split and berry abscission are the primary disorders which shorten the storage potential of export 'Thompson Seedless' table grapes. This study was conducted to determine the effects of cold storage duration at -0.5°C, and an elevation in temperature to 10°C after various periods at low temperature, on the development of berry split and berry abscission in 'Thompson Seedless' table grapes.

MATERIALS AND METHODS

The trial was conducted in 1998 on 'Thompson Seedless' from a vineyard in the De Doorns area in the Western Cape, South Africa. The trial block was planted in 1974, on Richter99 rootstock. The vines were trained onto a slanting trellis system with a 1.8 x 2.7m spacing, and irrigated by means of a sprinkler irrigation system. The recommended gibberellic acid (GA_3) sprays for 'Thompson Seedless' were applied (Wolf *et al.*, 1991). This comprised three sprays of 5ppm during flowering, and three sprays of 20ppm when the berries reached a diameter between 4

and 8mm. The vines were girdled when the berries were between 4 and 6mm in diameter. Normal cultural practices were followed as far as irrigation, fertilisation, cluster preparation, pest and disease control and foliage management were concerned.

'Thompson Seedless' grapes were harvested with total soluble solids (TSS) of 16.9°Brix and titratable acidity (TA) of 0.59%. The grapes were harvested between 6 and 7 a.m., when the average pulp temperature of the grape berries was 16.6°C. The grapes were packed in 4.5 kg closed-top corrugated fibre board cartons, with individual bunches in plastic carrybags, and the entire carton content enclosed in a non-perforated low density polyethylene liner with a thickness of 20µm. A 'Uvasys' sulphur dioxide (SO_2) generator sheet (supplied by Grapetek, South Africa) to control decay was enclosed in each of the polyliners, positioned on top of the grapes. Polyethylene liners containing the grapes and the 'Uvasys' SO_2 sheet were folded, closed with gum tape and placed under passive cooling within 3 hours after packing. Samples were cold stored at -0.5°C and a relative humidity (RH) of 83% for 0, 1, 2, 4 and 8 weeks. After storage at -0.5°C, the grapes were either stored for another two or five days at -0.5°C, or the grapes were transferred to 10°C and 88% RH for two or five days. The polyethylene bags were kept closed during the different storage periods.

For measurement of TSS and TA at harvest, 50 randomly selected berries from five randomly selected grape bunches were used. The berries were juiced in a liquidiser and filtered. The percentage soluble solids of the filtrate, expressed as degree-Brix, was measured using a bench top Atago Palette PR100 digital refractometer with automated temperature compensation. Because the acid fraction of the total soluble solids is usually very small compared to the sugar fraction, the soluble solids were considered as the sugar content. TA was determined by titrating a 10g aliquot of juice with 0.1N NaOH to a pH end-point of 7, using an auto Metrohm 665 Dosimat titrator. Pulp and ambient temperatures were recorded with a probe connected to a Kane-May 22 digital thermometer, while RH was measured using a whirling hygrometer to determine the wet and dry bulb temperatures. RH was then read from a psychrometric chart.

Fruit quality was assessed at ambient temperature (±24°C), after the different weeks at -0.5°C, as well as after the additional two or five days at -0.5°C or 10°C. Grapes were examined for berry split and berry abscission. Berry split was recorded as such when any split, whether restricted to the epidermis, or deeper into the mesocarp, was visibly evident. Artificial berry split due to external stresses from manual or mechanical sources, e.g. berry split caused by excessive pressure on the fruit during lidding, was not taken into account. Regarding berry abscission, all the berries with "dry" scars remaining in the carry bag on removal of the bunch,

were recorded. Berries that were torn loose due to improper handling or berry split around the pedicel end, were not recorded. All of the grapes in each carton were examined. The incidence of each defect was expressed as a percentage of the total grape mass per carton.

The experimental design was completely randomised. Each treatment was replicated five times, with one carton constituting a single replicate. Data were subjected to an analysis of variance using the General Linear Means (GLM) procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

The incidence of berry split increased with prolonged storage at -0.5°C (Figure 1). This increase was often significant. A linear function ($y = 0.58x - 1.14$) with a R^2-value of 0.97 described this increase in berry split with storage at -0.5°C (Figure 2). During the first week of storage at -0.5°C, the incidence of berry split was very low (Figure 1). A rapid increase in berry split commenced from the second week of storage at -0.5°C. After approximately 8 weeks of storage at -0.5°C (day 55), no further increase in berry split occurred. Transferring grapes from -0.5 to 10°C consistently resulted in a rapid increase in berry split. Even after only two days of storage at 10°C, an increase in berry split relative to levels at -0.5°C was evident at each transfer. Grapes stored for 0, 1, 2 and 4 weeks at -0.5°C followed by 5 days at 10°C, showed a significant increase in berry split, compared to grapes stored only at -0.5°C for the same period. After 8 weeks there was no significant difference in berry split between grapes stored only at -0.5°C, and grapes stored for 8 weeks at -0.5°C followed by 2 or 5 days at 10°C. The levelling off of berry split after 8 weeks at -0.5°C possibly signifies the stage at which most berries which were susceptible to split, had in fact developed the disorder. This is supported by the fact that transfer of grapes to 10°C after 8 weeks at -0.5°C did not result in a further significant increase in split, whereas the increase following transfer was significant at all earlier stages. Although there were sometimes significant differences in the occurrence of loose berries, especially for grapes only stored at -0.5°C, no definite trend was observed that could be ascribed to storage period at -0.5°C, or to an elevation in storage temperature from -0.5°C to 10°C (Figure 3).

The various phases from harvest through packing and cooling are associated with moisture loss through the process of transpiration. Transpiration refers to internal water loss through stomata, the cuticle or lenticels (Salisbury and Ross, 1985). In thermodynamic terms, the air that encloses the grape bunches inside the non-perforated bag can be defined as the surroundings of the grapes (Wills *et al.*, 1998). The grapes are separated from the surroundings by a

boundary layer that envelops each grape berry. In this layer, the water vapour pressure is approximately in equilibrium with that of the produce itself. The driving force for transpiration is the difference in water vapour pressure between the inside of the fruit and its surroundings (Mitchell, 1978). At the onset of cooling, the grapes were at a temperature of approximately 16.6°C, while the surroundings were at a temperature of -0.5°C. Water vapour pressure increases rapidly with temperature, therefore the grapes had a higher vapour pressure than the surroundings. Consequently, the grape berries would have lost moisture by means of transpiration, due to the vapour-pressure difference that existed between the grapes and the surroundings. During the cooling process, the moist air that surrounds the grapes beyond the boundary layer is cooled, and dew point is reached when the air becomes saturated with water vapour, and a RH of 100% is reached. With further cooling, the plastic liner is the first surface on which condensation takes place, as it is in direct contact with the cold air in the room. The berry takes longer to cool down as its high heat capacity prevents a rapid decline in temperature (much slower than the decrease in temperature of the air inside the bag). The inner surface of the liner is in contact with the grapes, therefore free moisture develops on the fruit surface due to condensation from the inner side of the liner. Due to solutes (i.e. sugars) present in the grape berries and because of moisture loss at the onset of cooling, the grape berries exhibit a lower water potential than the water on the fruit surface. Thus, water diffuses back into the berries in response to this water potential gradient, from the boundary layer, through the cuticle into the epidermal cells. An increased turgor pressure develops in the epidermal cells. According to Considine and Kriedemann (1972), Wade (1988) and Sekse (1995), turgor pressure plays an important role in fruit splitting. Osmotic entry of excessive water into the epidermal cells causes rupture of the cells. These results suggest that with prolonged storage at -0.5°C, the number of berries that absorb excessive water increase, resulting in increased levels of berry split.

The increase in berry split over time in storage at -0.5°C was almost linear. A consequence of establishing such a relationship between the incidence of berry split and a quantifiable factor such as days of storage at -0.5°C, is the facility for predicting the potential incidence of the disorder based on data collected during early storage. This has commercial value, since accurate prediction of berry split can be used to segregate grapes according to berry split risk. To reduce the risk, it will be possible to sell high risk product first.

When the storage temperature is raised from -0.5°C to 10°C, the temperature of the moist air surrounding the grape bunches increases more rapidly than the temperature of the grape berries. The warmer air closest to the grape berries in the boundary layer is therefore cooled down by the colder grape berries. As the moist air cools down, dew point is reached at a certain

temperature and condensation is initiated. Therefore, additional to the condensation that occurs at the onset of cooling, more free moisture develops on the grapes when the storage temperature is raised. Consequently, more moisture is available to be absorbed osmotically by the grapes. An increase in turgor pressure inside the grape berries due to excessive water absorption, can aggravate the problem of berry split considerably. As opposed to this, the rate of water absorption rather than the amount of water, can be the predominant factor causing the berry split. According to Verner (1937), Cline *et al.* (1995) and Belmans and Keulemans (1996), cracking of cherries appeared to be related more to the rate of water absorption than to the quantity of water that accumulates. It is postulated that the rate at which water is distributed within the berry (i.e. between cells) can also determine the amount of berry split. The possibility exists that only the superficial cells actually absorb the water, therefore leading to excessive turgor in those cells only, and the cells further in from the epidermal cells may not receive any of the absorbed water before splitting occurs due to a slow rate of distribution of the absorbed water. It is suggested that this postulation is the reason why berry split levels on 'Thompson Seedless' grapes increase significantly upon transfer to higher storage temperatures, as was the case in this study.

Although not shown in this study, it is evident that different populations of grapes have a different berry split and berry abscission potential. It is still uncertain what all the pre-harvest factors are which predispose grapes to berry split, and therefore no strategy exists to guarantee a population of grapes completely resistant to berry split. Consequently, to rapidly solve the berry split problem which has a serious negative impact on consumer confidence and the price of the product, research should concentrate on ways to control moisture in the grape cartons, i.e. moisture absorbing materials and/or perforated bags.

CONCLUSIONS

Two storage related factors have a significant influence on the incidence on berry split in 'Thompson Seedless' grapes during cold storage, *viz.* the duration of storage at -0.5°C, as well as an increase in temperature after low temperature storage. Berry split increased almost linearly with prolonged storage at -0.5°C. An elevation of storage temperature from -0.5°C to 10°C any time during the cold storage period, further aggravated the split problem. Consequently, the reduction of berry split in 'Thompson Seedless' table grapes during cold storage requires (a) the shortest possible cold storage period, and (b) good temperature management throughout distribution, from initiation of cooling until the final point of sale.

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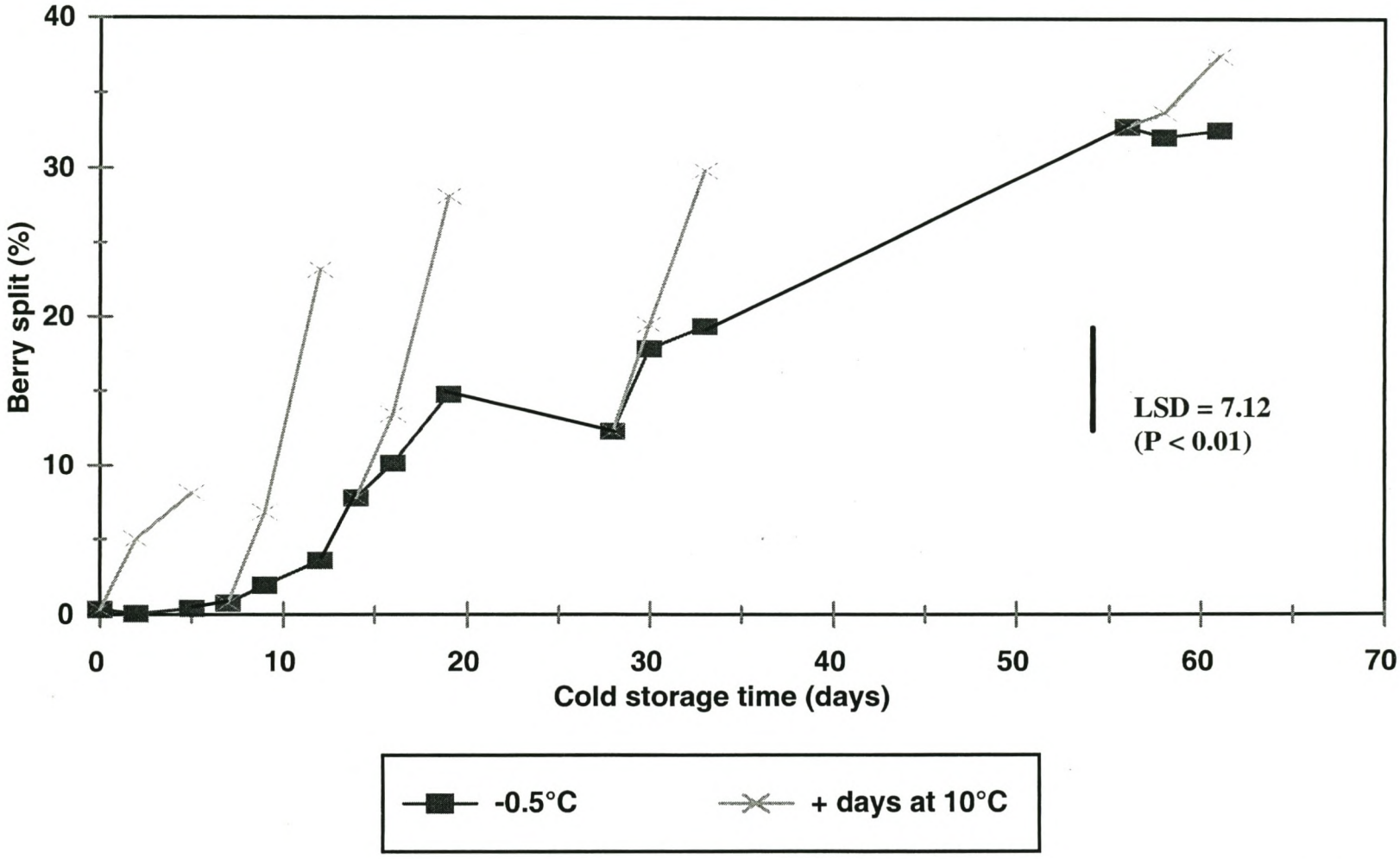

Fig. 1 : The effect of storage time at –0.5°C, and an elevation in temperature to 10°C after 0, 1, 2, 4 and 8 weeks, on the development of berry split in 'Thompson Seedless' table grapes.

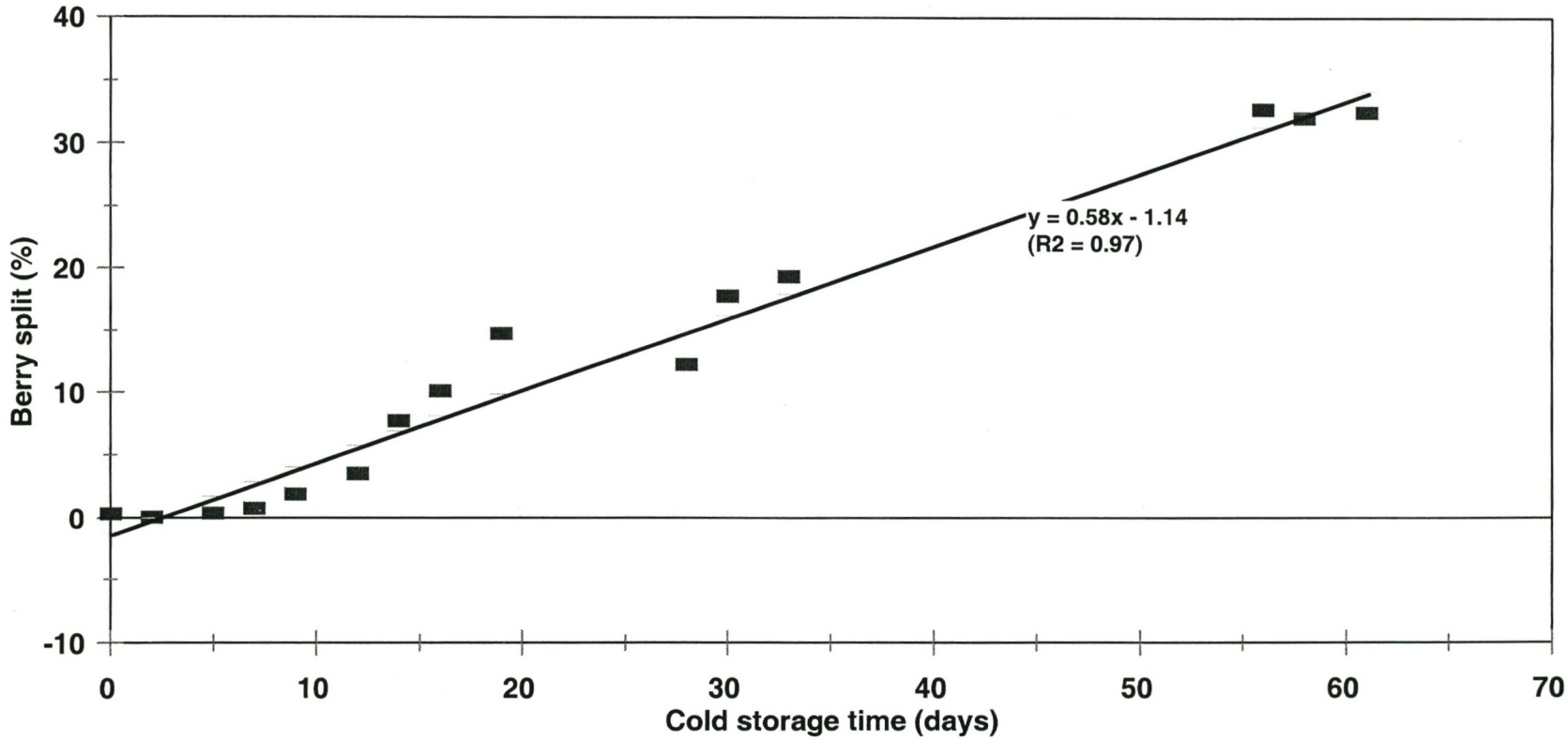

Fig. 2 : Regression analysis on berry split over storage time at –0.5°C for 'Thompson Seedless' table grapes.

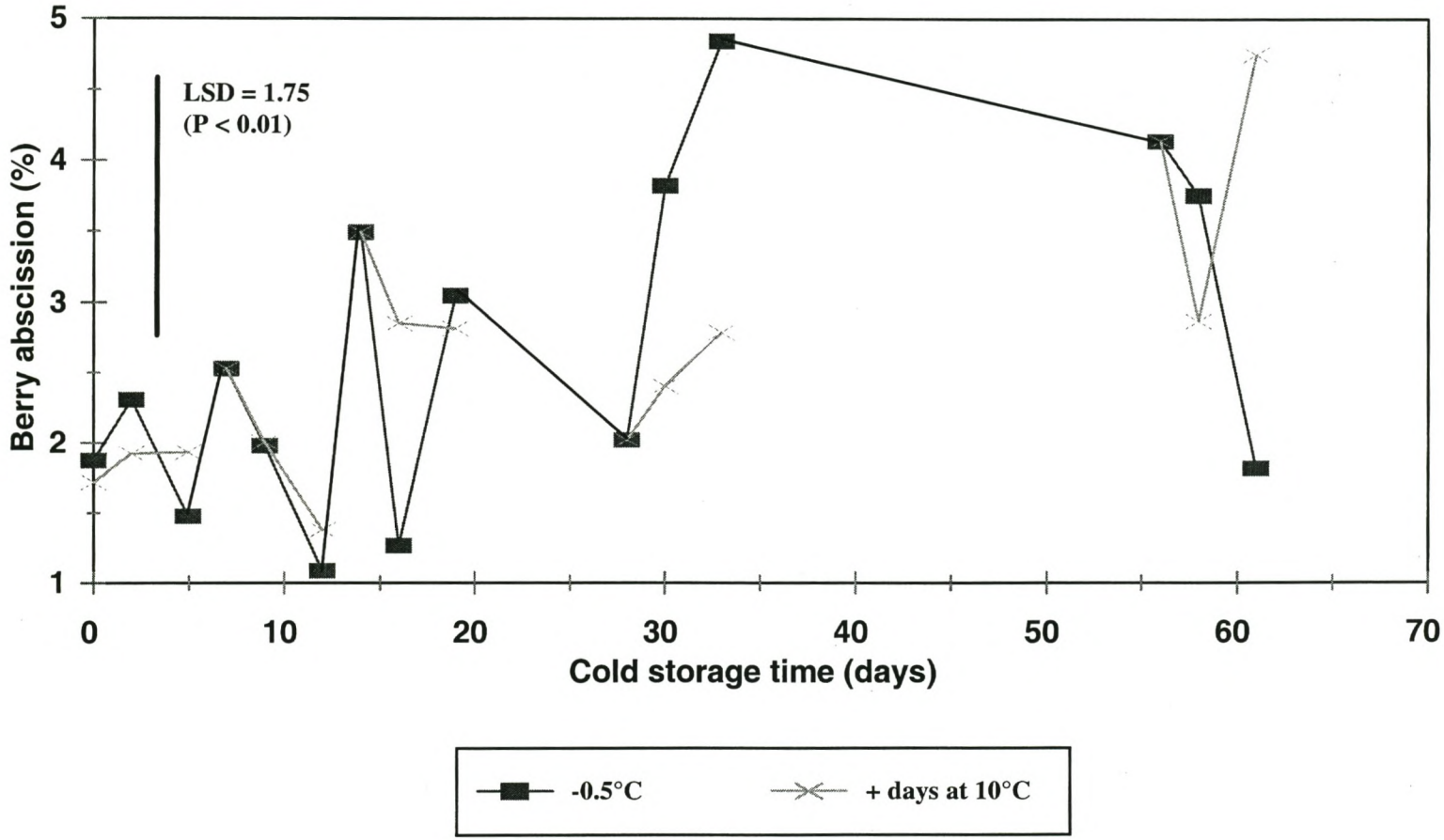

Fig. 3 : The effect of storage time at –0.5°C, and an elevation in temperature to 10°C, after 0, 1, 4 and 8 weeks, on the development of berry abscission in 'Thompson Seedless' table grapes.

PAPER IV - Berry abscission in 'Waltham Cross' table grapes : changes in abscission related factors during berry development and the influence of the application of pre-and postharvest ethylene inhibitors.

BERRY ABSCISSION IN 'WALTHAM CROSS' TABLE GRAPES : CHANGES IN ABSCISSION RELATED FACTORS DURING BERRY DEVELOPMENT AND THE INFLUENCE OF THE APPLICATION OF PRE-AND POSTHARVEST ETHYLENE INHIBITORS

ABSTRACT

During the 1999 season, changes in total soluble solids (TSS), titratable acids (TA), pedicel diameter, berry diameter, berry mass and fruit removal force (FRF) were determined at bi-weekly intervals, from 27 until 111 days after full bloom (DAFB), for 'Waltham Cross' table grapes. At each assessment stage, grape bunches were detached and held in the dark at ±25°C for 80 hours. Thereafter, moisture loss, FRF, berry abscission potential as well as the percentage berries that abscised, were determined. During stage I and II of fruit growth (27 to 54 DAFB), TSS did not change significantly, while TA increased during this period. The FRF increased significantly during this early stage of berry development, indicating a strengthening of the abscission zone tissue. During stage III (after 54 DAFB), a decline in FRF occurred, which coincided with a perceptible increase in TSS and a decrease in TA. Berry mass increased significantly from 27 to 111 DAFB. Pedicel diameter increased significantly from 27 to 41 DAFB and then remained constant, while berry diameter increased significantly for the period 27 to 97 DAFB. Grapes sampled at 27 DAFB had a significantly lower FRF and significantly higher levels of berry abscission and moisture loss after the 80h-period in the dark, compared to grapes sampled at a later stage. At 27 DAFB, the abscission zone developed between the pedicel and the rachis, thereafter it developed between the pedicel and the berry. Although the FRF did not change significantly as the berries ripened from 83 to 111 DAFB, the abscission potential and the percentage berries that abscised were significantly higher for grapes harvested at 83 DAFB at a TSS of 12.3°Brix than for grapes harvested more mature, at a higher TSS level. Moisture loss correlated highly significantly ($P<0.0001$) with berry abscission, with a correlation coefficient of 0.84. Berry abscission also correlated highly significantly ($P<0.0001$) with abscission potential, pedicel and berry diameter, FRF at sampling, FRF after 80h and berry mass. In both 1999 and 2000, preharvest applications of ReTain™ (a derivative of aminoethoxyvinylglycine), which inhibits ethylene synthesis, did not reduce the incidence of postharvest berry abscission in 'Waltham Cross' table grapes . A postharvest treatment with EthylBloc® (1-methylcyclopropene),which inhibits ethylene action, only reduced berry abscission during the 1999 season.

KEYWORDS : 'Waltham Cross', berry abscission, fruit removal force, ReTain™, EthylBloc®

INTRODUCTION

During the development of the grape berry a number of profound physiological and biochemical changes occur. Three phases have been identified during berry development (Coombe, 1973). The duration and manifestation of each growth period varies according to cultivar and environmental conditions. Stage I is characterized by a rapid increase in berry size, firstly due to cell division, followed by cell enlargement (Coombe, 1976). During stage I, chlorophyll is the predominant pigment and berries display active metabolism, with high rates of respiration and rapid acid accumulation (Winkler *et al.*, 1974). The level of sugars remain almost constant and the berries remain very firm. Stage II is a slow growth phase. During this phase the berries lose chlorophyll and reach maximum acid levels. Stage III extends from the beginning of ripening until the grapes are fully ripe, and is characterized by a sudden change in appearance and composition of the berries. During this stage there is an acceleration of growth, a decrease in berry firmness and an increase in deformability, as well as an increase in glucose and fructose (Coombe, 1973). Furthermore, decreases in the concentration of organic acids, loss of chlorophyll from the skin, accumulation of anthocyanins and a decrease in respiration rate take place during this stage (Winkler *et al.*, 1974).

The development of abscission at the abscission zone of fruit can be quantitatively indexed by measurement of the fruit removal force (FRF) (Wittenbach and Bukovac, 1974). However, there is an apparent lack of literature regarding changes in FRF during the different phases of table grape berry development. Consequently, the association between FRF and the biochemical and physiological changes which occur during berry development are not known. The first objective of this trial was to determine how FRF is influenced by changes in abscission-related physiological factors during the development of 'Waltham Cross' berries.

There are several types of berry abscission that occur during storage and transit, namely : abscission from brittle or weak stems; abscission in which broken vascular strands are pulled from the berry ("wet drop"); and abscission in which an abscission layer is formed to separate the berry from the pedicel ("dry drop") (Winkler *et al.*, 1974). In 'Waltham Cross' table grapes, postharvest berry abscission results primarily from the formation of an abscission layer at the attachment of the berries (Boyes and De Villiers, 1933). The berries separate effortlessly and neatly from the pedicels, leaving a "dry" wound.

A substantial amount of literature supports the involvement of ethylene in abscission of fruit (Brown, 1997). Strong evidence exists that ethylene accelerates fruit abscission (Burg, 1968;

Brown, 1997). In many cases, the levels of natural ethylene, and/or its precursor, 1-amino-cyclopropane-1-carboxylic acid (ACC), are higher during periods of abscission (Burdon and Sexton, 1993). In such cases, the rise in ethylene production is thought to be responsible for promoting abscission. When the ripening-related increase in ethylene production was blocked by antisense ACC oxidase in cantaloupe melon fruits, the fruit abscission zone was not activated and no fruit abscission occurred (Ayub *et al.*, 1996).

Berry and Aked (1996) and Yun and Lee (1996a) suggested that the presence of ethylene after harvest may stimulate berry abscission during storage. Ge *et al.* (1997) found that the potential for ethylene release from 'Thompson Seedless' grapes during cold storage was high. Because of the low permeability of the cuticle (Barmore and Briggs, 1972), the pedicel-fruit abscission zone may be the least resistant to ethylene diffusion from the fruit. Wu *et al.* (1992) and Ge *et al.* (1997) reported that for harvested grapes, the ethylene production rates of the cluster stalk, including most of the pedicel, were markedly higher than those of the fruits, and showed climacteric-like changes. The rate of abscission was significantly reduced when grapes were stored at 0°C in the presence of an ethylene scrubber (Yun *et al.*, 1995; Yun and Lee, 1996a,b).

Various chemicals have been reported to delay, reduce or prevent the deleterious effects of endogenous and exogenous ethylene on fruit quality, including abscission. Aminoethoxyvinylglycine (AVG) can delay the initial development of an abscission zone (Ward *et al.*, 1999), or it can inhibit fruit abscission by suppressing ethylene biosynthesis (Nito, 1985). Spraying grape bunches before or during flowering with AVG inhibited flower and berry abscission in 'Kyoho' grapes (Kim *et al.,* 1999). ReTain™ is another recently developed commercial product, and is a derivative of AVG, which suppresses the production of ethylene (Mitcham *et al.*, 1998). Preharvest applications of ReTain™ delayed maturation of 'Bartlett' pears by inhibiting ethylene biosynthesis. For many plants, silver thiosulfate (STS) has been shown to reduce or prevent leaf and fruit abscission caused by ethylene (Hoyer, 1998; Veen, 1983). Reports exist where silver STS completely terminated abscission for periods of one to several weeks (Goszczynsla and Zieslin, 1993; Sexton *et al.*, 1995), implying that ethylene is essential for abscission to occur (Sexton, 1997). Because STS contains silver, which is considered as a potent environmental pollutant, its commercial use has some environmental restrictions (Serek and Reid, 1993). A gaseous compound, 1-methylcyclopropene (1-MCP), which inhibits ethylene action in plants by binding irreversibly to ethylene receptors, inhibited a range of plant responses to ethylene, including ethylene-induced ripening and flower abscission (Serek *et al.*, 1994). 1-MCP is commercially available as EthylBloc®.

Since postharvest berry abscission in 'Waltham Cross' grapes is a disorder which negatively affects quality, the second objective of this study was to establish if preharvest applications of ReTain™ or postharvest treatment with EthylBloc®, could reduce levels of berry abscission in 'Waltham Cross' table grapes.

MATERIALS AND METHODS

Trials were conducted during the 1999 and 2000 seasons in a 'Waltham Cross' (clone 22) vineyard in the Paarl area in the Western Cape, South Africa. The grapes were planted in 1994, on Richter 110 rootstocks, at a spacing of 3 x 1.5m. The vines were trained onto a double gable system and irrigated by means of micro-jet irrigation. Normal cultural practices were followed regarding irrigation, fertilisation, cluster preparation, pest and disease control and foliage management.

Experiment 1 : Changes in abscission-related factors during berry development

During the 1999 season, changes in pedicel diameter, berry diameter, berry mass and FRF were determined at 14 days intervals, from 27 until 111 days after full bloom, in the 'Waltham Cross' trial block.

For the measurements of pedicel and berry diameter, 9 randomly selected bunches in the vineyard were marked. On each bunch, five berries were labelled at random. For both measurements, the average of the five berries per bunch was considered a single replicate, with each of the nine bunches comprising a single replicate. Berry and pedicel diameter were measured using a Mitutoyo digital calliper.

For the measurement of FRF, an additional nine bunches were collected randomly from the vineyard at each sampling time. Abscission was quantitatively indexed by measuring the FRF. The FRF was measured on 5 berries randomly selected per grape bunch. The berries were detached, with their pedicels still attached. The average FRF-reading of the five berries per bunch was considered a single replicate, which was replicated nine times. The tensile force required to remove a grape berry from its pedicel was measured with a specially designed clamp attached to the Stable Micro Systems TA-XT2 texture analyser. The TA-XT2 was pre-set to apply a tensile force on the pedicel of the berry over a distance of 7mm. The force, in Newton, necessary to detach the pedicel from the berry, was measured directly after sampling.

Thereafter, the grape bunches were held in the dark at ±25°C for 80 hours to stimulate abscission, according to the method used for sour cherry (Wittenbach and Bukovac, 1973). After this period, the FRF was measured again, as well as the amount of berry abscission which occurred. All the berries found detached, as well as the berries that became detached when the bunch was shaken for 10 seconds, were recorded for berry abscission. The percentage mass of the abscised berries relative to the bunch mass was recorded as the percentage berry abscission. According to Wittenbach and Bukovac (1974), fruit abscission potential, i.e. the capacity of the fruit to form an abscission layer after being detached from the plant, can be indexed by the decrease in FRF during the 80 hour period. Therefore, berry abscission potential was indexed by the difference between the FRF at sampling and the FRF after 80 hours in the dark, expressed as a percentage of the FRF at sampling. Moisture loss during the 80 hour period was also determined. The nine grape bunches were weighed at sampling as well as after the 80 hour period. It was assumed that the loss in fresh mass during this period was mainly due to moisture loss. Moisture loss was presented as a percentage of the initial fresh mass measured at harvest.

Grape maturity at each sampling time was determined by measuring TSS and TA. For these measurements, three randomly selected bunches were removed from the vineyard at each sampling time. Fifty randomly selected berries from each bunch were juiced in a liquidiser and filtered. The TSS of the filtrate, expressed as degree-Brix, was measured using a bench top Atago Palette PR100 digital refractometer with automated temperature compensation. Titratable acidity, expressed as percentage tartaric acid, was determined by titrating a 10g aliquot of juice with 0.1N NaOH to a pH end-point of 7, using an auto Metrohm 665 Dosimat titrator. From the same three bunches, 100 berries per bunch were randomly selected and weighed to determine the average berry mass.

The experimental design was completely randomised with nine replicates for all parameters measured, except TSS, TA and berry mass measurements which were only replicated three times. All statistical analyses on the data were performed according to the General Linear Means (GLM) and correlation (CORR) procedures in the Statistical Analysis System (SAS) (SAS Institute Inc., 1990). In the case of correlations, parameters measured across sampling times were compared to levels of berry abscission, also across sampling times.

Experiment 2 : Pre-and postharvest applications of ethylene inhibitors

During the 1999 season, ReTain™ (Abbott Laboratories, SA) was applied either one, two or

three weeks prior to initiation of harvest, at a concentration of 5 or 10 grams per 10 litres of water. During the 2000 season, the same concentrations were applied only at one and two weeks before anticipated harvest maturity. The ReTain™-treated vines were sprayed in the morning using a hand-operated knapsack sprayer, and spray coverage of the bunches and leaves to runoff was ensured.

To determine if the ethylene inhibitor ReTain™ had any influence on maturation, maturity in terms of TSS, TA and berry skin colour (only in the 1999 season) was measured at harvest. Samples of 100 berries per replicate, with six single bunch replicates, were randomly collected per treatment for the abovementioned measurements. The TSS and TA were determined as described for experiment 1. A Nippon NR3000 colorimeter was used for objective skin colour determination. For each replicate, measurements were taken of five berries and the mean value determined. Colour was expressed in terms of hue angle (h°), L*-value and chroma (C*). The colour space coordinates a* and b* were used to calculate the h° and C* (colour intensity) values. Hue angle refers to the angle formed by the line from the origin to the intercept of the a* (x-axis) and b* (y-axis) coordinates, where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992). L* is a measurement of the lightness of the fruit and its value may range from black = 0 to white = 100. The amount of berry abscission (as described in experiment 1) that occurred at harvest, was also recorded.

On the day of harvest, half of the grapes of each preharvest treatment (6 bunches per preharvest treatment) were fumigated in a closed chamber (6 m^3) with 1.5g EthylBloc® in 30 ml buffer solution (0.9% NaOH/KOH) for 12 hours at ±25°C. EthylBloc® (Floralife, USA) is a powder that, when added to a buffer solution, releases the gas 1-methylcyclopropene (1-MCP). The other half of the grapes were also sealed in a closed chamber for 12 hours at ±25°C, but received no EthylBloc® treatment. After the treatment, the grape bunches were held in the dark at ±25°C for 80 hours, before determining berry abscission. During the 2000 season, the bunches were then closed in non-perforated polyethylene bags and stored for an additional 120 hours in the dark at ±25°C. The amount of berry abscission was again determined after this additional period of storage in the dark.

The experimental design was a randomised complete block, each block being replicated six times. Each individual plot consisted of four vines. The treatment design was a 7 x 2 factorial in 1999 and a 5 x 2 factorial in 2000. The treatments were combinations of two factors : 1) Preharvest sprays of ReTain™ : 5g ReTain™ applied 3 weeks before harvest (only in 1999), 10g ReTain™ applied 3 weeks before harvest (only in 1999), 5g ReTain™ applied 2 weeks

before harvest, 10g ReTain™ applied 2 weeks before harvest, 5g ReTain™ applied 1 week before harvest, 10g ReTain™ applied 1 week before harvest and the control; and 2) postharvest applications of 1.5g EthylBloc® in 30 ml buffer solution or no EthylBloc® treatment. A two-way ANOVA was used to analyse the data with the General Linear Means (GLM) procedure of the SAS program (SAS Institute Inc., 1990).

RESULTS AND DISCUSSION

Experiment 1 : Changes in abscission related factors during berry development

From 27 to 54 days after full bloom (DAFB), TSS did not change significantly, while TA increased during this period (Table 1). The FRF (at sampling), which measures the attachment of the pedicel to the berry, increased significantly ($P<0.0001$) during this early stage of berry development, indicating a strengthening of the abscission zone tissue. After 54 DAFB, a decline in FRF (at sampling) occurred, which coincided with a significant increase in TSS and a decrease in TA. As the berries matured from 83 to 111 DAFB, the force required to remove a berry from its pedicel did not change significantly. From 54 to 97 DAFB, the increase in TSS and the decrease in TA was significant. Berry mass increased significantly from 27 to 111 DAFB. The diameter of the pedicel only increased significantly from 27 to 41 DAFB, while berry diameter increased significantly for the period 27 to 97 DAFB.

At 27 DAFB, grape bunches had a significantly lower FRF at sampling and after 80h in the dark, compared to grapes sampled at a later stage (Table 1). These grapes also showed a high abscission potential of 75.0%. After the bunches had been subjected to 80 hours in the dark, almost all of the berries abscised (98.3%). At this stage, the abscission zone developed between the pedicel and the rachis. Grapes sampled from 41 to 111 DAFB, revealed significantly less berry abscission and during these stages of berry development, the abscission zone developed between the pedicel and the berry. As the FRF at sampling, and FRF after 80h, increased significantly from 27 to 54 DAFB, the abscission potential decreased. At 54 DAFB, FRF after 80h was even higher than FRF at sampling. However, after 54 DAFB, there was a decline in FRF, thus a lesser force was required to effect separation of the pedicel from the berry. At 83 DAFB, FRF (after 80h) decreased significantly, compared to grapes harvested at 69 DAFB or at 97 DAFB. This resulted in an unexpectedly high abscission potential and significantly more berries abscised, compared to the other sampling dates (excluding 27 DAFB). Moisture loss, as measured after the 80-h incubation period, was significantly higher for grapes

sampled at 27 DAFB, compared to grapes sampled at a later stage during berry development. Moisture loss correlated significantly ($P<0.0001$) with berry abscission, with a correlation coefficient of 0.84 (Table 2). Berry abscission also correlated significantly ($P<0.0001$) with abscission potential, pedicel and berry diameter, FRF at sampling, FRF after 80h in the dark and berry mass. However, moisture loss provided the best correlation with abscission.

With reference to the three stages of berry development as described by Coombe (1973, 1976), the period from 27 to 54 DAFB can be considered as part of stage I and II of berry development, with stage III commencing just after 54 DAFB. Additional to the sugar level that remained almost constant and the acid level that increased to its maximum, these two phases can also be characterised by an increase in FRF. Although the term veraison was used by French viticulturists to denote the change in colour of the skin of grape berries from green, it is convenient to use this term in a wider context to embody the group of developmental changes evident at this stage (Coombe and Bishop, 1980). These changes include renewed expansion in berry size, a reduction in firmness, a loss of chlorophyll, an accumulation of flavonoids in the skin, an increase in glucose and fructose, and a decrease in tartaric and malic acids. Based on these criteria, veraison occurred around 54 DAFB in the 'Waltham Cross' used in this study. The results also indicated that a decrease in FRF occurs at veraison, and that this decrease in FRF continues during stage III.

In fruit abscission, the cell wall changes associated with the development of the separation layer in the abscission zone, can involve hydrolysis or dissolution of pectin in the middle lamella, which results in loss of cementing effectiveness between contiguous cell walls, as well as degradation of all or part of cell wall substances, particularly cellulose and hemicelluloses (Baird and Webster, 1979). A reduction in cell wall pectin content, usually measured as uronic acid, accompanied by an increase in water-soluble pectin, occurs during ripening (Pilnik and Voragen, 1970). The enzymes most commonly associated with abscission include cellulases and pectinases, which hydrolyse cell wall constituents resulting in weakening of the cell wall, and several defense-related enzymes such as chitinase and 1,3-glucanase (Sexton *et al.*, 1985). The presence of the cell wall hydrolysing enzymes polygalacturonase and pectin-methylesterase in grape berry pulp may be responsible for the continuous decrease in total pectin substances during the ripening of grapes (Robertson *et al.*, 1980). Therefore, it is suggested that during ripening (stage III), there is an increase in the activities of cell wall-degrading enzymes that are responsible for the weakening of abscission zone tissue, and consequently a reduction in FRF.

Although the FRF (at sampling) did not change significantly as the berries ripened from 83 to 111 DAFB, the abscission potential and the percentage berries that abscised were significantly higher for grapes harvested at a TSS of 12.3°Brix than for grapes harvested more mature, at a higher TSS level. These results correspond with previous research done by Beyers (1936), who found that 'Waltham Cross' grapes harvested below 15°Brix are more susceptible to postharvest berry abscission than riper berries. Therefore, it is imperative to harvest 'Waltham Cross' grapes at optimum maturity, not only for better eating quality, but also to reduce the incidence of postharvest berry abscission.

Fabri and Betti (1982) distinguished between two abscission zones on the grape berry stem (pedicel) where abscission can occur. The one abscission zone is situated where the pedicel is attached to the bunch rachis and the other abscission zone is situated where the berry is attached to the pedicel. In this trial, at 27 DAFB, the abscission zone developed between the pedicel and bunch rachis. However, from 41 to 111 DAFB, the abscission zone developed between the pedicel and the berry. The same tendency was observed in citrus (Goren, 1981) and cherry fruit (Wittenbach and Bukovac, 1974).

At 27 DAFB, the grape berries had a significantly lower FRF, as measured at sampling and after the 80h period, as well as a significantly higher abscission potential and incidence of berry abscission after 80 hours in the dark, compared to grapes sampled at a later stage. Therefore, 'Waltham Cross' grapes at this stage of development appear to be very susceptible to berry abscission. Consequently, any adverse external factors such as moisture or heat stress, that prevail during this period, could possibly induce severe premature berry abscission. Grapes sampled at this stage of development also showed significantly higher levels of moisture loss during the 80h period in the dark, and a significant correlation existed between moisture loss and berry abscission. High levels of moisture loss are thought to cause water stress in the berries, resulting in accelerated production of ethylene (Berry and Aked, 1996). Since ethylene stimulates abscission (Burg, 1968), it is likely that moisture stress would increase grape berry abscission, thus explaining the significant correlation between these two factors.

From 27 to 54 DAFB, the FRF increased and the berry abscission potential decreased. It is probable that the activities of cell wall-degrading enzymes were low during this period, resulting in a strengthening of the abscission zone tissue. At veraison, which occurred at approximately 54 DAFB, the abscission potential of the grapes was at its lowest, and then increased over further development time. Thereafter, it is likely that the activity of cell wall-degrading enzymes increased after veraison, resulting in a weakening of abscission zone tissue. It is well

established that plant hormones are directly involved in the control of different processes which lead to abscission (Addicott, 1982). Goren (1981) found that auxin delayed an increase in cellulase and polygalacturonase activity and abscission in citrus. Enhanced ethylene production has been associated with increased levels of cell wall degrading enzymes such as cellulase and pectinase in the citrus fruit abscission zones (Rasmussen, 1973). When ethylene production does not correlate with abscission, ethylene responsiveness, which depends upon developmental stage (Hoyer, 1996), may be an important factor (Brown, 1997). It is suggested that changes in ethylene sensitivity are mediated by modulation of ethylene receptor levels during development (Payton *et al.*, 1996). Auxin appears to be an important regulator of ethylene sensitivity and fruit abscission (Brown, 1997). According to Goren (1981) and Osborne (1989), the level of endogenous auxin in the abscission zone has to be reduced below a certain threshold before ethylene can induce the physiological processes which leads to abscission. Therefore, it is speculated that during the period of 27 to 54 DAFB, the auxin levels in the abscission zone may be above the threshold for ethylene responsiveness. It is further speculated that after veraison, the auxin levels could possibly decline to levels below the threshold needed for ethylene to induce the physiological processes which lead to cell wall degradation.

'Waltham Cross' exhibited an inverse correlation between pedicel diameter and berries abscised. It is probable that this correlation is the result of the moisture loss which occurred at 27 DAFB, rather than the stage of the vascular tissue development, which is also known to influence abscission (Baird and Webster, 1979).

Experiment 2 : Pre-and postharvest applications of ethylene inhibitors

In both the 1999 and 2000 seasons, preharvest applications of ReTain™ did not delay maturation, as indicated by no significant differences in TSS, TA or colour development (only in 1999) between the treatments and the control, as measured at harvest (Tables 3 & 5). These results are contradictory to the findings of Mitcham *et al.* (1998) for 'Bartlett' pears, where preharvest applications of ReTain™ delayed maturation of the pears by inhibiting ethylene biosynthesis. However, one would expect to see some differences in response between a classic climacteric fruit like a 'Bartlett' pear and non-climacteric grapes where ethylene is believed to play a much less prominent role in fruit ripening. During the 1999 season, no berry abscission occurred at harvest. In 2000, low levels of berry abscission were evident at harvest, but no significant difference occurred between the different treatments. The ReTain™ sprays also had no significant influence on the levels of berry abscission which were evident after a

delay period of 80 or 200h in the dark (Table 4 & 6).

In 1999, the postharvest application of EthylBloc® resulted in a significant reduction in berry abscission after an 80h period that the grapes were held in the dark at ±25°C (Table 4). In 2000, EthylBloc® had no significant influence on the amount of berry abscission evident after the 80h delay period in the dark (Table 6). In both seasons, the incidence of berry abscission after the 80h period in the dark, was very low. It is doubtful that the 80h period at 25°C stimulated berry abscission. The delay period at ±25°C caused some moisture stress on the grapes, as was indicated by a visible deterioration of stem condition over the 80h delay period. According to Berry and Aked (1996), water stress can induce increases in ethylene production in plant tissues, and the presence of ethylene after harvest may stimulate berry abscission during storage. Wu *et al.* (1992) and Ge *et al.* (1997) reported that for harvested grapes, the ethylene production rates of the cluster stalk, including most of the pedicel, are markedly higher than those of the fruits, and show climacteric-like changes. It is suggested that the ethylene released mainly by the cluster stalk, can induce berry abscission, especially if the ethylene is released in a restricted space, e.g. inside a non-perforated bag. During the 80h period, the grapes were not enclosed in a bag, and exogenous ethylene could escape in the open space with minimum exposure to the pedicel-fruit abscission zone. During the 2000 season, in an attempt to induce more berry abscission, the grapes were closed in non-perforated polyethylene bags and kept for an additional 120h in the dark at ±25°C. During this period, the amount of berry abscission increased substantially, although no significant differences were evident between the different pre-and/or postharvest treatments (Table 6). The considerable increase in berry abscission during the period that the grapes were closed in non-perforated polyethylene bags, may be attributed to a build-up in ethylene concentration inside the bag, with resulting increases in abscission. On the other hand, one would also expect a build-up of carbon dioxide in the bag due to respiration, and high carbon dioxide levels inhibit the action of ethylene.

CONCLUSION

The grape berry abscission potential, as quantitatively indexed by the measurement of the FRF, showed significant changes during berry development of 'Waltham Cross' table grapes, from 27 to 111 DAFB. This showed that at certain stages of fruit growth, 'Waltham Cross' grapes are more prone to berry abscission. At 27 DAFB, when the berries had an average diameter of 6.6mm, the grape bunches showed a significantly higher potential for berry abscission, compared to grapes sampled at a later stage. 'Waltham Cross' has inherently straggly bunches

with bare shoulders, therefore, any abscission during berry development will aggravate the problem. Consequently, it is of vital importance that any adverse factors such as moisture stress be avoided, especially during the period when 'Waltham Cross' appear to be very susceptible to berry abscission. Of all the parameters measured, moisture loss showed the best correlation with abscission. Grapes harvested at a TSS of 12.3°Brix, 83 DAFB, had a significantly higher abscission potential than grapes harvested more mature. Therefore, by harvesting Waltham Cross grapes at optimum maturity, at a TSS of approximately 16.4°Brix, berry abscission can be reduced to a great extent. It was evident that at veraison, the metabolism of grape berries changes drastically, and additional to the rapid increase in sugars and the rapid decrease in acidity, a decrease in FRF occurs.

The preharvest sprays of ReTain™ showed no promise as a means to reduce postharvest berry abscission. The reduction of berry abscission by means of an EthylBloc® postharvest treatment was not very convincing over the two seasons that the trial was executed.

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Table 1 : Parameters measured at different stages of berry development in 'Waltham Cross' table grapes.

Examination parameter	Days after full bloom[1] 27	41	54	69	83	97	111	Prob>F[2]
TSS (°Brix))	5.5^{a}	4.3^{a}	4.2^{a}	7.7^{b}	12.3^{c}	16.4^{d}	17.4^{d}	0.0001
TA (%)	2.4^{c}	2.8^{d}	2.8^{d}	2.5^{c}	0.8^{b}	0.4^{a}	0.3^{a}	0.0001
Berry mass (g)	0.2^{a}	1.8^{b}	3.1^{c}	4.0^{d}	5.7^{e}	7.1^{f}	8.9^{g}	0.0001
Pedicel diameter (mm)	1.2^{a}	1.5^{b}	1.5^{b}	1.5^{b}	1.5^{b}	1.5^{b}	1.5^{b}	0.0001
Berry diameter (mm)	6.6^{a}	13.3^{b}	15.6^{c}	16.9^{d}	20.5^{e}	21.5^{f}	21.9^{f}	0.0001
Moisture loss (%)	6.3^{b}	0.7^{a}	0.4^{a}	0.8^{a}	0.3^{a}	1.2^{a}	0.1^{a}	0.0001
FRF (N) (at sampling)	5.1^{a}	8.9^{cd}	12.8^{e}	10.3^{d}	8.3^{bc}	7.9^{bc}	7.2^{b}	0.0001
FRF (N) (after 80 hours)[3]	1.23^{a}	7.56^{d}	13.26^{f}	9.70^{e}	4.50^{b}	6.01^{c}	5.58^{bc}	0.0001
Abscission potential (%)	75.0^{d}	14.2^{ab}	6.1^{a}	13.7^{ab}	43.7^{c}	23.9^{b}	22.9^{b}	0.0001
Berries abscised (%)	98.3^{c}	0.2^{a}	1.2^{a}	1.4^{a}	7.7^{b}	1.6^{a}	1.5^{a}	0.0001

[1] Variates in same row labelled with different superscripts indicate significant differences (P<0.01) according to LSD test.
[2] One-way ANOVA table with completely randomised design.
[3] Fruit removal force measured after the detached bunches were held in the dark at ±25°C for 80h.

Table 2 : Correlation coefficients (with significance levels in brackets) of different parameters measured during berry development in 'Waltham Cross' table grapes.

	% Berries abscised
Abscission potential (%)	0.66315 (0.0001)
Pedicel diameter (mm)	-0.61095 (0.0001)
Berry diameter (mm)	-0.76857 (0.0001)
Moisture loss (%)	0.84363 (0.0001)
FRF (N) (at sampling)	-0.51540 (0.0001)
FRF (N) (after 80h)	-0.63079 (0.0001)
TSS (°Brix)	-0.30795 (0.1744)
TA (%)	0.26007 (0.2549)
Berry mass (g)	-0.59106 (0.0048)

Table 3 : The influence of ReTain on maturity of 'Waltham Cross' grapes, examined directly after harvest in the 1999 season.

Examination parameter	ReTain concentration & application time							Prob>F [1]
	3w before harvest		2w before harvest		1w before harvest			
	5g	10g	5g	10g	5g	10g	Control	
TSS (°Brix)	18.2	16.0	18.3	16.6	16.8	17.6	19.3	0.233
TA (%)	0.4	0.5	0.3	0.4	0.4	0.4	0.4	0.078
Colour (L-value)	42.0	41.7	43.4	42.7	41.0	43.0	42.7	0.853
Colour (Hue-value)	107.6	117.6	124.5	130.4	104.2	124.3	128.8	0.423
Colour (Chroma-value)	10.6	10.7	13.8	13.9	9.9	12.9	13.0	0.427

[1] One-way ANOVA table with randomised complete block design.

Table 4 : The effect of ReTain and EthylBloc on the incidence of berry abscission in 'Waltham Cross' grapes, examined after 80 hours in the dark at ±25°C in the 1999 season.

Examination parameter	ReTain concentration & application time (A) [1]							EthylBloc (B) [2]		Prob>F [3]		
	3w before harvest		2w before harvest		1w before harvest							
	5g	10g	5g	10g	5g	10g	Control	Yes	No	A	B	AxB
Berry abscission (%)	0.00	0.00	0.27	0.00	0.13	0.38	0.20	0.04[a]	0.24[b]	0.201	0.031	0.236

[1] Data pooled across Factor B.
[2] Data pooled across Factor A. Values in same row, followed by different superscripts indicate significant (P<0.05) differences according to LSD test.
[3] Two-way ANOVA table with randomised complete block design for factor A (ReTain concentration/ application time) and factor B (EthylBloc).

Table 5 :The influence of ReTain on the maturity and quality of 'Waltham Cross' table grapes, examined directly after harvest in the 2000 season.

Examination parameter	ReTain concentration & application time					Prob>F[1]
	2 weeks before harvest		1 week before harvest			
	5g	10g	5g	10g	Control	
TSS (°Brix)	16.0	16.4	15.9	16.8	15.7	0.075
TA (%)	0.4	0.5	0.5	0.5	0.5	0.086
Berry abscission (%)	0.9	0.1	0.5	0.1	0.7	0.059

[1] One-way ANOVA table with randomised complete block design.

Table 6 :The effect of ReTain and EthylBloc on the incidence of berry abscission in 'Waltham Cross' grapes, examined after an 80 and 200 hour period in the dark at ±25°C in the 2000 season.

Examination parameter	ReTain concentration & application time (A)[1]					EthylBloc (B)[2]		Prob>F[3]		
	2 weeks before harvest		1 week before harvest							
	5g	10g	5g	10g	Control	Yes	No	A	B	AxB
Berry abscission (%)[4]	0.2	0.0	0.0	0.4	0.4	0.2	0.2	0.118	0.275	0.126
Berry abscission (%)[5]	18.9	16.1	15.6	18.7	19.1	16.7	18.6	0.162	0.102	0.234

[1] Data pooled across Factor B.
[2] Data pooled across Factor A.
[3] Two-way ANOVA table with randomised complete block design for factor A (ReTain concentration/ application time) and factor B (EthylBloc).
[4] Measured after 80 hours in the dark at ±25°C.
[5] Measured after an additional 120 hours in the dark at ±25°C (bunches were closed in a polyethylene bag).