Pre-harvest determination of bitter pit potential in apples.

By

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DECLARATION

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

Signature:…… Date:……...

SUMMARY

Bitter pit fruit in commercial consignments of apples still poses an economic threat to exporters from South Africa. Bitter pit develops pre-harvest, but gets progressively worse during storage and is only traceable once the lesions appear after storage. Accurate, early indications of bitter pit incidence will allow for remedial pre-harvest measures in the field, e.g. Ca foliar applications, to reduce the potential losses. Similarly, the automatic detection of a bitter pit fruit during packing will reduce financial losses by identifying unacceptable fruit before shipping.

Fluorescence imaging is a fast, non-destructive technique, able to evaluate numerous fruits individually. Results of pre-harvest imaging on apples to identify fruit susceptible to bitter pit showed that pitted fruit were correctly classified, but misclassification of non-pitted fruit with fluorescence imaging was still too high.

NIR-spectroscopy point meter readings could distinguish visible bitter pit lesions from healthy tissue. Important wavelengths associated with visible bitter pit were identified. This technique could also identify immature apples, more prone to bitter pit development. It could however not distinguish between bitter pit and non-pitted fruit when applied randomly on the calyx end of apples at harvest.

Pre-harvest foliar applications to increase fruit Ca content and reduce bitter pit incidence, is a standard practice world wide. External Ca uptake by fruit was monitored to determine the efficacy of applications during different stages of fruit development. Two periods of efficient uptake of external Ca were identified, *viz*., cell division and the last few weeks before harvest. Foliar Ca applications from 40 days after full bloom were more effective in increasing fruit

Ca content and reducing bitter pit incidence than at 80 days after full bloom, which was recommended previously.

Mineral analysis of fruit has been used with variable success to predict bitter pit prior to harvest. The possibility of increasing the accuracy of existing predictive models by using analysis of individual fruit rather than pooled samples, was investigated. By improving the normality of different mineral distributions and decreasing the overlap between pitted and non-pitted fruit classes, it was attempted to improve the reliability of predictions based on variable threshold values. The Ca distribution showed a variation between pitted and non-pitted classes, but still a significant overlap between classes reduced the accuracy of the predictive capacity of this distribution. Even though our results produced a correct classification of 85% for non-pitted fruit, which can be useful, this was still below the required tolerance, of less that 2%, expected on the market.

The effect of pruning and fruit bearing position on two-year-old wood on dry mass and Ca allocation of fruit was determined. ‘Golden Delicious’ fruit set was the lowest at the basal bearing position compared to the other positions evaluated and was contrary to expectations. Fruit in a terminal bearing position was superior to the basal position regarding total dry weight and fruit size. Distal wood possibly inhibited growth and set on the basal position via auxin distribution. Ca allocation differed between seasons and cultivars and could either be influenced by bearing position or presence or absence of re-growth.

Voor-oes bepaling van bitterpit voorkoms by ‘Golden Delicious’ appels in die Wes- Kaap.

OPSOMMING

Bitterpit vrugte in appelbesendings veroorsaak ’n ekonomiese risiko vir produsente in Suid-Afrika. Bitterpit ontwikkel voor-oes, maar raak progressief erger gedurende opberging en is slegs waarneembaar sodra letsels, na opberging, aan die oppervlak verskyn. Akkurate, vroeë aanduidings van bitterpitvoorkoms sal regstellende aksies voor-oes, bv. kalsium blaarspuite, toelaat. Soorgelyk, sal die outomatiese opspoor van bitterpitvrugte gedurende verpakking finansiële verliese verminder deurdat ongewensde vrugte voor verskeping geïdentifiseer en verwyder kan word.

Beeld-fluoressensie is ’n vinninge, nie-destruktiewe tegniek wat instaat is om vrugte individueel te evalueer. Resultate van voor-oes beeldopnames op appels om vrugte te identifiseer wat bitterpit potensiaal het, dui op ’n korrekte identifikasie van vrugte met bitterpit, maar te veel vrugte sonder bitterpit, word verkeerdelik geklassifiseer met die tegniek.

Punt-lesings met naby-infrarooi-spektroskopie kon onderskei tussen sigbare bitterpit letsels en ongeskonde weefsel. Belangrike golflengtes wat geassosiëer word met bitterpit letsels, is geïdentifiseer. Hierdie tegniek kon ook onryp appels, wat meer geneig is tot bitterpit ontwikkeling, identifiseer. Dit kon egter nie onderskei tussen bitterpit en nie-pit vrugte indien dit lukraak toegepas is op die kelkent van appels by oes nie.

Blaartoedienings van kalsium voor oes met die doel om die kalsiuminhoud van vrugte te verhoog en bitterpit voorkoms te verminder, is ’n standaard praktyd wêreldwyd. Kalsiumopname is gemonitor om die effektiwiteit van die blaartoedienings te bepaal gedurende die verskillende ontwikkelingstadia van die vrug. Twee periodes van effektiewe opname van kalsiumbespuitings is geïdentifiseer, nl. seldeling en die laaste paar weke voor oes. Blaartoedienings vanaf 40 dae na volblom was egter meer effektief in die toename in vrugkalsium, asook die vermindering van bitterpit, as die toedienings vanaf 80 dae na volblom wat kommersieël aanbeveel word.

Mineraal analise van vrugte word met ‘n wisselende mate van sukses toegepas om bitterpit voor-oes te voorspel. Die moontlikheid om die akkuraatheid van die huidige modelle aan te pas deur middel van individuele vrug ontledings in plaas van saamgestelde monsters, is ondersoek. Deur die normaliteit van die verskeie mineraal distribusies te verbeter en die oorvleueling tussen bitterpit en nie-pit klasse te verklein, is gepoog om die betroubaarhied van die voorspellings wat op veranderlike drumpelwaardes gegrond is, te verhoog. Die kalsium-verspreiding het ’n variasie getoon tussen die bitterpit en nie-pit klasse, maar die oorvleueling tussen die klasse het die akkuraatheid van die voorspellingskapasiteit van die verspreiding benadeel. Ten spyte van ons resultate van ’n korrekte klasifikasie van 85% vir die nie-pit klas wat nuttig kan wees, was die klassifikasie steeds minder akkuraat as die toelaatbare toleransie van minder as 2% wat die markte verlang.

Die effek van snoei en draposissie op twee-jaar-oud hout op droë gewig en kalsium allokasie in apples van ‘Golden Delicious’ en ‘Royal Gala’ is bepaal. ‘Golden Delicious’ vrugset was die laagste in basale draposisies teenoor die ander posisies wat ondersoek is en is in teenstelling met verwagtings. Vrugte in ’n terminale draposisie was beter daaraan toe in

terme van totale droë gewig en vruggrootte as die op basale draposisies. Distale hout het moontlik die groei en set op basale posissies onderdruk via ouksienverspreiding. Kalsium allokasies aan vrugte het tussen seisoene en kultivars gewissel en kon moontlik deur draposisie of die aan- of afwesigheid van hergroei beïnvloed word.

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CONTENTS

This dissertation presents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters, therefore, has been unavoidable. The different styles used in this dissertation are in accordance with the agreements of different journals used for submission of manuscripts from this dissertation.

INTRODUCTION

Bitter pit remains a serious physiological disorder in apple production, in spite of extensive research on the numerous factors involved in the development of this disorder. The natural occurrence of bitter pit in a commercial orchard or even a single tree, varies between seasons and with orchard management (Yuri, 1995; Le Grange *et al*., 1998a). Because of the detrimental effect of the presence of bitter pit fruit in export consignments, incidence of the condition has been reduced significantly, e.g. by adjusting orchard practices like applying foliar Ca during the season (Terblanche *et al*., 1975), managing crop load (Ferguson & Watkins, 1992) and summer pruning to reduce tree vigour (Terblanche *et a*l., 1974;1975). Nevertheless, with a tolerance of < 2% for export fruit (H. Griessel, Tru-Cape, AECI, Strand; personal communication) it is important to identify fruit prone to bitter pit correctly before export.

Calcium deficiency has been implicated as the most important factor in the development of the disorder (Ferguson *et al*., 1979; Simons & Chu, 1982; Perring, 1986; Raese, 1989; Cocucci *et al*., 1990; Failla *et al*., 1990; Siddiqui & Bangerth, 1993). Pre-symptomatic detection of this disorder could result in apples being marketed earlier or downgraded. At present, the only way of predicting bitter pit prior to harvest, is by destructive mineral analysis during the seasib, or forced early ripening at advanced fruit development (Retamales & Valdez, 2001). Therefore, it was decided to investigate non-destructive techniques for bitter pit detection.

Chlorophyll fluorescence is a non-destructive measurement technique with great accuracy and speed (DeEll *et al*., 1999). Fluorescence is sensitive to stress caused by changes in different environmental conditions like light intensity and drought (Abbott *et al*., 1993; Hakkam *et al*.,

2000; Strasser & Tsimilli-Michael, 2001). Biological changes due to stress conditions as well as normal fruit ripening or senescence, will lead to breakdown of chlorophyll and an increase in synthesis of antocyanins and carotenoids (Huybrechts *et al.*, 2003b). Song *et al*. (1997) found changes in chlorophyll fluorescence with ripening and senescence of apples. Abbott *et al.* (1993) also proved that it was possible to detect thermal injury with symptoms like pitting, water logging and surface discoloration with fluorescence, before the visible lesions occurred. The possibilities of fluorescence imaging as a diagnostic tool to detect physiological disorders in apples have already been illustrated with the 'Fluorescence Imaging System' (FIS) (Ciscato *et al*., 2001; Huybrechts *et al.*, 2002; Huybrechts, 2003; Huybrechts *et al.*, 2003a, b). The potential of FIS to determine bitter pit potential of a single fruit in a fruit sample at harvest, using chlorophyll fluorescence will be discussed.

Although bitter pit is initiated during the pre-harvest period in association with a calcium deficiency, symptoms normally develop progressively during storage. The defect may be identified as brown, corky, roundish lesions predominantly under the epidermis, mainly at the calyx end (Ferguson & Watkins, 1989; Lotz, 1996). Internal pit can also develop just below the skin and in the cortex, but is not externally visible (Ferguson & Watkins, 1989; Little & Holmes, 1999). Near infrared reflectance (NIR) spectroscopy has been used successfully to study internal quality and quality disorders of a variety of fruit species (Slaughter, 1995; Clark *et al.*, 2004; Lammertyn *et al*., 1998; Peirs *et al*., 2000; 2002; 2003, 2005). Further, several authors have found that bruises (Brown *et al*., 1974; Upchurch *et al*., 1990; Upchurch *et al*., 1991; Crowe & Delwiche, 1996; Xing *et al*., 2003) and frost damage (Upchurch *et al*., 1991) can also be identified within an apple by reflection measurements. So far, no spectroscopic application is known with respect to the detection of bitter pit lesions in apple fruit. The objective is to identify useful wavelengths in the NIR range to identify bitter pit.

This knowledge is a first step in developing a hyper spectral system to determine bitter pit potential on apples, non-destructively before commercial harvest.

Mineral analyses (destructive) of young fruit from 80 days after full bloom (DAFB) or fruit 2 - 4 weeks before harvest are often used to predict the occurrence of bitter pit at harvest (Martin *et al*., 1975; Wills *et al*., 1976; Ferguson *et al*., 1979; Terblanche *et al*., 1980; Waller, 1980; Drahorad & Aichner, 2001). Threshold values of these minerals and/or their ratios are applied to determine the potential of the sample to develop bitter pit (Wills *et al*., 1976; Ferguson *et al*., 1979; Waller, 1980; Drahorad & Aichner, 2001).

In South Africa, cases are frequently reported where predictions based on composite sample thresholds alone are unreliable. Fruit with relatively high Ca concentrations (> 5 mg Ca.100 g^{-1} FW) developed bitter pit, whereas fruit with lower Ca concentrations, did not (Terblanche *et al*., 1980; Le Grange *et al*., 1998b). This anomaly lead Le Grange *et al*. (1998b) to evaluated bitter pit and the mineral content of 'Braeburn' apples on an individual fruit basis. Their results showed a non-normal distribution for all minerals in pitted and non-pitted fruit, except for Ca content. However, the distributions overlapped, complicating the use of Ca concentration alone as a parameter to predict susceptibility to bitter pit. We will use 'Golden Delicious' apples to determine whether, by increasing the sample size from the few fruit (37 pitted and 29 non-pitted fruit) used by Le Grange *et al*. (1998b), the normality of the different mineral distributions could be increased and the overlap between pitted and non-pitted classes, decreased. By using individual apples instead of pooled data for mineral analysis, we aim to improve the accuracy of the prediction.

Foliar Ca applications are used to increase the fruit Ca concentration pre-harvest (Terblanche *et al*., 1970; 1974; 1975; Le Grange *et al*., 1998b). Various researchers (Quinlan,1969; Ferguson *et al.*, 1987; Cline *et al*., 1991; Casero *et al*., 2002; Schlegel & Schönherr, 2002) described the rapid uptake and penetration of Ca into fruit, mainly during the first four weeks after full bloom (wafb) or between six and 14 wafb, followed by a decline until harvest. Alternatively, Zavalloni *et al.* (2001) reported a continuous linear increase in Ca from about 40 days after full bloom (dafb) until harvest for different cultivars in Italy, confirming earlier findings (Rogers & Batjer, 1954; Wilkinson, 1968; Tromp, 1979; Jones *et al*., 1983; Tomala *et al*., 1989).

In South Africa, most results favour late season (from 70 dafb) Ca applications. For 'Golden Delicious', November (20 dafb) was found too early and February (harvest) too late for efficient control of bitter pit in the Elgin area (Beyers, 1963). January (90 dafb) was found the most efficient month to apply foliar Ca, with the first application in mid December (70 dafb) and the next two in January. Guidelines for bitter pit control for the South African industry include at least six, weekly foliar Ca applications from middle December (Kotze, 1987). Wooldridge & Joubert (1997) recently evaluated various products for bitter pit control on 'Golden Delicious' and recommended four Ca applications from beginning of December (55 dafb) until harvest with 10 day intervals.

A survey (Lötze & Theron, 2003) involving commercial 'Golden Delicious' orchards in the Western Cape, revealed that more Ca applications (9-10) were not necessarily associated with less bitter pit. According to the same survey, only 40 percent showed bitter pit where applications started between 40 and 80 dafb. Where applications occurred after 80 dafb, only 17 percent of the orchards had bitter pit, confirming Kotze's (1987) recommendation for

applications from 70 dafb. This contrasted with experimental data (Lötze & Theron, 2005) that suggested higher absorption of $Ca(NO_3)_2$ 40-80 dafb than after 80 dafb. Therefore the decision to re-evaluate the effectiveness of early season applications versus late applications of $Ca(NO_3)_2$ to reduce bitter pit in 'Golden Delicious'.

Reynolds *et al.* (2005) described the effect of dormant pruning and quality of bearing two-year-old units on the mean dry weight of 'Packham's Triumph' pears. Mean dry weight of the pears were higher on shorter and thicker bearing units, compared to longer and thinner units. In apples, distal shoot pieces on longer shoots were reported to inhibit bud growth (Cook *et al.*, 1998; Cook & Bellstedt, 2001) and could be related to the export of auxin by the distal shoot piece (Bangerth, 1989). Improved set were influenced by leaves enhancing cytokinin delivery to the terminal position due to transpiration (Cook & Bellstedt, 2001). This instigated a trial with 'Golden Delicious' and 'Royal Gala' fruit, on basal and terminal on spur bearing positions of two-year-old wood, with and without re-growth, to determine how these factors influence dry weight and calcium allocation in apple fruit.

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Evaluation of pre-harvest prediction models for bitter pit incidence

1. Introduction

Bitter pit occurrence in apples is highly erratic. The pit incidence is known to vary between cultivars, seasons, orchards and, even between fruit from the same tree (Le Grange *et al.*, 1998; Yuri, 1995). It is imperative to correctly identify fruit prone to bitter pit before export in order to prevent economical losses due to rejections later in the market. The earlier in the season bitter pit potential can be identified and quantified, the higher the possibility to reduce the incidence. A prediction model will enable accurate estimations on expected bitter pit potential in an orchard. This will enable either measures to increase the fruit Ca level if the prediction is made before harvest, or alternatively a change in marketing strategy to reduce bitter pit expression, if the prediction is only made at harvest.

Present prediction methods use a range of variables with varying accuracy. Existing pre-harvest prediction methods and models for bitter pit incidence were therefore studied to evaluate the accuracy and required inputs for selection of the most suitable option to apply commercially under local conditions.

2. Existing Models

2.1 Historical data

The first approach is to analyse the bitter pit history of an orchard. Accordingly, the orchards can then be classified or ranked at farm level according to bitter pit incidence (N. du Toit, Two-a-Day, Elgin; O. Bergh, CAF, Strand; personal communication). This approach

will however not be feasible with young orchards. An existing database on annual bitter pit per incidence per orchard is a prerequisite.

2.2 Maturity enhancement

Two types of ethylene treatment, applied just before harvest to accelerate maturity and bitter pit expression in fruit, were introduced in South Africa by Eksteen *et al*. (1977). Fruit samples were typically collected 14 days before expected harvest for treatment. The Ginsburg method ripened apples with 1% Acetylene gas, and the Bangerth method, immersed fruit in a water solution containing 0.2 % Ethephon. After treatment, fruit were kept at 20°C and RH 90%. The Ginsburg method determined the final bitter pit potential within 14 days, thus at harvest, whereas the Bangerth method produced results within 10 days, four days before expected harvest. Both methods provided a 50% estimation of the final bitter pit incidence for 'Golden Delicious' apples (Eksteen *et al*., 1977). The estimations were also applicable for 'Cox's Orange Pippin', but not for 'White Winter Pearmain', emphasising the influence of cultivar. Pouwer (1974) used the Ethrel method as well. His results showed a correlation of 0.7 between the prediction and actual bitter pit. He also indicated that this method may over estimate bitter pit. However, these methods are easy to perform and the results are available in a short period. On the other hand, there are arguments against these maturity enhancement methods. The Bangerth method is followed on a commercial scale in South Africa (P. De Vries, Hortec PTY. Ltd., Ceres, South Africa, personal communication), but no local information is available regarding the accuracy or validity. The method combines internal ethylene levels and mineral condition of the fruit that cause the bitter pit like symptoms (Retamales *et al*., 2000b). A physiological over mature state is created for the fruit when the test is performed (Retamales *et al*., 2000b). The outcome could thus be

influenced by the variation in maturity of the sample, which is commonly found. This method needs to be re-evaluated scientifically for various regions and different cultivars for consecutive seasons to determine the accuracy in prediction for local conditions.

2.3 Vegetative growth

General visual observations associated with bitter pit prone orchards include vigorous vegetative growth, light cropping and large fruit. Retamales & Valdes (1996) found a correlation of 0.38-0.50 between shoot length and bitter pit and recommended the inclusion of this parameter in a predictive system. It requires low cost accumulation of data, is an uncomplicated measurement and producers already associate it with bitter pit in the field. Vegetative growth as parameter was also included in a model developed in New Zealand (Turner & Hill, 1998). These variables could however not predict bitter pit in isolation and can at best be added to increase the accuracy of existing models.

2.4 Mineral Nutrition

2.4.1 Physiological Infiltration

Fruit infiltration with magnesium (Mg) just before harvest, is the preferred prediction method of several researchers (Burmeister & Dilley, 1991, 1993; Tomala *et al*., 1993; Burmeister & Dilley, 1994; Retamales & Lepe, 2000; Retamales *et al*., 2000a, 2000b**;** 2001; Piestrzeniewicz & Tomala, 2001b). Hopfinger & Poovaiah (1979) reported morphologically bitter pit like symptoms a week after fruit were infiltrated by 2% $MgCl_2$. Burmeister & Dilley (1994) showed positive correlations (R^2 = 0.86, 0.73) between percentage incidence of Mg-induced pits with percentage incidence of bitter pit after storage for ‘Northern Spy’ apples.

Retamales & Lepe (2000) attained a correlation of 0.93 with Mg-induced predictions for bitter pit in 'Braeburn' performed 40 days before harvest. Bitter pit probability was determined based on bitter pit-like symptoms that occurred within 10-15 days, after infiltration for two minutes under vacuum with $MgCl_2$, sorbitol and a surfactant **(**Retamales *et al*., 2000b; Retamales & Valdes, 2001). According to the commercial application in Chile (Retamales *et al*., 1996, 2000b), samples (40 fruit per sample) can be tested as early as 60 days before harvest, but the accuracy of the prediction (not mentioned) increases closer to harvest. This allows time for additional Ca-applications, if needed. Retamales & Valdes (1996) found a higher correlation for 'Braeburn' for Mg-infiltration (0.87) than for mineral analysis based predictions (0.40). Methodological factors like repeated use of the $MgCl_2$ solution, or the fact that penetration of the cortical tissue by $MgCl_2$ was only 2-3mm, did not affect the accuracy of the prediction of bitter pit with the Mg-infiltration method (Retamales *et al*., 2001). Most of these reports compared favourably with other existing prediction methods. Nevertheless, there has to be an adjustment to the model per cultivar, area and sampling date. The sampling procedure is important and may alter results if taken incorrectly. Non-uniform fruit size, bruised fruit and uneven fruit numbers in samples will affect the accuracy of the prediction.

2.4.2 Mineral analysis

Mineral analysis as a prediction tool has various potential problems. Differences in results for the same fruit samples analysed in different laboratories have been reported (Holland *et al*., 1975; Marcelle, 1990a). The sample size and position of fruit within a tree further complicates the accuracy of the results of such samples (Ferguson *et al*., 1979). In addition, the time of sampling, tissues being sampled and interpretation of these results as

well as extrapolation of the results to orchard levels, can potentially reduce the accuracy of this method. Success in accurate prediction of the number of pitted fruit varied however. In spite of all of the above, this is still the most widely accepted method for bitter pit prediction.

Ca content of apple peel samples (10 fruit per sample) has been correlated with mid season (July, Northern Hemisphere (NH)) leaf Ca by Drake *et al*. (1974) with varying results. Results were promising when compared to correlations between leaf and whole fruit Ca, but too much variation was caused by crop load. They found correlation coefficients of 0.0669 and 0.533 between yield and peel Ca in two consecutive years for 'Baldwin' apple samples, emphasising the underestimated contribution of crop load on Ca content on this possible relationship.

Shear (1972) reported a regression coefficient of 0.49 for a prediction of cork spot in 'York Imperial', using only the Ca concentrations of fruit cortex at harvest in a 2^{nd} degree polynomial regression. The prediction with Ca concentrations of leaves, collected two weeks prior to harvest, had a regression coefficient of 0.52, also with a 2^{nd} degree polynomial regression. He did mention the effect of environmental factors on the accuracy of these equations in predictions, e.g. drought early in the season, which may alter the Ca concentration of fruit more than that of leaves. Under these circumstances, the accuracy of the prediction using the leaf Ca only, will result in an under estimation of cork spot. Similar inaccuracies could develop when these equations are used in isolation with regard to factors influencing fruit size.

These findings were confirmed with correlations between bitter pit and Ca concentrations from leaves and fruit samples for 'Cox's Orange Pippin' (Sharples, 1980). Shear's (1972) best correlations (R^2 = 0.73) were also found between Ca concentration of fruit at harvest and bitter pit, although the mineral composition of fruit only accounted for

50% of the bitter pit variation. Seasonal differences occurred frequently, emphasising the dependency of these predictions on factors other than Ca concentration only.

Van der Boon (1980) did extensive research on the prediction of bitter pit for 'Cox's Orange Pippin' at harvest. Using a multi linear regression, a significant correlation (0.60 – 0.75) was found between percentage external bitter pit and the ratio (K + Mg)/Ca of fruit, the leaf/fruit ratio and uniformity of cropping during three seasons. The same ratio for leaves also sampled at harvest, gave lower, but significant correlations as well. The second best correlations, though much lower, was found between the fruit Ca content and bitter pit. These relationships deteriorated when very low external bitter pit was experienced the following season, and could not be used for prediction. Threshold levels for the ratios, as well as Ca content (42 – 52 $mg.100g^{-1}$ fruit dry weight), were established, but the slope of the fitted regression line between the ratio and bitter pit differed yearly. A factor for yield was added, but did not stabilise the line. A multivariate analysis including a fruit/leaf ratio and an index for regular bearing with the fruit mineral ratio increased the correlation coefficient (0.69-0.81 for three seasons). It should be considered in future models. Although temperature data was not included in the model, a general relationship between mean daily temperatures from August (NH) and bitter pit was evident (Van der Boon, 1980).

Investigations with mineral analysis of leaf (end of shoot elongation), fruitlet (beginning July, NH) and fruit samples (50 fruits/leaves per sample) (at harvest) (Tomala *et al*., 1993), gave the highest correlation coefficients for linear regressions between bitter pit incidence and the K/Ca ratio for cultivars 'Cortland', 'Gloster' and 'Spartan'. Cultivar differences were prominent, with correlation coefficients for fruits, varying between 0.45 and 0.64. Differences in correlation coefficients were also found in the K/Ca ratio between the three parameters within each cultivar. This work was continued with 'Jonagold' (Piestrzeniewicz & Tomala, 2001a). More variables (40) were included in this prediction at first, namely

mineral contents of the soil, leaf, fruitlet and fruit samples at harvest, as well as maturity parameters internal ethylene concentration, firmness, total soluble solids, starch index and mean fruit weight. When these variables were used to predict bitter pit incidence as forced by infiltration (0.2 M $MgCl_2$ + 0.4 M sorbitol solution), the highest percentage (84%) sound fruit could be determined with a correlation coefficient of 0.96. Due to the practicality, the number of variables was reduced considerably to the leaf ratios K/Ca and Mg/Ca and the number of spots caused by the Mg infiltration. Still an acceptable R^2 = 0.79 was achieved. The relationship between Mg infiltrated apple symptoms and actual bitter pit after storage, showed a coefficient of determination of 61%. Again, cultivar differences were a prominent factor when predicting bitter pit incidence. Although a satisfactory result was obtained by reducing the variables to three with this method, the effect of the lower R^2 will only become evident once the sample's prediction is extrapolated onto the orchard (Piestrzeniewicz & Tomala, 2001a).

Johnson & Ridout (1998) combined the mineral analyses data of fruitlets (mid-July, NH), fruit (20 fruit per sample) at harvest and leaves (mid-August, NH) with meteorological data to improve the prediction of bitter pit incidence. The multiple regression equation confirmed a better correlation between Ca and K content in fruit, than fruitlet samples, and bitter pit incidence. Leaf Ca was considered a significant explanatory variable. Rainfall and temperatures made a valuable contribution to the model, as was suggested earlier by Van der Boon (1980). Results for bitter pit prediction under regular atmosphere (RA) versus controlled atmosphere (CA) storage varied in respect of weather data inclusion. The best correlation coefficient for prediction of bitter pit under normal air storage was 66.7%, with only 39.2% in CA storage. Thus the prediction must be adapted according to storage system.

The chemical composition of pitted and non-pitted fruit tissues of 'Cox's Orange Pippin' was analysed for specific ratios for three fruit tissue types: pitted, non-pitted and

'original' (Askew *et al*., 1960). Although very high correlations were attained between incidence of bitter pit and the values of ratios between the constituents of fruits from one region (Annesbrook), it was not consistent with findings from another region (Mountere Hills). As a consequence, the diagnostic value of these ratios, Mg/Ca, Mg/P, K/Mg K/Ca and K/N was reduced when fruit from different regions, typically found in commerce, were concerned.

Wills *et al*. (1976) compared results regarding Ca concentration thresholds for bitter pit predictions from New Zealand to those from the rest of the world. They reduced the number of fruit per analysis sample to five from the preferred 10-20 to minimise fruit-fruit variation. It was emphasised though, that this is only possible in uniform orchards. Fruit on spurs were generally bigger, with less Ca and more bitter pit, than fruit on laterals. Fruit with less than 2 mg Ca/100 g FW were always very susceptible to pit, but other values gave varied results in susceptibility between cultivars, areas and seasons. Ca gave the best correlation with bitter pit from all minerals tested, but the K/Ca ratio was more highly correlated with pit. Cooper & Bangerth (1976) also favoured a prediction for bitter pit in apples with the Mg/Ca ratio. As this ratio decreased, fruit behaved as Ca deficient fruit, irrespective of the cause, being it a lack of Ca or increase in Mg.

According to Bramlage *et al*., (1985) a different approach was proposed by Steer. Mineral concentrations of the fruit at harvest as well as fruit size, harvest data and tree age were used to predict the risk factors per orchard with a procedure of arbitrarily ranking of fruit lots. Scores were assigned to ranges of whole fruit concentrations of Ca and N and ratios of K/Ca, P/Ca and Mg/Ca. The mineral scores were then multiplied by factors according to differences in fruit size, harvest date and tree age. The predicted score was the sum of the values for Ca, N, K/Ca, P/Ca and Mg/Ca. No information was available regarding accuracy or correlations. A data base with sufficient information covering a wide range of possibilities

will be required to derive reliable factors for parameters like fruit size, harvest date and tree age per cultivar and production region.

Bramlage *et al.* (1985) adapted this procedure for 'McIntosh' in Massachusetts, by adjusting the mineral ranges to their conditions, arbitrarily modifying the score assignments to their samples and excluding the factors for fruit size, harvest date and tree age due to standardisation of their samples. Unfortunately bitter pit data was meaningless as a result of low frequencies. Nevertheless, mineral analysis data of the cortex showed that Ca was the element with the biggest, and K the smallest effect on other post-harvest defects. Using the nutrient score, a consistent pattern of risk potential was observed, although incidence varied amongst the seasons. The best results were achieved with predictions based on regression equations. It only required the Ca content and was a reliable predictor of senescence breakdown across the full range of Ca concentrations (Bramlage *et al.*, 1985).

Autio *et al*. (1986) used whole fruit analysis of 'Cox's Orange Pippin' to predict various storage disorders. The data was analysed using a stepwise multiple regression and then subjected to a regression procedure. A prediction equation (ln (%bitter pit +1) based on the Ca and K concentration of fruit accounted for 49% of the variance in bitter pit. Using Ca concentration only in a different equation increased the prediction in bitter pit variance to 55%. When this equation was applied to later data, including an additional cultivar, 'Bramley's Seedling', the highly significant correlation was improved from r = 42 to 60 (Autio *et al*., 1986). Once again an adequate data base will be required.

Ferguson *et al.* (1979) used the actual Ca concentration as the only characteristic to predict whether fruit will develop bitter pit. They found an acceptable association between the predicted and actual bitter pit when fruit were classified into five Ca categories. Subsequent research led to emphasis on the effect of sampling procedure on the prediction accuracy.

Using the K/Ca ratio Waller (1980) proposed, at least 30 fruits were needed for mineral analysis for a 95% confidence limit of 10%. He proposed K/Ca values in excess of 30:1 for 'Bramley's Seedling' or 'Cox's Orange Pippin' in RA storage to identify pit prone fruit. This is based on sampling four weeks before harvest to rank orchards, with a follow up analysis at harvest of a proportion of the orchards to increase the accuracy of the prediction. Correlations of 0.7 were achieved in two consecutive seasons. A bitter pit risk table quantified the number of times in 10 that bitter pit will exceed 10 percent with specified K/Ca ratios varying from less than 19 to higher than 48 (Waller, 1980).

A combination of threshold values based on mineral analysis of fruit can be used to determine the proneness to bitter pit for commercial predictions (Terblanche *et al.*, 1980). The values for P and P/Ca were different for 'Granny Smith', 'Golden Delicious' and 'Red Delicious' cultivars. The value ranges must be adapted if the analysis is done earlier than at harvest. No validation results of these predictions are available.

The superior results with fruit sample versus leaf sample mineral analyses for bitter pit prediction, was confirmed for England (Holland, 1980). The best results were achieved with the ratio (K+Mg)/Ca ($R^2 = 0.56$) above single nutrient (Ca $R^2 = 0.49$) fruit analysis just before harvest. K/Ca also provided acceptable accuracy ($R^2 = 0.56$). A regression between K/Ca and growth and crop load, rendered no significant differences between these orchards, thus these parameters were of little relevance when using the K/Ca ratio for predictions. This ratio facilitated the prediction of bitter pit risk, as well as ranking of orchards according to bitter pit.

In addition to mineral analysis variables, climate variables were also included in a study by Johnson & Ridout (1998) for 'Cox's Orange Pippin'. The multiple regression model included leaf and fruit Ca, in addition to rainfall and daily temperatures in July and September (NH), for bitter pit prediction in RA storage. The incidence of bitter pit under these

conditions could be predicted with 67% accuracy. This prediction renders application in more regions with different cultivars.

Interesting work by Brooks (2001) combined mineral analysis of fruit samples with factors including cultivar, region, season and cultural practices, to develop a computer program, Megalab. This dynamic system was reported to be adapted world wide to predict the bitter pit risk per orchard. Unfortunately no information is available regarding the accuracy of the model. According to the author, it is already commercialised and internet operated in New Zealand (Turner & Hill, 1998). Turner & Hill (1998) reported that bitter pit developed predominantly where fruitlet Ca content was low, that was confirmed in fruit with low Ca content at harvest as well. The study was carried out with selected plots that were not randomly distributed. The actual threshold levels could therefore not be determined statistically.

Crop load affects the mineral concentration and thus bitter pit. A variation in crop load significantly altered K, Ca, Ca/Mg and Ca/K of 'Cox's Orange Pippin' fruit (Ferguson & Watkins, 1992). Although adding crop load as a variable on its own was not significant, the Ca x crop load interaction was significant. The study also indicated that light cropping trees produced fruit with less Ca, higher K and a higher bitter pit incidence, regardless of fruit size.

3. Universal factors influencing accuracy

3.1 Sampling

The natural variation in bitter pit incidence in a 'Golden Delicious' orchard was quantified by Visser & Pienaar (1976). Fruit were harvested, stored and analysed for bitter pit symptoms. The bitter pit percentage varied from 8.8 to 79.8 % per box (105 boxes in total). This large discrepancy in bitter pit incidence was in spite of no significant differences

between the seven plots (blocks), thus a homogenous orchard. The majority of variation in bitter pit incidence was therefore due to within tree variation. Some of the variation was as a result of variation in maturity, often found in bulk samples, with less mature fruit being more susceptible to bitter pit. The rest of the variation was as a consequence of natural variation in susceptibility to bitter pit found in fruit on different bearing positions and fruit sizes within a tree. The influence of within tree variability in a bulk sample of fruit for analysis can be minimised by the correct sampling procedure (Visser & Pienaar, 1976).

Ferguson & Triggs (1990) quantified the effect of fruit size on bitter pit incidence with a 5% increase in pit for each 10g increase in fruit weight (‘Cox’s Orange Pippin’). Fruit in the upper part of the tree are more susceptible to bitter pit. When the crop tends to be in the upper parts of the tree, samples from these parts should be included to get an accurate prediction. Finally the principle to pick less fruit from more trees was emphasised by the much bigger fruit-to-fruit variation within a tree, than for fruit between trees. Two diametrically opposed tissue samples per fruit were analysed which reduced fruit variability satisfactory.

To reduce sample variation, Marcelle (1990b) proposed practical measures when sampling in the orchard. These included sampling fruit deliberately from bearing positions prone to bitter pit, sampling bigger fruit normally on the sunny side, picking fruit from the inside and the outside of the trees and avoiding trees next to pollinators to reduce the crop load effect.

For individual fruit, when using single tree populations, there is a significant positive relationship between fruit size and Ca concentration (Broom *et al*., 1998). Within a tree, a significant positive relationship was also found between fruit size and Ca concentration in a vertical and radial zone. However, positional effects have been reported to negate these relationships for fruit analysis of individual fruit, within a single tree population (Perring &

Jackson, 1975). Factors such as fruiting wood type and leaf area of spur buds give rise to a population of variable fruit size/Ca concentration relationships within a tree. These differences in fruit size and Ca concentration are typically found in bulk samples and should be incorporated when data is interpreted.

3.2 Interpretation

Given an acceptable analysis method for the prediction model, the interpretation of the data still remains. For mineral analysis, the seasonal influence on threshold levels and mineral composition are well documented (Autio *et al*., 1986; Marcelle, 1990b). This accounts for some researchers' preference to work with ratios instead of single mineral levels (Marcelle, 1990b). Variation between cultivars also necessitates different values to be established.

Once the accuracy of the method is determined, an accurate extrapolation of these results needs to be done for the whole orchard. This should be done in conjunction with the history of the orchard concerning bitter pit potential, as well as current season's information on crop load, vegetative growth and meteorological data. Only then can the acceptability of the prediction be determined.

4. Future Possibilities

A future prediction method should be capable of determining bitter pit before visual lesions appear. Ideally, if bitter pit fruit are characteristically different from non-pitted fruit, and these differences can be identified and quantified, it should be possible to build an image of a 'typical' bitter pit fruit using e.g. the 'Eigenface' approach (Muller, 2002). Such a

compound fruit value can then be compared on an individual fruit basis to classify fruit into classes of bitter pit prone, or not. New technology e.g. non-destructive techniques like Near Infra Red - or Fluorescence Imaging can be explored for this application.

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Investigating Fluorescence Imaging as Non-destructive Method for Pre-harvest Detection of Bitter pit in Apple Fruit (*Malus domestica* Borkh)

Abstract

Bitter pit in apples still causes significant losses, especially in the export markets of 'Golden Delicious' apples from South Africa. Orchard practices to reduce the possibility of bitter pit are followed, as well as destructive methods to predict the probability thereof, but the occurrence of bitter pit is still unacceptably high. Fluorescence imaging is a fast, non-destructive technique, able to evaluate numerous fruit. Results of pre-harvest imaging on apples to identify fruit with bitter pit potential showed that pitted fruit were correctly classified, but misclassification of non-pitted fruit with fluorescence imaging is still too high.

Keywords: apples, bitter pit, classification, fluorescence imaging

1. Introduction

Bitter pit is a physiological disorder in apples that occurs throughout the apple industry and is especially prominent in 'Golden Delicious' in South Africa (Le Grange *et al.*, 1998a; b; Wooldridge, 1999). Though management practices to minimise the risk exist and are applied, bitter pit still poses an economical threat to the producer, because is not always visually detectable on the fruit surface at harvest or packing. It develops as a progressive defect during storage. Development is mainly in the cortex at the calyx end of the apple as a cavity with corky cells (Lotz, 1996). It is visible on the fruit surface (epidermis) as roundish, subtle dark green or brown bitter pit lesions. When these lesions are already visible in the orchard, it is frequently referred to as orchard pit versus storage pit, which is identical, but develops post

harvest during storage. Internal pit is described as corky lesions in the cortex, not externally visible (Ferguson & Watkins, 1989; Little & Holmes, 1999), in contrast to clearly visible external pit. Calcium deficiency has been implicated as the most important factor in the development of the disorder (Ferguson & Reid, 1979; Simons & Chu, 1982; Perring, 1986; Perring & Pearson, 1987; Raese, 1989; Cocucci *et al.*, 1990; Failla *et al*., 1990; Siddiqui & Bangerth, 1993). Pre-symptomatic detection of this disorder could result in apples being marketed earlier or downgraded. At present, the only way of predicting bitter pit prior to harvest, is by destructive mineral analysis or forced early ripening late during fruit development (Retamales & Valdez, 2001).

The most accurate method that predicts bitter pit potential, is a fruit mineral analysis two weeks before harvest (Wills *et al*., 1976; Marmo *et al*., 1985; Autio *et al*., 1986; Ferguson & Watkins, 1989). Destructive mineral analysis of fruit is conducted on a small fruit sample of 20 - 25 fruit per orchard. Segments are taken from these fruit and analysed for macro nutrients, especially calcium and potassium content. Then the bitter pit potential of the orchard is predicted. Unfortunately, at this stage, there is no time for remedial actions like additional calcium sprays. To date, the only possible actions with late predictions (two weeks prior to harvest) is to apply a post-harvest calcium-drench or to change the market strategy and/or the intended storage duration of the fruit. The question though is identifying single fruit with bitter pit potential in a sample that contains predominantly fruit free of bitter pit (Le Grange *et al*., 1998b). With the composite mineral analysis, there is no guarantee that all the fruit in the orchard prone to develop bitter pit, can be identified.

Techniques to estimate fruit maturity and quality, non-destructively would be useful. Chlorophyll fluorescence is a non-destructive measurement technique with great accuracy and speed (DeEll *et al*., 1999). Fluorescence is sensitive to stress caused by changes in different environmental conditions like light intensity and drought (Abbott *et al*., 1993; Hakkam *et al*.,

2000; Strasser & Tsimilli-Michael, 2001). Biological changes due to stress conditions as well as normal fruit ripening or senescence, will lead to breakdown of chlorophyll and an increase in synthesis of antocyanins and carotenoids (Huybrechts *et al.*, 2003b). Song *et al*. (1997) found changes in chlorophyll fluorescence with ripening and senescence of apples. Nedbal *et al*. (2000) found that a decrease in the variable fluorescence Fv, defined as Fv (= F_m-F_o), or F_v/F_m, where F_m the maximum and F_o the minimum fluorescence yield, indicated stress in plants. Stress is indicated by a decrease in the variable fluorescence Fv, defined as (F_m-F_o), or in quantum efficiency of PSII, $\varphi_{Po} = F_v/F_m$, where F_m the maximum and F_o the minimum fluorescence yield (Nedbal *et al*., 2000). Several studies show that chlorophyll fluorescence can be used to measure the efficiency of the photosynthetic apparatus and indirectly the physiological status of plant material (Song *et al*., 1997; Mir *et al*., 1998; DeEll *et al.*, 1999; DeEll & Toivonen, 2000).

Until recently, most of these measurements mentioned for apple ripening and senescence were performed with point-source chlorophyll fluorescence techniques (Song *et al*., 1997; DeEll & Toivonen, 2000). Disadvantages of such a system include the absence of spatial resolution and consequent inability to detect local fruit or leaf surface differences and heterogeneity. Therefore, new techniques were developed to address these disadvantages like incorporating a camera based system for imaging photo-oxidative stress in leaves and studying sink-source transition (Meng *et al.*, 2001), and a kinetic imaging fluorometer to monitor fluorescence emission parameters (Nedbal *et al.*, 2000). These systems are not transportable and cannot be used in field studies. A recently developed transportable fluorescence imaging system (FIS) overcomes both problems (Ciscato, 2000). The FIS includes a very sensitive CCD-camera to measure fluorescence of samples with a lower chlorophyll content e.g. maturing fruit, and the system is portable. Fruit quality can thus be

interpreted by studying fluorescence from the fruit surface using imaging technology, even under field conditions.

The possibilities of fluorescence imaging as a diagnostic tool to detect physiological disorders in apples have already been illustrated (Ciscato *et al*., 2001; Huybrechts *et al.*, 2002; Huybrechts, 2003; Huybrechts *et al.*, 2003a; b). With their equipment, the procedure of dark adaptation was also eliminated and measurements could be performed under light conditions. Abbott *et al.* (1993) also proved that it was possible to detect thermal injury with symptoms like pitting, water logging and surface discoloration with fluorescence, before the visible lesions occurred.

In this paper we report on follow up research based on preliminary findings by Huybrechts *et al*. (2002) where bitter pit 'Braeburn' apples were identified using FIS. The potential of FIS to determine bitter pit potential of a single fruit in a fruit sample at harvest, using chlorophyll fluorescence will be discussed.

2. Materials and methods

2.1.1 Plant material and treatment

'Golden Delicious' samples were harvested from a commercial farm in Vyeboom, in the Western Cape, during 2002/03 and 2003/04. The trial fruit received none of the standard Ca-sprays during the season, resulting in an undesirably low Ca-status in the fruit and were harvested pre-optimum (immature at harvest) to increase the bitter pit potential even more. This resulted in 30% of the fruit developing bitter pit during storage. The fruit were of similar size and visually of uniform appearance. The pre-optimum harvest date was determined by analysing similar fruit samples destructively for maturity status. As expected under South

African conditions, all fruit were not at the same maturity stage, but 75% tested pre-optimum. At harvest, all fruit were visually free of bitter pit.

2.1.2 Data recorded

Fluorescent imaging at harvest entailed two images on one side per fruit towards the calyx end. The area where the imaging was performed was blemish free and indicated with a permanent marker following the imaging (Plate 1a). This was done to ensure all information collected corresponded with the imaged area. The marker had no influence on the development of bitter pit and was applied to all fruit.

After imaging, fruit were digitally photographed and then stored at -0.5°C for ten weeks to allow bitter pit symptoms to develop. Fruit with bitter pit were classified into different bitter pit categories e.g. single and multiple lesions. Digital photographs of the fruit were taken again after storage for visual control.

2.2 Fluorescence imaging (FI)

2.2.1 The Fluorescence Imaging System (FIS)

The system takes two 8-bit images of 310 kB of the fruit: a low light intensity fluorescence image (LI) and a high light intensity fluorescence image (HI) after illumination with respectively actinic (600 µmol m^{-2} sec^{-1}) and saturating light intensities (2600 µmol m^{-2} sec^{-1}). Actinic light will thus provide the steady state and saturated light the maximum fluorescence yield under light adapted conditions. The LI-image and the HI-image

correspond, respectively with the steady state (F_S) and maximum fluorescence (F_M) defined in the fluorescence nomenclature by Genty *et al.* (1989).

The FIS used in this experiment is a transportable prototype (Ciscato, 2000). It consists of an excitation, imaging and control unit.

The excitation unit illuminates the fruit surface over an area (20 cm^2) with two different light intensities: a lower actinic (600 µmol m^{-2} sec^{-1}) and higher saturating light (2600 µmol m^{-2} sec^{-1}). A blue cut-off low pass glass filter (Schott BG-39) mounted in front of the lamps provides the blue actinic light. To ensure only the red/far-red region of the electromagnetic spectrum where chlorophyll fluorescence is located is detected by the camera, a red cut-off high pass filter (B+W 092 Schneider Kreuznach) is fitted on the CCD camera of the detection unit. It is necessary in order to image only fluorescence and to completely exclude any light reflected from the sample surface. The transmittance spectra of both of the filters used in the system do not overlap.

2.2.2 Processing of the fluorescence images

The images were digitally processed by a three-step procedure to enable a quantitative interpretation thereof. First, the dark signal was subtracted in order to correct for inherent camera properties. Secondly, a correction procedure was applied for differences in excitation light intensity across the camera field. A third step was performed to correct for the irregular shape of the apples. Masking the images minimised the curvature effect of the apples. These corrections were performed by a freeware student version of Khoros Cantata (Khoral Research Inc, Albuquerque, NM, USA). In Plate 2 the steps are illustrated on an apple image. After correction, a SA-pseudo colour scale (named after the *Scientific American* that first

described it) was fitted to enhance the visual effect and highlight details of the fluorescence image. Plate 1b shows the initial and final sizes of the image.

2.2.3 Analysis of the results of the fluorescence images

A fluorescence image consists out of 256 x 256 pixels. The frequency of the intensity values (pixel values) of the image is presented in a histogram. The number of pixels of a particular value is represented by a frequency. In our case, the number of gray-scale levels of the 8-bit image corresponds with 256 of the x-axis in the image histogram, representing the fluorescence intensity with pixel values as relative units. The data can also be presented as an integrated histogram with the difference being that the y-values represent the probability of a pixel to have a value less or equal to x, instead of equal to x.

A CDF (cumulative distribution function) describes fluorescence distribution in terms of relative frequency as a mathematical function. The difference between a CDF and histogram of the same data set is that the histogram values are summed as the fluorescence intensity increases starting on the left axis at 0% and ending at 100% at the right axis. It can be used very well to determine if the fluorescence data follows a normal distribution by looking at any given percentile e.g. 25^{th} or median for a specific data set. The percentile values carry information about the average intensity of fluorescence emission, the maximum and how the intensity values are spread around the average. If the pixel value of the CDF is significantly lower (more towards 256), it reveals an area of lower fluorescence. This may be an indication of fruit with pit in our study. Alternatively, when the slope of the CDF (gradient) is flatter, it could indicate more heterogeneous fluorescence thus a possibility of pitted fruit (Huybrechts *et a*l., 2002).

If the fluorescence values are not normally distributed, the image histogram presents an asymmetrical shape. Therefore, Strasser *et al.* (1995) suggested use of the median, quartiles, minimum and maximum to present fluorescence data instead of the mean and standard deviation of the histogram. The R- statistical package calculates the mean and standard deviation of the distribution to obtain values to simulate a normal distribution with the same mean and standard deviation (see section on statistical analysis). This simulation is then applied to do a normality test, a linear regression is calculated and the slope and intercept of the regression line and the R^2 are extracted. This normal probability plot provides information regarding the physiological condition of the sample.

For further analysis additional parameters were obtained by calculating the Phi, VI and RFD values using pixels from the HI (maximal) and LI (steady state fluorescence) – images as described by Huybrechts (2003a). PHI = (HI-LI)/HI describes the efficiency with which light that is absorbed by chlorophyll, is used by the photo system in a light adapted state. RFD = (HI-LI)/LI indicates the photosynthetic quantum conversion. VI = HI/LI also indicates the photosynthetic quantum conversion, but has higher values than RFD.

3. Statistical analysis

The reduced data sets of 256 points were simplified further by describing each image with variables using the R- statistical package (Huybrechts, 2003a) and the NLIN procedure (in SAS). These analyses provided various additional parameters that described the images. These parameters were then applied to attempt differentiating between classes of fruit with and without bitter pit.

3.1 R-Statistical package

R-Stats (R-gui) enabled the presentation of the ASCII data, that is usually an asymmetrical shape, into 10 variables: median, quartiles 1 and 3, minimum, line intercepts and three gradient coefficients for each CDF (Littell, 1989). With the NLIN procedure the 256 variables of CDF of one image (M-state) were thus presented as five variables in total.

A stepwise analysis (PROC STEPDISC) was preformed to determine the most important variables from the potential 36 obtained by Rgui and NLIN, for each of the different images. Thereafter these variables were used in a discriminant maximum, mean, standard deviation, intercept, slope and R^2. The original histograms, derived from the pixel values per image, with non-normal distribution, are thus transformed and recalculated as new histogram that are normally distributed. The deviation from normality is one indication of the physiological conditions of the fruit. This procedure was followed for only three fluorescence parameters: Maximal-, Phi- and Vi-images.

3.2 Statistical Analysis System (SAS)

The NLIN METHOD = DUD procedure of the Statistical Analysis System (SAS program) (SAS Institute Inc., 1999-2001). SAS was used to obtain two analysis (PROC DISCRIM) in the SAS program to determine whether the variables could be used to distinguish between fruit in different classes e.g.: fruit without bitter pit versus fruit with bitter pit lesions. Finally the data obtained from the stepwise analysis were further subjected to the canonical analysis (CANDISC) in order to aid in the visual interpretation of group differences.

3. Results

Plate 3 shows a 'Golden Delicious' fruit imaged at harvest (2003/04) before any visual bitter pit lesions and the same fruit after three months' storage, with bitter pit within the imaged area.

Plate 4 shows the analysed fluorescence images of five fruit from 2003/4 at harvest, with corresponding digital photographs after storage. Fig 1 shows an example of the CDFs for fruit from (a) 2002/03 and (b) 2003/04. The graphs show the clear separation of randomly chosen fruit into the different classes of pitted and non-pitted fruit when the averages of 10 fruit were compared in (a) and five single fruit in (b). In case of individual images (CDF-curves), it is not always possible to discriminate between non-bitter pit and bitter pit fruit (Fig 2). This can be explained partly by the normal biological variability in a fruit population. Although some fruit showed bitter pit symptoms, the further physiological status of the fruit was unknown to us. With individual fruit evaluation, FIS may also detect other physiological differences between fruit in a group causing misclassification.

In Fig 2 though, the lack of consistency in the complete separation between the two classes, pitted and non-pitted fruit, with CDF for data of eight individual fruit (chosen at random) from 2002/03 is shown. This tendency seems to be prevalent for most fruit (450) analysed during the last two seasons (data not shown) when all fruit were included in this assessment.

For statistical analysis, fruit were classified into bitter pit or no-pit (2002/3) and no-pit, one lesion and multiple lesion (2003/4) classes. The most important parameters for each season (Table 1) were selected on R^2-values from the stepwise results and a discriminant analysis was performed on the separate datasets. Even though these variables could not be referred to specific physiological characteristics of the fruit, it could be applied successfully to

discriminate between the groups. The Chi-square values for these discriminant analysis results are given in Table 2. The Chi-Square values were significant for the two scenarios at the 0.001 level, indicating heterogeneity of the within covariance matrices and resulting in quadratic discriminant functions being computed.

A summery of the discriminant classification results is presented in Table 3. Using a quadratic discrimination function gave a good (75-100%) classification of fruit with bitter pit (BP). In contrast, classification of the non-pitted fruit into the NO class was poor with 50.5% and 13.5% depending on the classification used for analysed. FIS was able to discriminate to a higher or lesser degree between pitted and non-pitted fruit. At present, FIS is unable to discriminate between and quantify factors that induce stress and physiological disorders. The unsatisfactory result (50.5% to 13.5%) of the discriminant classification of the non-pitted fruit into the NO-class, may be a consequence of this discrepancy. In this study we concentrated on bitter pit, but environmental and physiological factors may induce physiological changes in fruit that concomittedly influence the fluorescence behaviour of the fruit.

Results from the CANDISC procedure are summarised in Table 4. Groupings of CANDISC agreed with the results from the DISCRIM classifications of the fruit. Fruit with no pit could not be separated from groups with pit in all instances and had an unacceptable high misclassification rate. As mentioned before, this is probably due to physiological differences between no-pitted fruit causing further undefined classification. Classifying fruit into single and multiple lesions, lesions within the image and not or bitter pit and pit with lenticel spot did not improve the misclassification of the non-pitted fruit. This confirmed that other factors than lesions are involved in bitter pit classification and that was recorded by FIS. Thus an increase in the number or appearance of the lesions did not improve the classification based on fluorescence. Presentation of the CANDISC results in Fig 3(a) and (b) illustrates the lack of clear distinction between the non-pitted and pitted groups. It also emphasises the

inability of the first canonical correlation (r = 0.1781 for 2002/3 and r = 0.07786 for 2003/4) to describe the main class differences satisfactory. The first canonical proportions were 0.7622 (2002/3) and 0.6865 (2003/4). This emphasised the probability that some of the fruits that have been considered "good quality" having no pit, were indeed not necessarily so. This highlights the problem defining a good reference system (BP and NO) with applicable, compound definition of such fruit.

Standardised canonical coefficients for the pooled within-class CANDISC procedure are presented in Table 4. Fig 4 presents the variation between the two seasons as well as between the different classes according this canonical classification.

4. Discussion

Previous work by Huybrechts *et al*., (2002) revealed that fluorescence image regions with lower fluorescence are associated with bitter pit in selective cases. The slope of the CDF as well as the x-axis intercepts varied between the two fruit. Nevertheless, the distinction between pitted and non-pitted fruit of our total sample was not all as clearly defined according to CDFs. Our results showed that, when the averaged CDF of pitted fruit was compared to the average of images from fruit free of bitter pit, it was possible to show a visible difference between the classes (Fig 1a, b). The distinction was even possible between averaged images of fruit with one, multiple and bitter pit lesions, and fruit with no pit. Pitted fruit tended to display lower fluorescence than non-pitted fruit. In these cases, it seemed that fruit with bitter pit could be identified into a separate class using FIS when comparing averages.

However, in the majority of cases, on a single fruit basis, separation in groups was not satisfactory. Results from 2002/03 and 2003/04 indicated that fluorescence of the non-pitted fruit still did not always vary significantly from that of the pitted fruit, although good

classification (+ 75%) of pitted fruit was achieved. With the stepwise analysis (SAS) not all parameters chosen during the two seasons to classify fruit on fluorescence images, could be standardised. Discriminant analysis of the fruit into various classes describing pitted fruit *versus* non-pitted fruit only confirmed the lack of consistency. The heterogeneity of the fruit in the non-pitted sample is partly blamed for this phenomenon. The immature picking may enhance natural variation in the non-pitted fruit. In an attempt to do a simple discrimination between the groups, the canonical results only confirmed the poor classification of non-pitted fruit.

The fluorescence results in this paper were interpreted as being indicative of bitter pit *per se*, but it may be a reflection of a combination of a reaction of fruit physiology, maturity and bitter pit. The varying results, with the non-pitted fruit, emphasises the heterogeneity that still existed between individual fruit in a sample in spite of measures taken to guarantee homogenous samples of uniform fruit with the same treatments. The fact that we did not have access to non-destructive technology to ensure that the fruit were also homogenous according to internal parameters, could have accounted for misclassification of non-pitted fruit due to unquantified internal or physiological factors. If these factors e.g. varying maturity could have been quantified non-destructively and combined with the fluorescence results, accuracy in classification could have increased.

4. Conclusions

Fruit harvested immature are more prone to bitter pit than fruit harvested at physiological maturity. Recent work by Huybrechts *et al*., (2003a; b) assessing apple quality and storage potential with the FIS, presented positive results concerning identification of various apple maturity stages after a storage period using chlorophyll fluorescence imaging.

In this case, the whole fruit was affected by the change in maturity. These experiments were also performed on fruit after harvest, like most on this topic in literature, when chlorophyll degradation and a decrease in fluorescence have been triggered.

The experienced heterogeneity of fruit samples during the two seasons and possibility to use the FIS to identify immature (pre-optimum) fruit prone to bitter pit, will be evaluated. The aims will be i) to enhance the classification of pitted fruit by including maturity data, as well as ii) to determine if the unsatisfactory classification of the non-pitted fruit are enhanced by a heterogeneous physiological maturity.

Future research to address the influence of fruit maturity on fluorescence of bitter pit fruit will be conducted to assess the physiological effect in more detail. Using fluorescence imaging and statistical processing of the data, as has been done here, is already an advance on the approach used by DeEll & Tiovonen(2000), Song *et al*. (1997), and others (cfr introduction) where they used point measurements, dark adapted conditions and did not report their results statistically.

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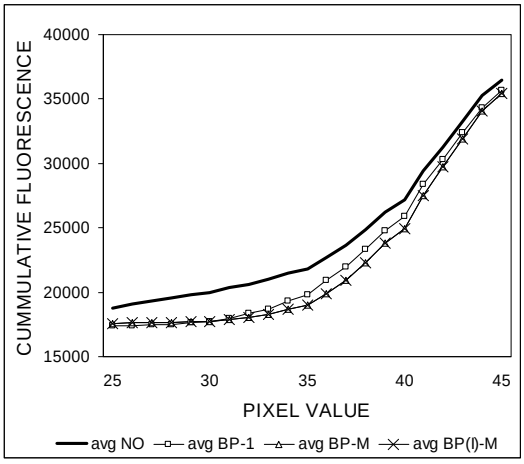
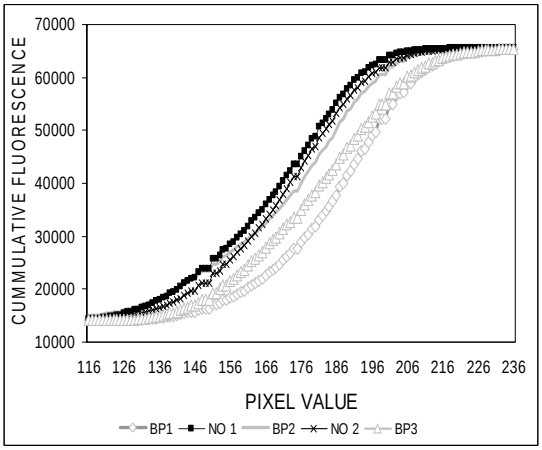
Figures





a) b)

Figure 1 Good separation with CDF's of fruit with (a) averages of 10 no bitter pit fruit (NO) versus all 30 fruit with bitter pit (BP), with classes pit in the image (BP(I)), multiple pits (BP-M) and only one pit per fruit (BP-1) for 2002/03 and (b) individual fruit, 2 without bitter pit (NO) versus 3 bitter pit (BP) 2003/04. The x-axes (0-256) were shortened to emphasise fluorescence values.

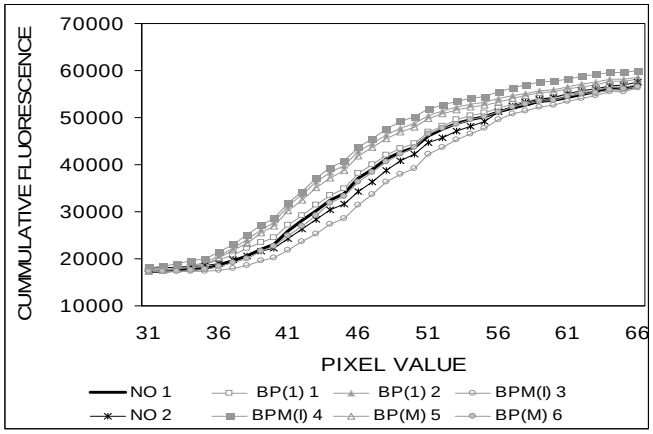
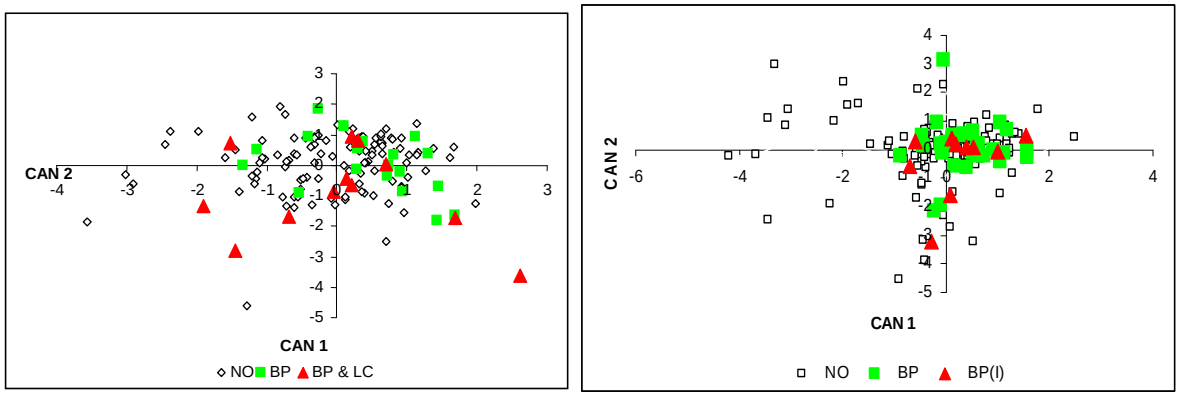


Figure 2 CDFs indicating no clear separation between eight individual fruit in the different classes of pitted (BP 1-6) and non-pitted fruit (NO 1 & 2) (2002/03). The x-axis pixel values were reduced from 0-256 to 31–66 to emphasise the fluorescence values.



a) b)

Figure 3 Discriminant between fruit with no bitter pit (NO), fruit with bitter pit (BP) and fruit with pit (BP) and lenticell lesions (LC) using the data in a canonical discriminant analysis for 2003/04 (a). Discriminant between fruit with no bitter pit (NO), fruit with bitter pit (BP) and fruit with (BP(I)) in the image for 2002/03 (b).

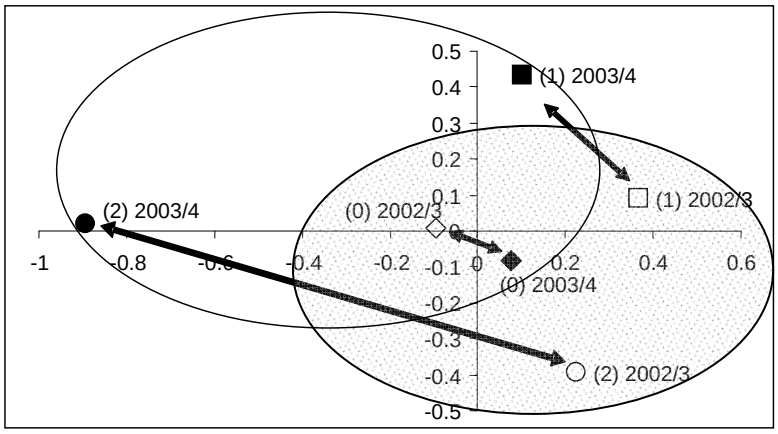


Figure 4 Canonical discriminant analysis of different classes , fruit with no bitter pit (0), fruit with bitter pit (1) and fruit with pit and lenticell lesions or in image (2) for 2003/04 and 2002/03, showing the spacial variation between seasons (circled) as well as between similar classes.

Tables

Table 1 Summary of Stepwise Analysis on 2002/03 an 2003/04 data for best classification results.

Variable	R-Square	F Value	Pr > F	Variable	R-Square	F Value	Pr > F
Selected variables 2002/03				Selected variables 2003/04			
A-slope	0.0038	0.34	0.7153	Phi-R^2	0.0102	0.72	0.4867
Phi-average	0.0038	0.34	0.7115	M-slope	0.0124	0.88	0.4152
A- R2	0.0039	0.35	0.7076	M-R^2	0.0126	0.9	0.4086
Phi-median	0.0039	0.35	0.7048	M-intercept	0.0127	0.91	0.4056
A-average	0.0047	0.42	0.659	Phi-slope	0.0131	0.94	0.3947
Vi-std error	0.0049	0.44	0.6465	Phi-intercept	0.0159	1.14	0.3227
Vi- max	0.0051	0.46	0.6338	Vi-intercept	0.0171	1.22	0.2974
Phi – quart 3	0.0105	0.95	0.3905	Vi-slope	0.0185	1.33	0.2679
A-min	0.012	1.08	0.3421	Vi-R^2	0.0185	1.33	0.2675
				Phi-max	0.0222	1.6	0.2048

Table 2 Chi-Square values for the test of homogeneity of within covariance matrices.

Season	Chi-Square	DF	Pr > ChiSq
2002/03	1013.354432	90	<.0001
2003/04	310.360610	110	<.0001

Table 3 Summary of the Discriminant Analysis for Calibration Data using Quadratic Discriminant Function for (a) 2002/03 data and (b) 2003/04 data.

a)

From CLASS	In class 0 (no pit)	In class 1 (bitter pit)	In class 2 (pit in image)	Tot
0	19	89	33	141
	13.48	63.12	23.40	100
1	0	23	7	30
	0.00	76.67	23.33	100
2	0	0	10	10
	0.00	0.00	100.00	100
Total	19	112	50	181
	10.50	61.88	27.62	100

b)

From CLASS	In class0 (no pit)	In class 1 (bitter it)	In class 2 (pit & lenticell)	Tot
0	56	41	14	111
	50.45	36.94	12.61	100
1	0	20	1	21
	0	95.24	4.76	100
2	0	3	9	12
	0	25	75	100
Total	56	64	24	144
	38.89	44.44	16.67	100

Table 4 CANDISC procedure results for the pooled within-class standardised Canonical coefficients for 2003/04 (a) and 2002/03 (b)

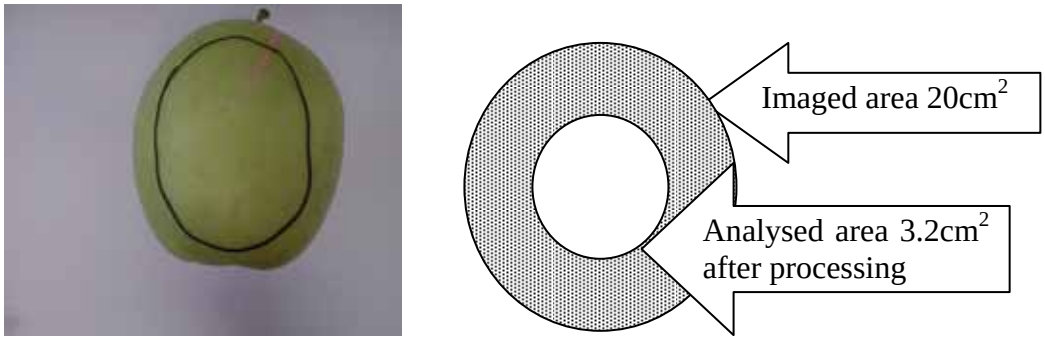
a)

Class Means on Canonical Variables		
CLASS	Can1	Can2
0 (no pit)	-0.0943662811	0.0086148145
1 (bitter pit)	0.3682001335	0.0911869238
2 (pit in image)	0.2259641630	-.3950296562

b)

0 (no pit)	0.0769037980	-0.0845542526
1 (bitter pit)	0.1036876124	0.4349171097
2 (pit & lenticell)	-0.8928134534	0.0210218943

Plates



a) b)

Plate 1 (a) Identification of exact area on the apple where imaging was done, immediately after imaging, at harvest, with a marker and (b) the imaged area used for analyses.

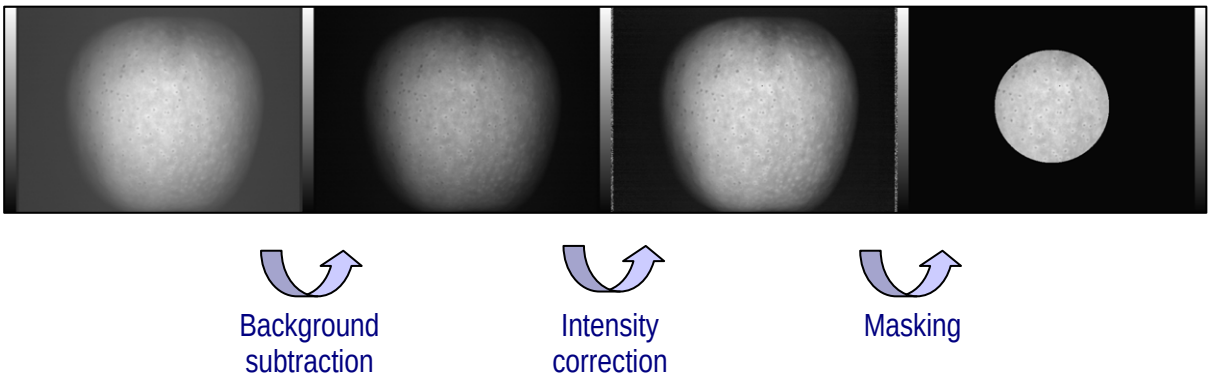


Plate 2 Processing of an apple fruit chlorophyll fluorescence image: first cropping, then background subtraction, intensity correction and masking

a) b)

Plate 3 Digital photographs of the same apple taken a) at harvest and b) after storage and bitter pit development.

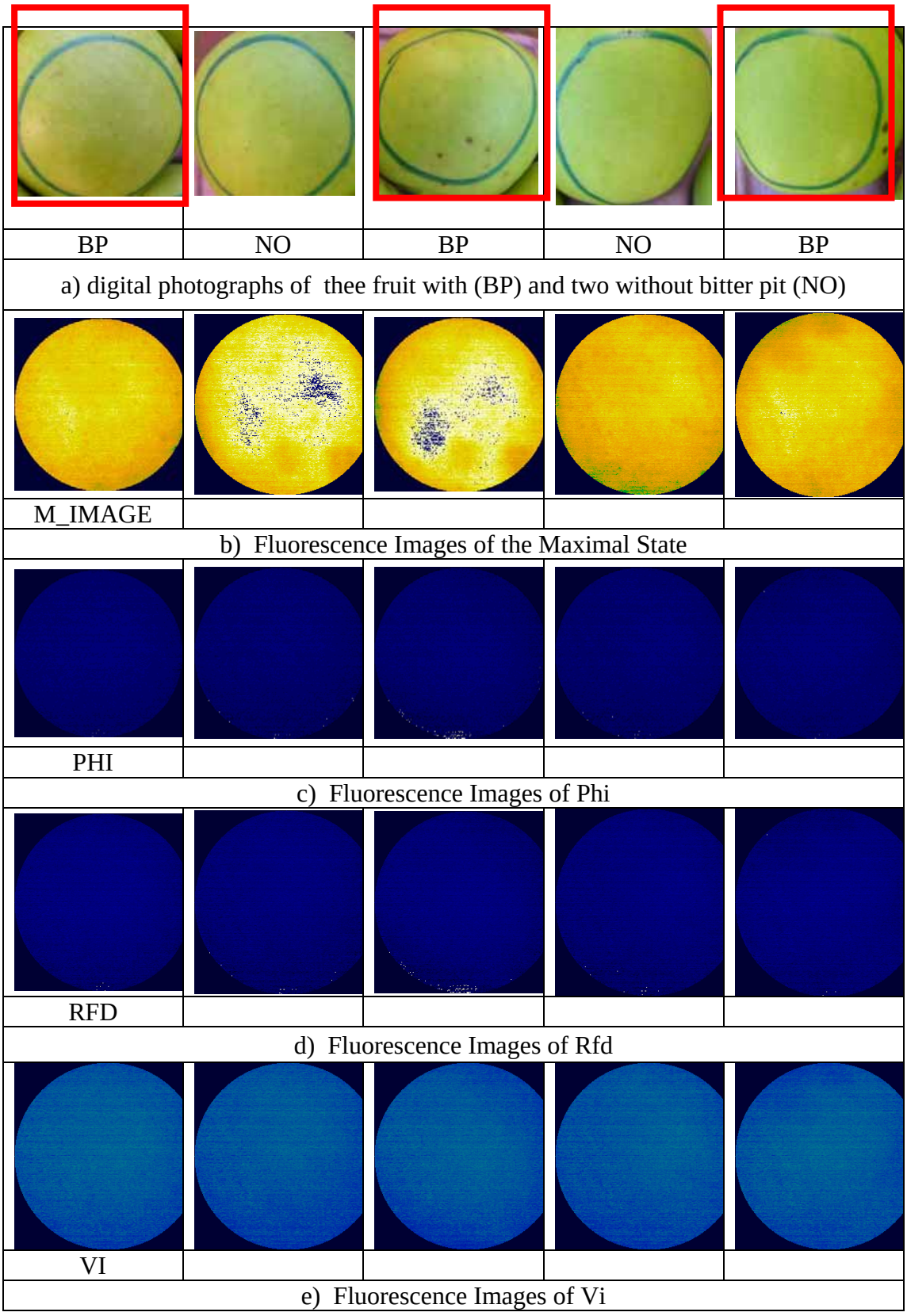


Plate 4 Digital photographs of fruit after storage (a) with corresponding Fluorescence Image at harvest time, displayed as four different parameters (Images b-e) for random fruit, with (BP) and without bitter pit (NO), showing no difference between the two classes (2003/04 data)

Potential of NIR-spectroscopy to predict bitter pit development in apple fruit

Summary

Bitter pit is a physiological disorder in apples which is predominantly attributed to a calcium deficiency. This disorder develops post harvest and can, in certain years, result in large losses. To reduce the risk of bitter pit, techniques are being developed to identify fruit prone to bitter pit, before storage. NIR-spectroscopy point meter readings could distinguish visible bitter pit lesions from healthy tissue. There were significant differences between the spectra of healthy and pitted areas once the lesions were visible. Important wavelengths associated with visible bitter pit were identified as well. Destructive and non-destructive results regarding maturity parameters were correlated. Fruit with more or less advanced maturity could be classified with NIR-spectra. This technique could identify immature apples, more prone to bitter pit development. NIR-spectroscopy point meter readings could however not distinguish between bitter pit and non-pitted fruit when applied randomly on the calyx end of apples at harvest.

Keywords

physiological disorder - non-destructive techniques - spectra - wavelengths

Introduction

The South African deciduous fruit industry is an export driven industry that mainly focuses on apple export to countries in the northern hemisphere. During transport and storage, progressive physiological disorders may arise, among which bitter pit is very prominent. Although the average proportion of pit-affected fruit in the Western Cape is only 0.4% for

'Golden Delicious' (WOOLDRIDGE, 1999), which seems insignificant, individual producers may approach figures of up to 2% following export. This is unacceptable in terms of export regulations and such produce is either declined or the producer fined by exporters for exceeding the bitter pit threshold which is often zero percent (personal communication, H. Griessel, Tru-Cape, Somerset West). South African growers are therefore interested in techniques to identify bitter pit prone fruit prior to commercialisation.

Although bitter pit is initiated during the pre-harvest period in association with a calcium deficiency, symptoms normally develop progressively during storage. The defect may be identified as brown, corky, roundish lesions predominantly under the epidermis, mainly at the calyx end (FERGUSON AND WATKINS, 1989; LOTZ, 1996). Internal pit can also develop just below the skin and in the cortex, but is not externally visible (FERGUSON AND WATKINS, 1989; LITTLE AND HOLMES, 1999). Pre-harvest factors enhancing bitter pit development include excessive vigour of the tree, a light crop load, immature fruit characterised by a low soluble solids content, a high firmness and a high starch content, large fruit from young bearing trees and insufficient Ca-uptake (COCUCCI *et al*., 1990; FAILLA *et al*., 1990; FAUST AND SHEAR, 1968; FERGUSON AND REID, 1979; LE GRANGE *et al*., 1998a; b; PERRING, 1986; PERRING AND PEARSON, 1987; RAESE, 1989; SIDDIQUI AND BANGERTH, 1993; SIMONS AND CHU, 1982).

Presently, only two physiological infiltration methods (forcing maturity using ethylene or magnesium solutions) are used to predict the potential of bitter pit occurrence, but both techniques are destructive (RETAMALES, 2000). However, the use of a non-destructive technique to detect bitter pit potential on individual fruit at an early stage would create the opportunity to remove such fruit prior to commercialisation.

Near infrared reflectance (NIR) spectroscopy has been used successfully to study internal quality and quality disorders of a variety of fruit species (see e.g., SLAUGHTER, 1995;

LAMMERTYN *et al*., 1998; PEIRS *et al*., 2000; 2002; 2003a; 2003b, 2005; CLARK *et al.*, 2004; SARANWONG *et al*., 2004). Advantages of NIR-spectroscopy include its measurement speed and non-destructive nature, so that it potentially can be used for online grading purposes. Further, several authors have found that bruises (BROWN *et al*., 1974; UPCHURCH *et al*., 1990; UPCHURCH *et al*., 1991; CROWE AND DELWICHE, 1996; XING *et al*., 2003) and frost damage (UPCHURCH *et al*., 1991) can also be identified within an apple by reflection measurements. BROWN *et al*. (1974) investigated wavelengths in the 700 to 2200 nm range and UPCHURCH *et al*. (1990) in the 400 to 1012 nm range. Bruised areas in apples caused a reduced NIR-reflectance in 500 to 800 nm (PEIRS *et al*., 2002) and 700 to 2000 nm region (BROWN *et al*., 1974) as a result of cell wall destruction, that increase the scattering of the radiation in the tissue. LU (2003) showed that the spectral region between 1000 nm and 1340 nm was most appropriate for bruise detection. KLEYNEN *et al.* (2003) concluded that all studies found significant wavelength bands above 700 nm in detecting skin defects of apple, which suggested that it was important to scan beyond the visible spectrum into the NIR-region. So far, no spectroscopic application is known with respect to the detection of bitter pit lesions in apple fruit.

The objective of this paper was to identify useful wavelengths in the NIR-range to identify bitter pit. This knowledge is a first step in developing a hyperspectral system to determine bitter pit potential on apples, non-destructively before commercial harvest.

Materials and methods

Plant Material

Braeburn apples were selected for this experiment, due to high susceptibility of this cultivar to bitter pit under South African conditions. The optimal harvest date for long-term storage is at

an average 40% to 60% starch breakdown for ‘Braeburn’ (Starch conversion chart, Capespan, PO Box 505, Bellville, 7535, South Africa).

125 fruit were harvested on three dates (27-02-2001; 13-03-2001; 27-03-2001) from a commercial orchard in Ceres (Western Cape, South Africa) and transported to the lab. The apples were randomly selected from the trees, picked at shoulder height, not more than one meter from the outside. Fruit with similar sizes were selected. End trees and rows were avoided. NIR-reflectance spectra were acquired within 2 days from all samples.

After storage at 0°C and under normal air conditions for 41 to 67 days, fruit were removed from cold storage and evaluated for lesions. Bitter pit was characterised as a superficial lesion, single and/or multiple lesions (Fig.1a), darker than the surroundings. Lesions included the dark ‘lenticel spot’ (Fig.1b). Lenticel spots are darker and more pronounced than the more subtle lesions described as bitter pit lesions, but still associated with calcium deficiency and corky cells. Where no bitter pit was detected after three months' storage, the fruit was peeled and cut diagonally in thin rings at the calyx end to determine if internal pit was present (Plate 1c). Regardless of whether pit was internal and/or external, fruit was classified as having bitter pit. If no pit was found within the three months' storage period, fruit was classified as having no bitter pit.

NIR-spectroscopy

NIR-spectra were measured using a Fourier transform spectrophotometer (InfraProver, Bran and Luebbe, Germany) in the range 4056-9936 cm^{-1} (1002 to 2495 nm) at an interval of 24 cm^{-1}. All spectra were acquired at room temperature under a black cover to avoid influences of external radiation. As temperature is known to affect the spectral measurements of apples

(PEIRS *et al*., 2002), in experiment 2 (see further) fruit was removed from cold storage at least 8 hours prior to ensure measurements were carried out at room temperature.

Experimental design

Experiment 1: Spectroscopic evaluation of local measurements on bitter pit lesions after storage

The aim of this experiment was to identify specific features in the spectra unique for pitted lesions. These features should represented frequencies at which the NIR-scattering and absorption properties are different for healthy and bitter pit affected tissue. Measurements were taken directly on all the visual lesions and on adjacent non-pitted tissue (as close as possible to the visual measurement area) at various positions on the same fruit for 3 apples. The number of lesions varied per fruit.

Experiment 2: Applying point meter readings of NIR-spectroscopy to detect/identify bitter pit prone fruit

Bitter pit development might be related to a general, rather than localised changes in physiological state of the fruit. The hypothesis of this experiment was that such a general change would also affect the NIR-reflectance properties of the tissue. NIR- readings were therefore performed in the calyx area on 125 fruit prior to storage and visible bitter pit detection, to determine if random point readings could identify bitter pit prone fruit in a random sample at harvest. After 41 to 67 days of storage the fruit were inspected for bitter pit occurring on the surface, as well as in the cortex, under the peel.

Data analysis

The data were analysed using principal component analysis (PCA) with Matlab vs. 7.0.1 (The Mathworks, Natick, USA). Prior to further processing, second derivative spectra were calculated using a Savitsky-Golay filter of order 3 and interval width 21 (NÆS *et al*., 2004). Also log(1/*R*) and Kubelka-Munk transforms, in combination with first and second derivatives were attempted, but the best results were obtained with second derivatives of the raw spectra. The PCA was carried out on the covariance matrix.

Results and discussion

Experiment 1: Spectroscopic evaluation of measurements on bitter pit lesions after storage

The second derivative spectra of healthy tissue and bitter pit or lenticel spot lesions are shown in Fig. 2. As expected from the high water content of apple fruit, the spectra are similar to a typical water NIR-reflectance spectrum.

The PCA score plot of the two first principal dimensions is shown in Fig. 3. PC1 and PC2 account for 78% and 16% of the total variance, respectively. Lenticel spots and adjacent tissue are separated along PC2, whereas bitter pit spots and adjacent tissue are separated along PC1.

The loadings of PC1 and PC2 are shown in Fig.4. PC1 seems to be dominated by typical water bands at 1160, 1417, 1830 and 1930 nm. The pitted lesions probably caused an increase in light scattering due to dehydration compared to healthy tissue. Findings from BROWN *et al*. (1974) also indicated that the average reflectance is less for bruised than unbruised apples at wavelengths between 700 to 2200 nm. In bruised areas, water replaces the intercellular air spaces in the plant tissue and causes a decrease in NIR-reflectance of these areas (WOOLLEY, 1971).

The separation between the groups improved when only one single fruit was considered (Fig. 5), with an explained variance of 85% and 11% of the total variance for PC1 and PC2, respectively. However, the number of lenticel spot spectra was too small to draw any conclusions.

Experiment 2: Applying point meter readings of NIR-spectroscopy to detect /identify bitter pit prone fruit

NIR-spectroscopy point meter readings measure a limited area (10mm diameter circle) per measurement. This feature may limit applications to detect bitter pit lesions that are not only smaller in diameter, but are also irregularly distributed and generally invisible at harvest. However, it is known that bitter pit lesions show deviating physiology characteristics, such as disintegrated plasmic material, higher starch content and increase of organic acids of pitted cells (BANGERTH, 1973). Hence, it would be possible to detect those bitter pit prone fruit based on the physiological characteristics of the affected areas, which can also be measured by the point measurements, if done exactly on the bitter pit lesion.

The PCA score plot of the second derivative reflectance spectra ‘Braeburn’ apples immediately after harvest is shown in Fig. 6. There is no difference between apples which developed bitter pit after storage and which did not. PC1 and PC2 account for 84% and 10% of the total variance, respectively. Apparently, the random point meter readings on the calyx end of the fruit could not be used to classify the fruit into a bitter pit prone or no pit class. As the possibility of bitter pit occurring as well as the exact position of the lesions should it occur was unknown at harvest, the inability of NIR-spectroscopy to identify the bitter pit prone fruit could be due to not being able to take measurements on the affected areas. The physiological changes within the lesion at this early stage of maturity (pre-optimum) may need alternative

methods to NIR for detection such as NIR-hyperspectral imaging. With NIR-hyperspectral imaging, an imaging spectrophotometer records a hyperspectral image data cube instead of collecting single spectra at one spot (PEIRS *et al*., 2003c). GUYER AND YANG (2000) used such a system to classify cherries according to the type of defect. A NIR-hyperspectral imaging system could measure the whole calyx area to determine either pitted lesions or a physiological difference in the pitted fruit.

Note that variations in fruit maturity like starch breakdown, skin colour and total soluble solids between fruit can readily be detected with NIR-spectroscopy point meter readings, but this is normally be performed closer to optimum maturity with a larger area showing these changes (PEIRS *et al*., 2000).

Conclusions

NIR-reflectance spectra of healthy and bitter pit and lenticel spot affected tissue were compared. By multivariate analysis it was possible to see significant differences between the spectra of healthy and pitted areas once the lesions were visible. The differences in water status and accompanying cell structure of the pitted lesions enabled NIR-spectroscopy to differentiate between visible lesions and healthy areas. Spectral threshold values for bitter pit lesions were identified. Nevertheless, NIR-spectroscopy point meter readings were unsuitable for single measurements needed to include large areas of fruit that are bitter pit prone, at harvest. We are currently investigating the usefulness of NIR-hyperspectral imaging techniques to this end.

Acknowledgements

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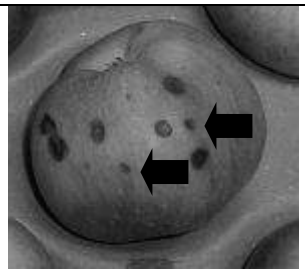
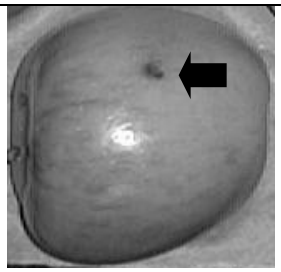
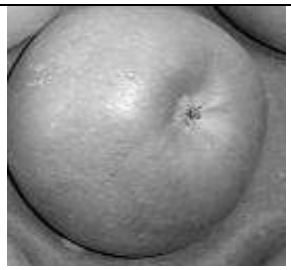
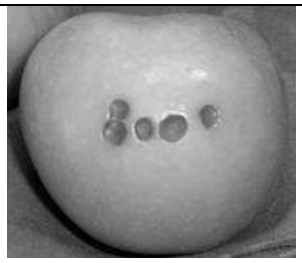
(a)			
(b)			
(c)			

Fig. 1 (a) Extreme bitter pit lesions in ‘Braeburn' apples after storage - multiple lesions, a single lesions and no pit; (b) classification of fruit with bitter pit into bitter pit and lenticel spot; (c) fruit with external bitter pit symptoms showing with even more internal lesions when peeled.

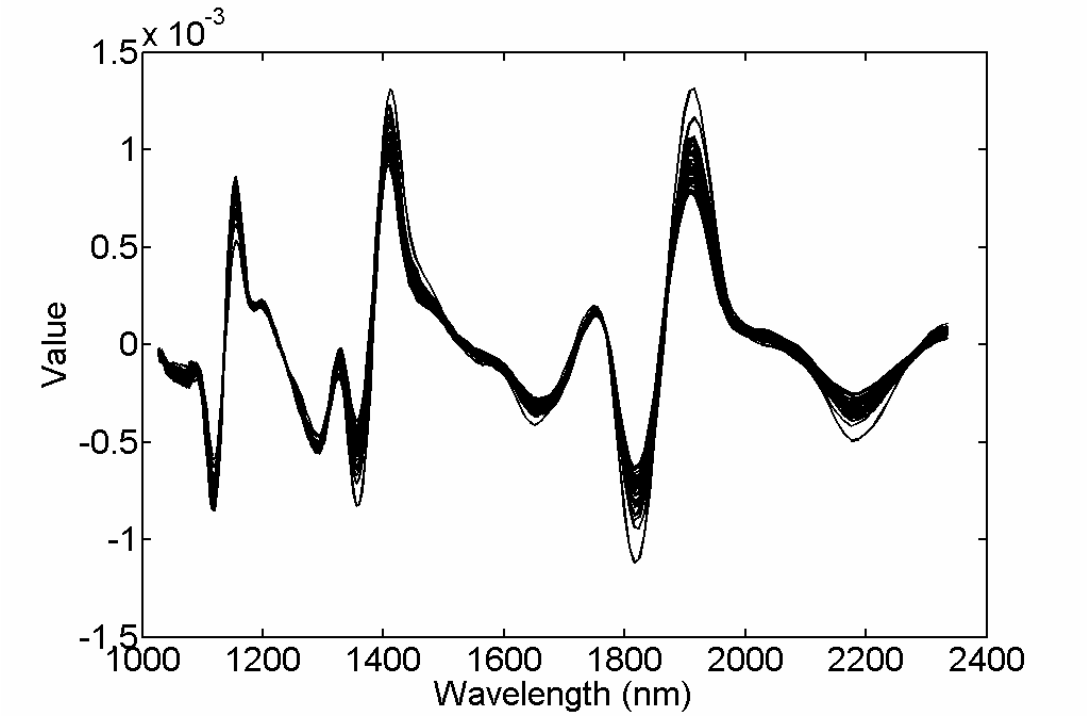


Fig. 2 Second derivative of Kubelka-Munk transformed spectra of bitter pit and lenticel spots and adjacent unaffected tissue.

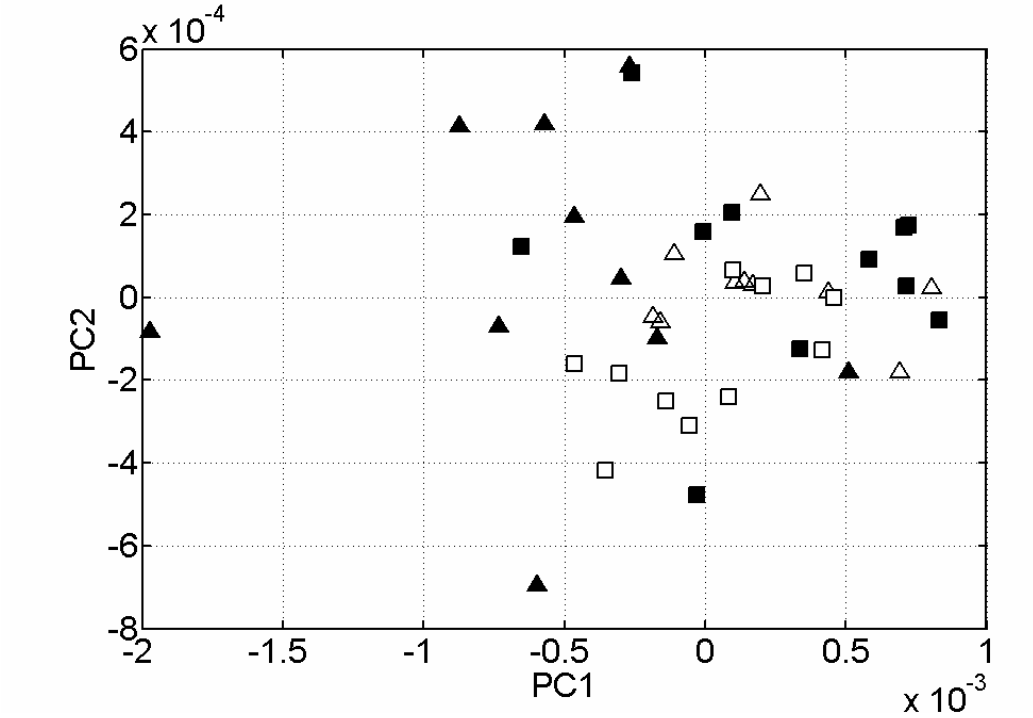


Fig. 3 PCA score plot of the second derivative reflectance spectra of three Braeburn. Lenticel spots (■) and adjacent tissue (□) are separated along PC2, whereas bitter pit spots (▲) and adjacent tissue (△) are separated along PC1. PC1 and PC2 account for 78% and 16% of the total variance, respectively.

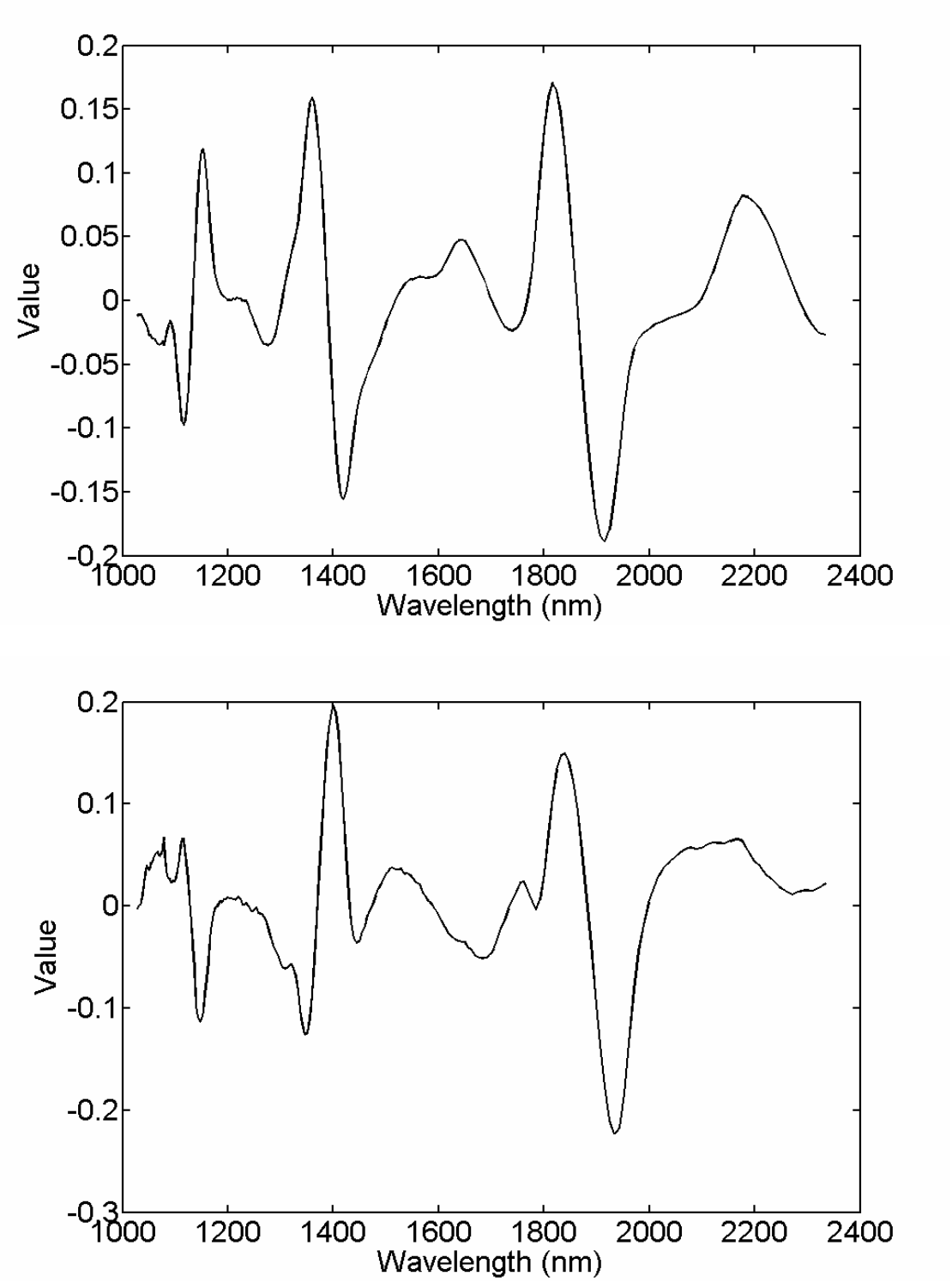


Fig. 4 Plot of the first (a) and second (b) loadings. The PCA analysis was carried out on the second derivative reflectance spectra of bitter pit and lenticel spots and adjacent unaffected tissue.

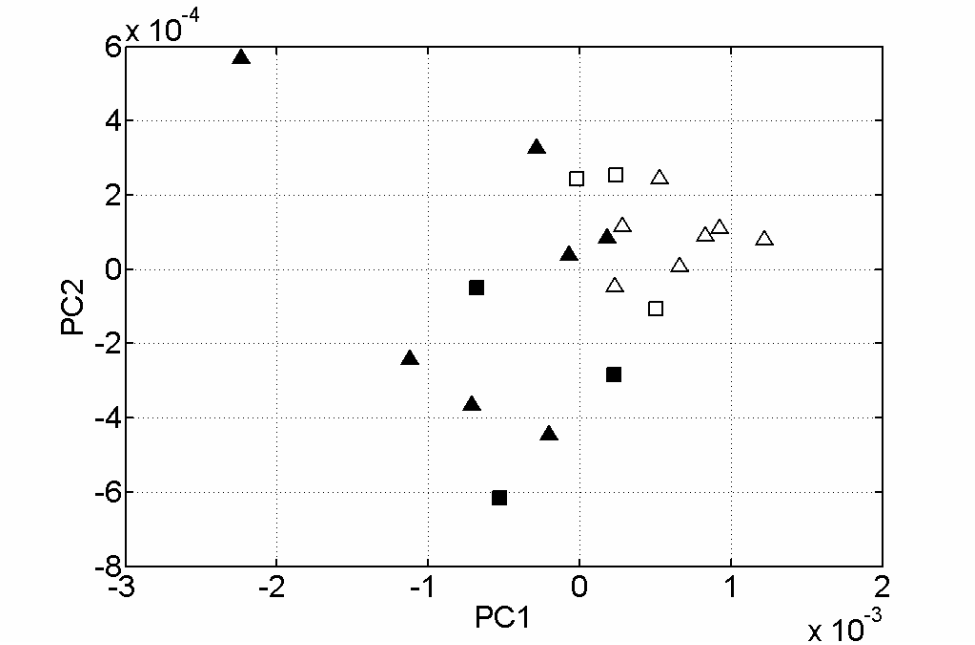


Fig. 5 PCA score plot of the second derivative reflectance spectra of one Braeburn apple. There are too few measurements of lenticel spots (■) and adjacent tissue (□), but, as in Fig.4, bitter pit spots (▲) and adjacent tissue (△) are separated along PC1. PC1 and PC2 account for 85% and 11% of the total variance, respectively.

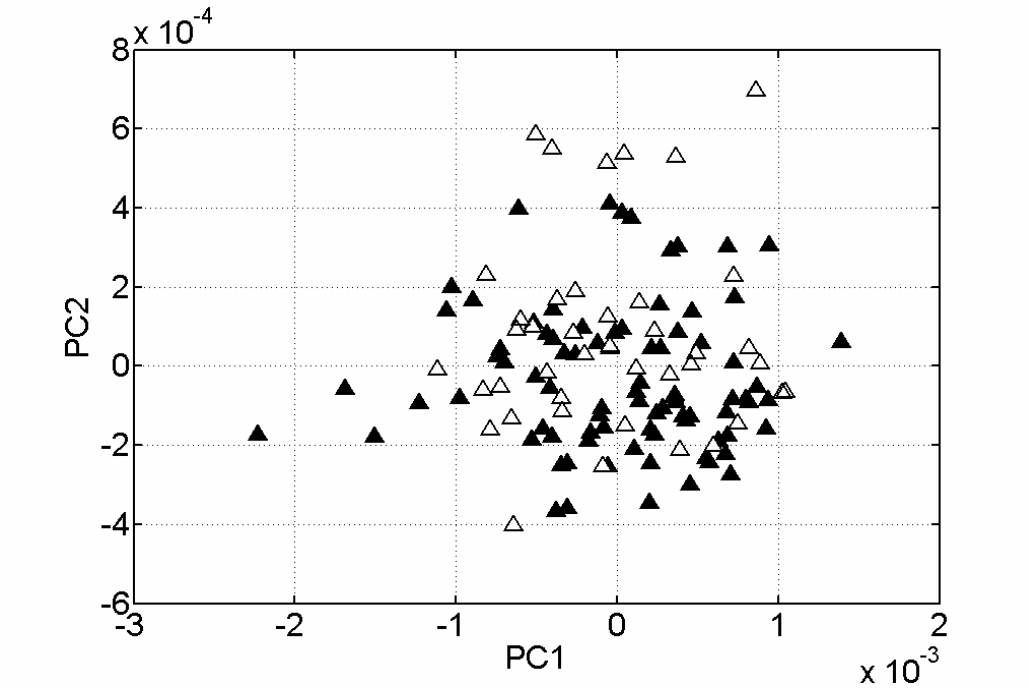


Fig. 6 PCA score plot of the second derivative reflectance spectra Braeburn apples immediately after harvest. There is no difference between apples which developed bitter pit after storage (▲) and which did not (△). PC1 and PC2 account for 84% and 10% of the total variance, respectively.

Evaluating the effectiveness of pre-harvest Ca application for bitter pit control in 'Golden Delicious' apples under South African conditions.

Abstract

Pre-harvest foliar applications to increase fruit calcium (Ca) content and reduce bitter pit incidence, is a standard practice world wide. We re-evaluated the effectiveness of early season applications versus late applications of $Ca(NO_3)_2$ to reduce bitter pit in 'Golden Delicious', under the present environmental conditions of the Western Cape. Two periods of efficient uptake of external Ca were identified: during cell division and the last few weeks before harvest. Foliar Ca applications during mid season (from 40 days after full bloom) were more effective in increasing fruit Ca content and reducing bitter pit incidence, than later applications. Thus it is important to apply the bulk of the $Ca(NO_3)_2$ during the first few weeks after cell division to maximise the Ca content of 'Golden Delicious' apples.

Introduction

Bitter pit in apples is closely associated with the Ca content of the fruit (Ferguson *et al*., 1979; Perring & Pearson, 1987; Ferguson & Watkins, 1989). Relatively high fruit Ca content at harvest can reduce the incidence of this disorder considerably. Except for applying Ca to the soil and employing optimal orchard practices, foliar Ca applications pre-harvest to increase the Ca content of fruit and reduce bitter pit are widely used (Beyers, 1963; Terblanche *et al*., 1970; Ferguson *et al*., 1987; Hewett & Watkins, 1991; Yuri *et al*., 2002).

Results with foliar Ca applications vary however, from highly successful cases, to a substantial number with little effect on Ca content of fruit or bitter pit control (Carbo *et al*., 1988; Hewitt & Watkins, 1991; Le Grange *et al*., 1998). Various researchers (Quinlan , 1969; Ferguson *et al*., 1987; Faust, 1989; Cline *et al*., 1991; Casero *et al*., 2002; Schlegel &

Schönherr, 2002) described the rapid uptake and penetration of Ca into fruit, mainly during the first four weeks after full bloom (wafb) or between six and 14 wafb, followed by a decline until harvest. Alternatively, Zavalloni *et al.* (2001) reported a continuous linear increase in Ca from about 40 days after full bloom (dafb) until harvest for different cultivars in Italy. This confirmed earlier findings by Rogers & Batjer, (1954), Wilkinson (1968), Tromp (1979), Jones *et al*. (1983) and Tomala *et al*. (1989) who reported fruit Ca uptake until harvest. These differences may be due environment and genotype.

To determine the best time to apply foliar Ca to reduce bitter pit, Stahly (1986) induced bitter pit with 2,3,5-triiodo-benzoic acid (TIBA) sprays. TIBA inhibits the basipetal transport of the auxin, indole-3-acetic acid (IAA) and reduces the acropetal transport of Ca (Stahly, 1986). He concluded that the first Ca applications (35, 55 and 77 dafb) did not increase fruit Ca content as much as the later applications (77, 98 and 119 dafb), but that the first treatments were significantly more effective in reducing bitter pit. A reduction in IAA was followed by a reduction in Ca, after applications of TIBA during the early phases of cell division and expansion, in nectarines (Wand *et al*., 1991) and avocado (Cutting & Bower, 1989). These experiments indicate a relationship between Ca transport and cell division and expansion, partly by demand, but also possibly due to a signal from increased IAA for additional Ca influx into the sink, due to the spontaneous IAA increases in response to fruit growth.

Zavalloni *et al*. (2001) were able to increase the fruit Ca in **'**Golden Delicious' at harvest by 20 percent with foliar applications of $CaCl_2$. Ten applications were made ranging from before bloom, at petal drop, and at 20 day intervals thereafter until 15 days before harvest. In Spain, Casero *et al*., (2002) also working on 'Golden Delicious', substantiated the increase in Ca absorption (mg Ca per fruit per day) from the beginning of fruit growth (20 mg Ca per fruit per day) until a maximum was reached about 60 dafb for treated and untreated fruit (approx. 80 mg Ca per fruit per day). Thereafter, the absorption declined before it

increased slightly again, before harvest. This trend caused no significant differences in Ca content between treatment and control during the first part of the season, but an increase in Ca absorption during the second part due to Ca applications, resulted in a higher Ca content of the treated fruit.

In Chile, Venegas (1994) evaluated time and number of Ca applications on 'Granny Smith'. Foliar applications of $CaCl_2$ were made fortnightly. There was a significant difference in percentage bitter pit after storage between the treatments with three and six versus nine applications, with more applications resulting in less bitter pit. All applications started at 42 dafb. No significant differences were found when he applied the same number of sprays at different times, starting from 42, 83 and 130 dafb and combinations thereof. Yuri *et al*. (2002) applied six Ca sprays on 'Braeburn', splitting applications in three sprays three to eight weeks after full bloom and three between 120-150 dafb. Applications after full bloom had a greater influence in increasing Ca levels of the fruit than the late applications.

In Spain, increasing fruit Ca content was more successfully achieved by increasing the frequency of the applications from every 20 to every 10 days, than by advancing the date of the first foliar application by 10 days from 40 dafb to 30 dafb (Carbo *et al*., 1998).

In British Columbia, recommendations are four to five foliar applications ($CaCl_2$) at 10 day intervals from mid July (approximately 40 dafb) (Neilsen & Neilsen, 2002). Results on timing of spray applications showed that at least five post bloom sprays were required to increase fruitlet Ca concentration (64-67 dafb) in 'Fuji','Jonagold' and 'Gala'. Where three applications of $CaCl_2$ were given within the first month after full bloom, no increase in fruit Ca concentration was observed. In contrast, four to five late season applications (weekly sprays in August) of $CaCl_2$ always resulted in an increase in fruit Ca concentration at harvest. The later Ca applications were more effective in increasing the absolute fruit Ca uptake than the early applications. However, when elimination of bitter pit was the purpose, early

applications provided complete control, whereas late applications controlled bitter pit only after cold storage, but was only moderately effective as far as controlling pit at harvest. Late applications in July were also more effective than those in August for elimination of bitter pit in 'Braeburn', a variety that is susceptible to tree pit, before harvest. The conclusion was to apply early season foliar Ca where the disorder has an early season origin (Neilsen & Neilsen, 2002).

Under South African conditions, most results favour late season (from 70 dafb) Ca applications. For 'Golden Delicious', November (20 dafb) (SH) was found too early and February (harvest) too late for efficient control of bitter pit in the Elgin area (Beyers, 1963). January (90 dafb) was found the most efficient month to apply foliar Ca, with the first application in mid December (70 dafb) and the next two in January. Terblanche *et al*. (1970, 1974, 1975) proved that effective control of bitter pit was possible when bitter pit incidence was less than 16 percent in the orchard, with three to five foliar Ca applications at fortnightly intervals from middle December for 'Golden Delicious'. When higher bitter pit percentages occurred, the efficiency of control with foliar applications decreased. Guidelines for bitter pit control for the South African industry include at least six, weekly foliar Ca applications from middle December (70 dafb) (Kotze, 1987). Wooldridge & Joubert (1997) recently evaluated various products for bitter pit control on 'Golden Delicious' and recommended four Ca applications from beginning of December (55 dafb) until harvest with 10 day intervals.

Although in this research no difference was made between bitter pit visible on the tree (tree pit) and only visible after storage (storage pit), there has been a local report stating that early season foliar applications are more effective in reducing tree pit, opposed to late season applications being more effective in reducing storage pit in 'Golden Delicious'(Ginsburg, 1962).

A survey (Lötze & Theron, 2003) involving 91 commercial apple orchards in the Western Cape revealed that, for 'Golden Delicious', more Ca applications (9-10) were not necessarily associated with less bitter pit than the standard six applications. More than 50 percent of the orchards (19), where the first application date was before 40 dafb, experienced bitter pit. Nevertheless, there are consultants presently recommending Ca applications from as early as petal drop. According to Ginsburg (1962), New Zealand growers applied $Ca(NO_3)_2$ bi-weekly from petal drop successfully to reduce bitter pit on 'Cox's Orange Pippin', during the 1960's. No information about the number of sprays was related, thus the effect could have either been the number of applications, or the timing.

According to the same survey, only 40 percent, where applications started between 40 and 80 dafb showed bitter pit. Where applications occurred after 80 dafb (mid Dec), only 17 percent of the orchards had bitter pit, enforcing Kotze's (1987) recommendation for applications from 70 dafb. This contrasted with experimental data (Lötze & Theron, 2005) suggesting higher absorption of $Ca(NO_3)_2$ 40-80 dafb than after 80 dafb. Therefore the decision to re-evaluate the effectiveness of early season applications versus late applications of $Ca(NO_3)_2$ to reduce bitter pit in 'Golden Delicious', under the present environmental conditions of the Western Cape.

Materials & Methods

Ca application trials were conducted at the same site during three consecutive seasons (2002/3-2004/5). We selected mature, uniform, bearing 'Golden Delicious' trees on a commercial farm in a bitter pit prone orchard. In all three trials, Calnitro ($Ca(NO_3)_2$) was used as Ca source, but the timing and number of sprays varied between trials (Table 1). Agral® was used as surfactant the first two seasons, but omitted during the last season. Ca was applied with a hand gun until run off (high volume). Buffer trees were used between

treated trees. Mineral analyses on individual fruit were done by a commercial laboratory (Bemlab Pty Ltd, Strand). The whole fruit without the pips and core was analysed.

During the first season (2002/03), weekly fruit samples were collected to establish Ca uptake patterns. This was used to establish the grouping of the treatments, expressed according to phenological stage, to compare the different seasons. Early season was defined as applications up to 40 dafb (mainly cell division phase), mid season 40 - 80 dafb (end of cell division and beginning of cell expansion), and applications starting after 80 dafb (cell expansion & end of shoot growth) were defined as late season. Under our conditions, the growing season for 'Golden Delicious' is approximately 140 days.

During 2002/03 the standard rate of 122g $Ca(NO_3)_2$ per 100L water was applied weekly from 40 dafb (Table 1). In 2003/04 the rate of the first applications was reduced (36g $Ca(NO_3)_2$ per 100L water) to minimise phytotoxicity on the leaves, but all treatments received an equal amount of $Ca(NO_3)_2$ that was applied bi-weekly. Table 1 gives a summery of the $Ca(NO_3)_2$ applied per tree per week during the three seasons. From 30 dafb, two litre spray solution instead of one was applied per tree due to the increase in leaf area.

During 2004/05 weekly applications were made with the standard rate, except for the early-late application (EL9). For this treatment, where a combination of five weekly early and four weekly late applications were used, the initial applications were again at the reduced rate (36g $Ca(NO_3)_2$ per 100L water). The intention was to apply similar Ca , but management changes resulted in Mid and Late 7 receiving 17.1 g and EL9, 16.4 g active Ca (Table 1).

Statistical analysis

Trials were conducted as randomised complete block designs with 10 replicates per treatment and two to three trees per plot. Analysis was done using the Statistical Analysis System (SAS) programme (SAS Institute Inc, 1999-2001). Analyses of variance were

performed with the general linear model (GLM) procedure. In additional, single degree of freedom, orthogonal, polynomial contrasts were used where applicable. The correlation coefficients between the Ca concentration of fruit at harvest and the volume of $Ca(NO_3)_2$ applied during the phases I and II for each season was determined with the regression procedure (PROC REG) in SAS.

Due to the skew distribution of bitter pit percentages (0 - 35 %), a logit transformation was performed on the bitter pit percentage data for analysis, within each season, to stabilise the variance and equalise the ranges (Snedecor & Cockran, 1997).

Results and Discussion

2002/2003

The Ca content increased naturally in fruit without any foliar Ca applications (Fig. 1), from about 40 to 70 dafb (phase I), followed by a period of no increase of about 10-14 days, before the final increase started at 84 dafb for approx. six weeks (phase II), confirming previous results (Rogers & Batjer, 1954; Stahly & Benson, 1982). We did not monitor changes in Ca content before 40 dafb. However, the increases after 40 dafb were much more pronounced in fruit receiving twelve foliar Ca applications, than with the natural trend observed in the control. The Ca content per fruit could be increased significantly ($P < 0.0001$), and bitter pit reduced significantly, by applying Ca foliar sprays (Table 2). The percentage increase in Ca content during phase I was more dramatic (100%) than that of phase II (50%) (Lötze & Theron, 2005). Phase II was approximately two weeks longer. This confirms previous work by (Vang-Petersen, 1980; Faust, 1989; Schlegel & Schönherr, 2002) who reported the primary and rapid uptake and penetration of Ca into fruit occurs during the first four to six weeks after full bloom. However, in terms of Ca content of the fruit, the increase in total Ca content in the fruit was higher during phase II than phase I. The fruit

surface is much smaller during phase I than II, explaining in part the higher total Ca increase during phase II (Ferguson *et al*., 1987). It must also be noted that the period of increase in Ca uptake during phase II (four weeks) is slightly longer than during phase I (three weeks).

2003/04

Significant differences were found in Ca content of fruit at harvest between the different treatments (Table 3). Early 8 fruit had significantly more Ca than Mid 8 fruit, and both treatments were significantly higher than the control. The Early 6 applications, starting from petal drop, did not increase the Ca content of the fruit significantly above that of the control. The highest fruit Ca content was found with treatment Early 8, followed by the second highest in Mid 6. Mid 6 however, was not significantly different from Early 8 and Mid 8 regarding fruit Ca content. It was significantly higher than the control and Early 6.

These results indicated that the Early 6 applications were ineffective in increasing the Ca content even though the same amount of Ca than in Early 8 was applied, because it was applied at the incorrect time, too early (before 40 dafb). Early 8 was highly effective in contrast with the Early 6, that received the same amount of Ca – suggesting that the later applications, after 40 dafb, are partly responsible for the difference. Mid 6 was not significantly different from Early 8, which can be explained by the overlap during phase I between the treatments. Mid 6 and Mid 8 did not differ significantly either, also with an overlap during phase I.

Bitter pit occurrence was reduced significantly by all treatments when compared to the unsprayed control (Table 3). The control had the highest average bitter pit percentage (15.7 %), followed by significantly less in the Early 6 (3.5 %) and Early 8 (1.6 %) treatments. The Early 8 treatment had the lowest bitter pit percentage (1.6 %), but this did not differ significantly from with Mid 6 (3.0 %) and Early 6 (3.5 %). The Ca applied in these three

treatments was very similar during phase I (108-122 g). Thus, although the Ca content of the last three treatments was different, the control of bitter pit was comparable, indicating that the overlap in timing of the Ca applications influenced the control of bitter pit, or that the threshold value for Ca required to prevent bitter pit, has been achieved with all three treatments. A contrast confirmed a statistical difference only between the control and treatments for bitter pit. No statistical differences were found between number or time of treatments.

Hence, a trial was conducted the following season to determine how efficient early applied Ca (before 40 dafb) is. The time of $Ca(NO_3)_2$ applications started at petal drop (early) and at 40 dafb (mid), varying the number of sprays (six & eight) and reducing the total $Ca(NO_3)_2$ applied.

2004/2005

The third trial on the same site again, on different trees, with slightly different treatments, resulted in significant differences in Ca content per fruit at harvest for treatments Mid 7 and Late 7 versus Early-Late (EL9), but not between EL9 and the control (Table 4). The highest fruit Ca content in Mid 7 confirmed results from 2003/04 that indicated phase I as the most suitable time to increase fruit Ca with foliar applications. Late 7 had the second highest Ca content with the first spray dates also within phase I, although it did not differ significantly from Mid 7. In the EL9 treatment, only one application fell within phase I (Table 1).

A contrast confirmed a significant difference between the treatments and control (0.0165) (Table 4). Although there was a significant difference in the final Ca content per fruit when foliar applications started after 40 dafb (Mid) when compared to an earlier date (petal fall), no significant difference could be established between applications after this date,

Mid 7 versus Late 7 (0.1024). However, a significant difference in Ca content was found in a contrast between the EL9 and Mid 7 versus Late 7 (0.0012) treatments (Table 4).

Concerning bitter pit, the only statistically significant difference existed between the Mid 7 season application with less bitter pit, and the other treatments, including the control (Table 4). No significant differences could be found between the control and treatments, Mid 7 versus Late 7, or EL9 and Mid 7 versus Late 7 treatments. Relatively low incidences of bitter pit were observed in this season (± 3.5 %) compared to the previous seasons. In this trial, the highest fruit Ca content corresponded with the lowest bitter pit percentage after storage.

A regression model with Ca concentration in the fruit and amount of $Ca(NO_3)_2$ applied during phases I (40 – 74 dafb) and II (84-120 dafb), confirmed the best correlation between these variables (Ca concentration and $Ca(NO_3)_2$ applied) and application time, for phase I (r^2 = 0.44, $p < 0.0001$) (Table 5). The Early and EL applications showed no correlation with phase II due to one and none applications, and the phase I correlation was also inferior to the applications during Mid and Late season. Phase II regressions with all the data (All) resulted in the same coefficient than for phase I, due to the applications being spread across both phases, when all application data was considered. Mid and Late data, showed the highest correlation coefficients (0.4188) of all application times (treatments), as well as the highest coefficient with phase I data. The best fit was achieved changing from a linear to a 3rd degree polynomial equation. There was an increase in Ca concentration of fruit with an increase in Ca applied, for the Mid and Late treatments for three seasons. Lesser and insignificant correlations were found for Mid and Late applications during phase II, as well as for Early and Early-Late applications in phase I (Table 5).

General

In contrast with previous researchers (Beyers, 1963; Beyers & Dempers, 1967; Terblanche *et al*., 1974), this information favours the following hypothesis: foliar Ca applications during phase I (mid season) are more effective in increasing fruit Ca content for 'Golden Delicious' than applications during phase II under South African conditions and also resulted in better bitter pit control.

When the total applied $Ca(NO_3)_2$ after 40 dafb increased, it was followed by an increase in fruit Ca in most cases (Fig. 2a). To determine whether the volume of $Ca(NO_3)_2$ applied in phase I and II contributed equally to this trend, the data for the phases were split (Figs. 2b & c). The trend showed that product volumes applied in phase I (mid season at 40dafb) is the main contributor to the final Ca content per fruit as was confirmed by the regression co-efficient. Thus it is important to apply the bulk of the total of $Ca(NO_3)_2$ during the first few weeks after cell division (mid season) for the maximum increase in Ca content per fruit.

Conclusion

According to Lötze & Theron (2005), mid season applications increased Ca content more efficiently on a percentage increase base, than late season applications. If the presented results of the three seasons are viewed collectively, mid and late season foliar applications were the most efficient in increasing final Ca content of fruit at harvest. Fruit that received foliar Ca for about four weeks from 40 dafb consistently showed a higher fruit Ca content at harvest than fruit where applications did not occur during this period, indicating a higher level of efficiency in foliar applied Ca during this period. Late applications seemed to be effective due to the first few applications starting during phase I. Both treatments were also associated with less bitter pit compared to fruit of the control treatments.

These results were compared for different seasons and with only one product ($Ca(NO_3)_2$). It does show a trend that should be addressed in future trials. In contrast to present recommendations to start Ca applications late in the season (Dec) or very early (before 40 dafb), these trends points towards foliar applications on 'Golden Delicious' during phase I (Nov), to be more effective in increasing fruit Ca content early in the season and decreasing bitter pit incidence.

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Table 1. Summary of the $Ca(NO_3)_2$ (g) applied per tree per week for the three different seasons.

									Phase I *				Phase II *									
$Ca(NO_3)_2$ applied/tree			1l	1l	1l	2l	2l	2l	2l	2l	2l	2l	2l	2l	2l	2l	2l	2l	2l	Total $Ca(NO_3)_2$ applied / season	Phase I * 3 weeks	Phase II * 4 weeks
Season	Treatment	DAFB	7	14	21	28	35	42	49	56	63	70	77	84	91	98	105	112	119			
200203	Ca application from 40 dafb (mid)	M12						2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	29.3	7.3	9.8
200304	Ca application from 100 % petal drop (early)	E6	0.36	1.08		2.44		2.44		2.44		2.44								11.2	2.4	0.0
200304		E8	0.36	0.36		0.72		0.72		2.44		2.44		2.44		2.44				11.9	2.4	4.9
200304	Ca application from 40 dafb (mid)	M8						0.72		0.72		0.72		0.72	2.44	2.44	2.4	2.4		12.7	0.7	5.6
200304		M6						0.72		2.29		2.44		2.44		2.44		2.4		12.7	2.2	4.9
200405	Ca application from 40 dafb (mid)	M7						2.44	4.88	2.44	2.44	2.44	2.44							17.1	9.8	2.4
200405	Ca application from 80 dafb (late)	L7							2.44		2.44	2.44	2.44	2.44	2.44	2.44				17.1	4.9	9.8
200405	Ca application from 100 % petal drop(early) & Beginning 80 dafb (late)	EL9		0.36	0.36	1.08	2.35		2.44						2.44	2.44	2.4	2.4		16.4	2.4	4.9

* Phase I is approx. 40 to 74 dafb; Phase II is approx. 84 to 120 dafb

Table 2. Anova results of the effect of Ca-applications on bitter pit control during 2002/03.

TREATMENT	mg Ca/ 100g FW	BP %	BP Log
Untreated (control)	2.20	21	-1.04 a*
Ca applications M12 (treatment)	4.94	3	-3.42 b

* Means with the same letter are not significantly different

Table 3. Mean Ca (mg) per fruit at harvest in 2003/04. Mean separation in columns using LSD (5%).

Treatment	Mean Ca/fruit at harvest (mg)	% Bitter pit *	% Bitter pit
Early 8	3.54 a	-3.92 c	1.6
Mid 8	2.87 b	-2.90 b	5.1
Mid 6	3.09 ab	-3.62 c	3.0
Early 6	2.02 c	-3.61 c	3.5
No Ca applications	1.87 c	-1.86 a	15.7
Significance level:	*Pr > F*	*Pr > F*	
Treatment (4 d.f.)	*0.0001*	*0.0001*	
Contrasts:	*Pr > F*	*Pr > F*	
Control (no) vs treatments	<0.0001	<0.0001	
Mid (40 dafb) vs Early (petal drop) season	<0.0001	0.3920	
6 vs 8 applications	0.5048	0.3771	

* Logit % bitter pit = LOG ((Bitter pit fruit +0.5)/ (Total no. of fruit – Bitter pit fruit +0.5))

Table 4. Mean Ca (mg) per fruit at harvest in 2004/05. Mean separation in columns using LSD (5%).

Treatment	Mean Ca/fruit at harvest (mg)	* % Bitter pit	% Bitter pit
Mid 7	5.09 a	-4.89 a	0.59
Late 7	4.28 a	-3.65 b	2.41
Early & Late 8	2.90 b	-3.90 b	3.82
NO	3.09 b	-3.44 b	4.08
Significance levels:	*Pr > F*	*Pr > F*	
Treatment (3 d.f.)	*0.0002*		
Contrasts:	*Pr > F*	*Pr > F*	
1(NO) vs treatments	*0.0165*	*0.0678*	
Mid (40 dafb) vs Late (80 dafb)	*0.1024*	*0.5751*	
Early-Late (petal drop + 80 dafb) & Mid vs Late (80 dafb)	*0.0012*	*0.0689*	

* Logit % bitter pit = LOG ((Bitter pit fruit +0.5)/ (Total no. of fruit – Bitter pit fruit +0.5))

Table 5. Regression between Ca content at harvest and applied $Ca(NO_3)_2$ during phase I and II for the different application treatments.

Treatment	Phase I *		Phase II *		equation
	R^2	P	R^2	P	
Early & Early-Late	0.3014	0.0017	n. a	n. a.	linear
All	0.4125	0.0001	0.4125	0.0001	linear
Mid & Late	0.4188	0.0001	0.0001	0.9494	Both linear
Mid & Late	0.4418	0.0001			3rd degree polynome

* Phase I is approx. 40 to 74 dafb; Phase II is approx. 84 to 120 dafb

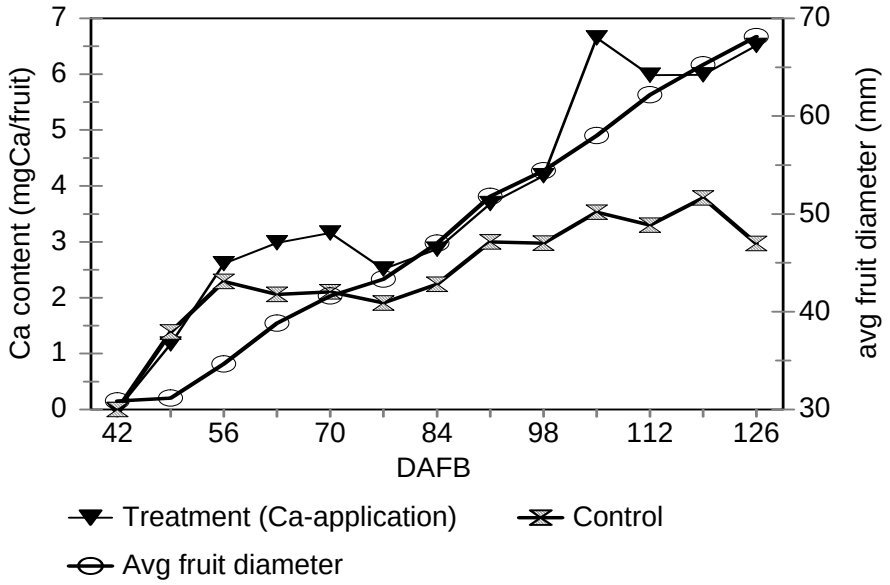

Fig 1 The seasonal increase (2002/03) in Ca content per fruit for the control (no foliar Ca application) versus the treatment (12 foliar Ca applications, starting 42 dafb).

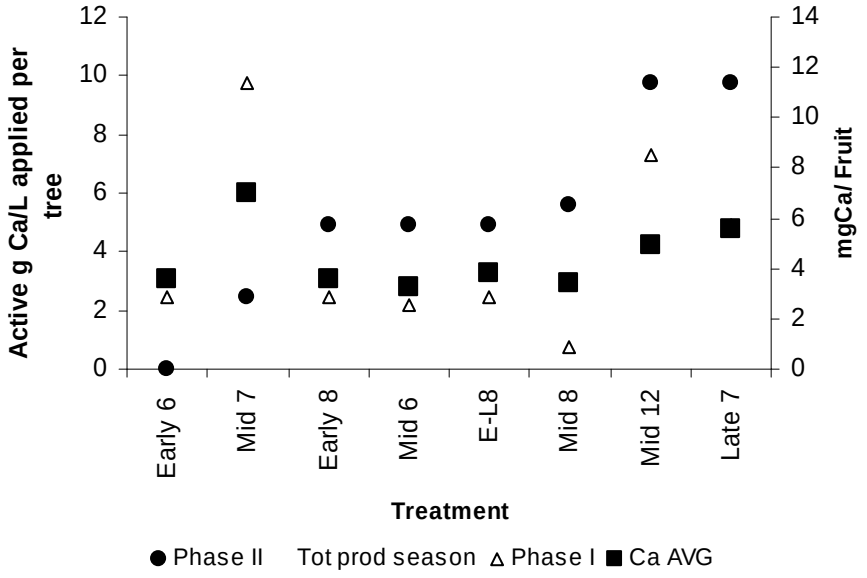

Fig 2a Total $Ca(NO_3)_2$ applied in phases I and II during the season versus average Ca content/fruit for each treatment.

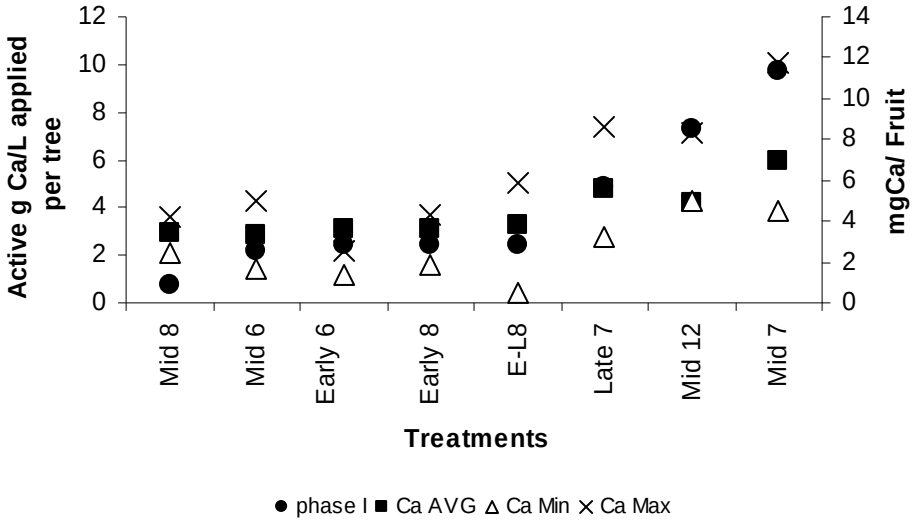

Fig 2b Total $Ca(NO_3)_2$ applied in phase I versus average, min. and max. Ca content/fruit for each treatment

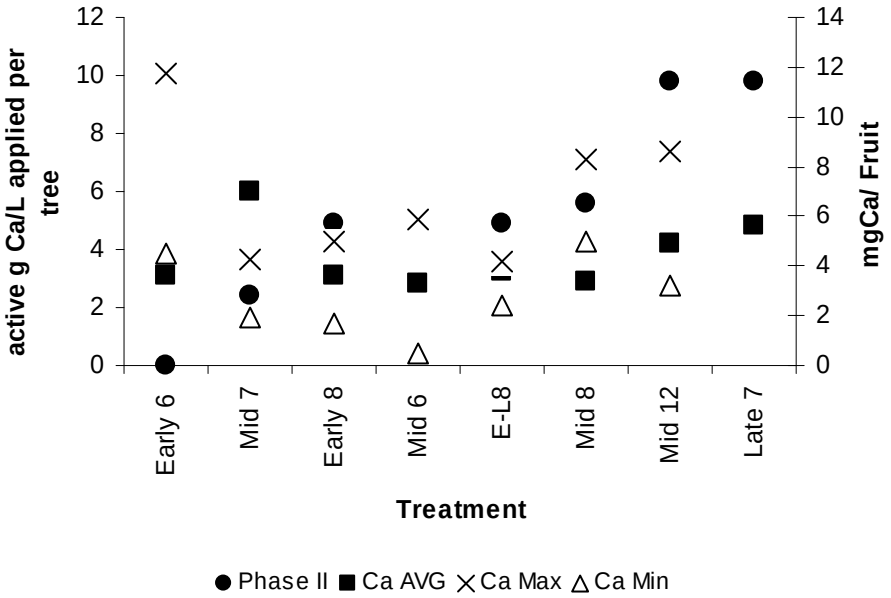

Fig 2c Total $Ca(NO_3)_2$ applied in phase II versus average, min. and max. Ca content/fruit for each treatment

Determining the probability of bitter pit in ‘Golden Delicious’ apples through the post harvest mineral content of individual fruit

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SUMMARY

Bitter pit fruit in commercial consignments of apples still poses an economic threat to exporters from South Africa. Mineral analysis of fruit has been used with variable success to predict bitter pit prior to harvest. The possibility of increasing the accuracy of existing predictive models by using analysis of individual fruit rather than pooled samples was investigated. By improving the normality of the distributions of the different minerals and decreasing the overlap between pitted and non-pitted fruit classes, we attempted to improve the reliability of predictions based on variable threshold values. Even though our model produced a correct classification of 85% for non-pitted fruit which can be useful, this was still below the required tolerance expected on the market which at present is less than 2% bitter pit in an overseas consignment. The classification for pitted fruit, 63%, was not satisfactory.

The natural occurrence of bitter pit in a commercial orchard or even a single tree, varies between seasons and with orchard management (Yuri, 1995; Le Grange *et al*., 1998). Because of the detrimental effect of the presence of bitter pit fruit in export consignments, incidence of the condition has been reduced significantly, e.g. by adjusting orchard practices like applying foliar Ca during the season (Terblanche *et al.*, 1975), managing crop load (Ferguson & Watkins, 1989) and summer pruning to reduce tree vigour (Terblanche *et al*., 1974,1975). Nevertheless, with a tolerance of < 2% for export fruit (H. Griessel, Tru-Cape, P.O. Box 3772, Somerset West; personal communication) it is important to identify fruit prone to bitter pit correctly before export.

The development of bitter pit is closely associated with the calcium (Ca) content of the fruit (Faust & Shear, 1968). Mineral analyses of fruit samples, including pitted and non-pitted fruit, have produced models with ranges of mineral concentrations typical of bitter pit and non-pitted fruit classes. Accurate prediction of the number of pitted fruit varied, however (Wills *et al*., 1976; Ferguson *et al*., 1979; Terblanche *et al*., 1980; Waller, 1980; Drahorad & Aichner, 2001).

Mineral analyses of young fruit from 80 d after full bloom (DAFB) or fruit 2 - 4 weeks before harvest are often used to predict the occurrence of bitter pit at harvest (Martin *et al*., 1975; Wills *et al*., 1976; Ferguson *et al*., 1979; Terblanche *et al*., 1980; Waller, 1980; Drahorad & Aichner, 2001). This method is based on the mineral content of a composite sample of 25-50 fruit in which the whole fruit, or representative sections thereof, are analysed with an emphasis on the Ca, potassium and magnesium concentrations, and the ratios of these minerals. Usually mean mineral concentrations [mg $100g^{-1}$ fruit weight (FW)] are used for prediction. Threshold values of these minerals and/or their ratios are applied to determine the potential of the sample to develop bitter pit (Wills *et al*., 1976; Ferguson *et al*., 1979; Waller, 1980; Drahorad & Aichner, 2001).

In 1976, Wills *et al*. compared results on thresholds of Ca concentrations for predicting bitter pit in apples from New Zealand with those from the rest of the World. They reduced the number of fruit analysed per sample from 10-20 to five to minimise fruit-to-fruit variation. However, in contrast with previous reports, they could not define general threshold values for Ca. Fruit samples with less than 2 mg $Ca.100g^{-1}$ FW were always susceptible to bitter pit, but samples with other levels of Ca varied in their susceptibility between cultivars, areas and seasons.

In South Africa, cases are frequently reported where predictions based on composite sample thresholds alone are unreliable. Fruit samples with relatively high Ca concentrations (> 5mg $Ca.100g^{-1}$FW) developed bitter pit, whereas fruit samples with lower Ca concentrations, did not (Terblanche *et al*., 1980; Le Grange *et al*., 1998). This anomaly lead Le Grange *et al*. (1998) to evaluated bitter pit and the mineral content of 'Braeburn' apples on an individual fruit basis. Their work was aimed at documenting distribution patterns of mineral concentrations around the mean. Their results showed a non-normal distribution for all minerals in pitted and non-pitted fruit, except for Ca content. Ca distributions for both populations were normal, but with a significantly lower mean for bitter pit fruit. However, the distributions overlapped, complicating the use of Ca concentration alone as a parameter to predict susceptibility to bitter pit. Le Grange *et al*. (1998) did, however, use relatively few fruit for these analyses (37 pitted and 29 non-pitted fruit).

We used 'Golden Delicious' apples to determine whether, by increasing the sample size, we could increase the normal distributions of the different minerals and decrease the overlap between pitted and non-pitted classes. In addition, by using individual apples instead of pooled data for mineral analysis, we aimed to improve the accuracy of the mineral analysis prediction model.

MATERIALS AND METHODS

Mineral distribution of bitter pit and non-pitted populations

Population 1: Mature, fruit-bearing 'Golden Delicious' apple trees (*Malus domestica* L. *Borkh*) in a bitter pit-prone orchard on a commercial farm (Graymead) in the Vyeboom area (32°53'S; 19°18'E) were selected for this experiment in the 2003-04 season. Fruit were harvested while immature (< 20% starch break-down) to increase their potential for bitter pit. The whole batch of fruit sampled will be referred to as a population of fruit. Fruit were stored for 3 months and then evaluated visually for the presence of bitter pit. Fruit were classified as either having (BP) or not having (NO) bitter pit symptoms, regardless of the number of lesions. All pitted fruit (310) were used, as well as randomly chosen non-pitted fruit (500). Mineral analyses were performed by a commercial analytical laboratory (Bemlab Pty. Ltd, Strand, South Africa) using individual whole fruit but without the pips, core and stalks. Fruit were washed with a 1% v/v HCl solution, then rinsed twice each with tap and deionised water to remove any superficial Ca residues. The standard preparation and analysis of samples was followed using the ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometer) procedure and a nitrogen analyser (W.A.G. Kotze, Bemlab Pty. Ltd, Strand, South Africa; personal communication). Results were expressed as concentration of each mineral 100 g^{-1} FW for N, P, K, Ca and Mg.

Population 2: In 2002-03, samples of 'Golden Delicious' apples from two different producers in the Vyeboom area (Population 2a and 2b) were selected from bins after storage. A random sample of pitted (75) and non-pitted (75) fruits of similar size were selected from each orchard. Mineral analyses were performed on individual fruit as above.

Statistical analysis

Data were analysed statistically using the Univariate procedure from the Statistical Analysis System (SAS) programme (SAS Institute, 1999-2001) to determine how mineral concentration values from pitted or non-pitted fruit were distributed around their respective means. Partial t-tests were performed to determine whether the differences between population mineral means were significantly different.

Discriminant and stepwise discriminant analyses were performed on the data, using the 'DISCRIM' and 'STEPDISC' procedures (SAS Institute, 1999-2001) to determine if the mineral composition of individual fruit could be used to classify fruit into classes with or without bitter pit. Variables were identified using a stepwise discriminant analyses based on the mineral contents of individual fruit and fruit weight. These selected variables were then used to discriminate between BP and NO classes. The selected variables were also used to determine how well it could discriminate when sub-set of only 25 fruit were used.

The data set was also evaluated using existing prediction models (Terblanche *et al*., 1980; Waller, 1980; Drahorad & Aichner, 2001) to determine the accuracy of the models using individual fruit data. According to Waller (1980) and Drahorad & Aichner (2001), a K:Ca ration of 30-35 is indicative of physiological problems, with serious disorders possible above 35. Terblanche *et al*. (1980) found that for 'Golden Delicious' apples, concentrations of N (46-60 $mg.g^{-1}$ FW) and Ca (4.5-5.0 $mg.g^{-1}$ FW) and a ratio (K+Mg):K (<30) indicated optimum fruit quality at harvest. Their threshold values for mineral concentrations and ratios in composite fruit samples were applied to our individual fruit analyses to separate the fruit into the classes BP and NO and compared to results from the discriminant analysis.

RESULTS AND DISCUSSION

Mineral distribution of pitted and non-pitted classes

Population 1: The distributions of all minerals analysed in fruit from 'Population 1' were slightly non-normal (W < 1) for both the pitted and non-pitted classes (data not shown). In agreement with Le Grange *et al.* (1998), the distribution of K for pitted fruit was located slightly to the right (i.e., at higher concentrations) from that of non-pitted fruit, and Ca to the left (i.e., at lower concentrations) of the non-pitted fruit. The distribution of Mg was similar in both classes, as reported before (Le Grange *et al.*, 1998). The distributions of P and N diverged from those of Le Grange *et al*. (1998), with the pitted fruit located slightly to the right (i.e., higher concentrations) of the non-pitted fruit.

Population 2: In most cases, except for Ca, all minerals analysed were slightly non-normally distributed in both pitted and non-pitted fruit from both producers in 'Population 2' (2a and 2b; data not shown). As with 'Population 1', these results agreed with previous findings for the distribution of K (Le Grange *et al.*, 1998) where the pitted fruit distribution was located slightly right (i.e., at higher concentrations) from non-pitted fruit distribution and distribution for Ca to the left (i.e., at lower concentrations) from non-pitted fruit. The distributions of P, N and Mg again differed from Le Grange *et al*. (1998), with the pitted fruit distribution located slightly to the right (i.e., at higher concentrations) of the non-pitted fruit for N and Mg, with similar distributions for P for both classes and both producers ('Population' 2a and 2b) in 'Population 2'.

As the results of the two experiments were very similar, data were pooled and the results from both populations are discussed. The normality of the distribution of all mineral elements analysed improved with an increase in fruit number sampled, but we still found varying degrees of positive 'skewness' for all minerals, except Ca (3.433; Table I). Distributions of N, K, Mg and P (Figure1A - D) were located to the right (i.e., at higher concentrations) for the pitted fruit, and Ca to the left (i.e., at lower concentrations) in the

pitted fruit (Figure 1E). These results differed from previous findings on 'Braeburn' apples (Le Grange *et al*., 1998), where pitted fruit could be distinguished from the non-pitted fruit by their K, Mg and Ca distributions. Our data showed additional differences in the distributions of N and P, which could be an influence of cultivar or sample size. Using mean values to predict fruit susceptible to bitter pit, based on these results, may result in errors, because all distributions overlapped to a high degree.

Partial t-tests of Satterthwaite (Snedecor & Cochran, 1989) for unequal samples confirmed significant differences ($P < 0.0001$) between the means of the mineral distributions between the two classes (results not shown).

Discriminant analysis to segregate pitted and non-pitted fruit in a population

The first analysis was applied to all data from 1,061 fruit in total, including both populations as classified into classes BP and NO. Using all variables (fruit FW, N, P, K, Ca and Mg concentration or Ca:N, Ca:K, (K + Mg):Ca and Ca:Mg ratios) in the 'DISCRIM' procedure, the positive classification for NO was 83.7% and for BP, 66.2% (results not shown). These variables were then entered into the 'STEPDISC' procedure and the procedure selected N concentration, (K + Mg):Ca ration and fruit FW (Table II). When these variables were applied to the 'DISCRIM' procedure, an improved correct classification of 84.9% was achieved for non-pitted fruit, with a miss-classification of only 15.1% (Table III). The correct classification of pitted fruit was 63.3%, showing a higher degree of miss-classification of pitted fruit into the non-pitted class (36.7%) than was the case for miss-classified non-pitted fruit (15.1%), as well as a slightly worse classification than was achieved with all 10 possible variables (66.2%). Clearly other factors not included in this analysis contributed in the development of bitter pit.

When analysing fruit from 'Population 1' only, additional variables K, and ratios Ca:K and Ca:N were selected (Table II) and improved the classification in both classes, compared to the classification when fruit from both populations were analysed together (Table III). In addition, the data set from 'Population 2' was analysed after separating it into the two producers' fruit (2a and 2b). Although the variables selected by 'STEPDISC' varied slightly between the producers (Table II), the classification did not improve in the non-pitted class when these other variables were chosen for analysis. In the pitted class, an improvement to 79% accuracy was achieved when these variables were included for analysis (Table III).

Table IV summarises the accuracy of different 'models' (i.e., our 'DISCRIM' variables; Waller (1980); or Terblanche *et al.,* (1980); or Drahorad and Aichner (2001)), when mineral analysis data of individual fruit were used for classification of fruit into the two classes, BP and NO. The correct classification of fruit into the respective classes according to our 'DISCRIM' variables, was superior in both classes (BP = 63% and NO = 85%) when compared to percentage fruit classified correctly by the other 'models', with the highest correct classification achieved for BP = 59% and NO = 65% (Table IV).

In Table IV, the average fruit mineral analyses of all pitted and non-pitted fruit are presented as well as the range of mineral analysis values that existed within each class. These were compared to the optimum values published by Terblanche *et al*. (1980) for 'Golden Delicious' fruit. Mineral concentration ranges of the two classes overlapped for all mineral concentrations and ratios selected. These ratios that Terblanche *et al*. (1980) used were the closest to the actual ranges that occurred in the classes. When the ratios of Terblanche *et al*. (1980) were however used on our data set, it was still less accurate than the variables selected by our 'DISCRIM' procedure as mentioned before (Tale IV).

Sub-sets of 25 randomly selected fruit, as used commercially for fruit mineral analyses, were used to test the 'DISCRIM' variables' results based on all fruit (Table VI).

Classification results varied between 100% classification in both classes to a poor 60% and 74% in another sub-set. The variation was found in sub-samples of equal numbers of BP and NO fruit, as well as more natural ratios of one to three BP with 22-24 NO fruit per sample. No trend in miss-classification could be established.

CONCLUSION

The distribution of minerals in apple populations with and without bitter pit, based on individual fruit analysis, showed significant differences between values for pitted and non-pitted classes for all minerals, but this masked the high degree/extent of overlap present between the classes in all cases. This overlap resulted in inaccuracies when mean mineral content values of fruit populations were used to predict the probability of bitter pit.

Mineral Ca analysis of individual fruit gave satisfactory results (85%) for the classification of non-pitted fruit, but not for pitted fruit (68%), even though it is still below the requirement of 100% accuracy required by the markets.

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TABLE I

Quantification of non-normal distribution of minerals analysed for all data for pitted (BP) and no-pitted (NO) fruit as described by 'skewness' and 'kurtosis'

Mineral	Class	'Skewness'	'Kurtosis'
Ca	NO	1.29	3.62
	BP	3.43	22.42
N	NO	0.53	2.62
	BP	1.95	13.35
K	NO	1.30	4.47
	BP	0.85	1.33
Mg	NO	1.21	3.38
	BP	0.74	0.88
P	NO	1.44	4.02
	BP	1.08	1.87

TABLE II

Variables selected with a stepwise discriminant analysis for fruit of pitted (BP) and non-pitted (NO) classes

	Step	Variable	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Squared Canonical Correlation	Pr > ASCC
Populations 1 and 2 Stepwise Selection Summary for all fruit	1	(K+Mg):Ca	0.1913	250.47	<.0001	0.8087	<.0001	0.1913	<.0001
	2	Fruit weight	0.0282	30.67	<.0001	0.7611	<.0001	0.2389	<.0001
	3	N	0.0106	11.32	0.0008	0.7530	<.0001	0.2470	<.0001
Population 1 only Stepwise Selection Summary	1	(K+Mg):Ca	0.2141	206.71	<.0001	0.7859	<.0001	0.2140	<.0001
	2	Fruit weight	0.0125	9.57	0.0021	0.7106	<.0001	0.2893	<.0001
	3	K	0.0068	5.19	0.0229	0.7058	<.0001	0.2942	<.0001
	4	Ca:N	0.0031	2.35	0.1260	0.7036	<.0001	0.2964	<.0001
	5	Ca:K	0.0048	3.60	0.0581	0.7003	<.0001	0.2997	<.0001
	6	N	0.0074	5.60	0.0182	0.6951	<.0001	0.3049	<.0001
Population 2 only Stepwise Selection Summary	1	(K+Mg):Ca	0.1678	60.07	<.0001	0.8322	<.0001	0.1678	<.0001
	2	N	0.0709	22.67	<.0001	0.7732	<.0001	0.2268	<.0001
	3	Fruit weight	0.0657	20.83	<.0001	0.7224	<.0001	0.2776	<.0001
	4	Mg	0.0098	2.92	0.0886	0.7153	<.0001	0.2847	<.0001

TABLE III

Classification of fruit into bitter pit (BP) and non-pitted (NO) classes by discriminant analyses (2002-03, 2003-04)

	CLASS		NO	BP
Populations 1 and 2 (all fruit)	NO	no. fruit	552	98
		% fruit	84.9%	15.1%
	BP	no. fruit	151	260
		% fruit	36.7%	63.3%
Population 1	NO	no. fruit	434	66
		% fruit	86.8%	13.2%
	BP	no. fruit	82	179
		% fruit	31.4%	68.6%
Population 2	NO	no. fruit	125	25
		% fruit	83.3%	16.7%
	BP	no. fruit	60	90
		% fruit	40.0%	60.0%

TABLE IV

Individual fruit classification into classes BP (bitter pit) and NO (non-pitted) according to different mineral analysis thresholds

	Classes	'DISCRIM' variables	[N][1] Terblanche *et al*. (1980)	(K+Mg):Ca[2] Terblanche *et al*.(1980)	K:Ca[3] Waller; Drahorad & Aichner	[Ca][4] Terblanche *et al*. (1980)
CORRECTLY	BP	63	2	59	50	22
CLASSIFIED	NO	85	65	39	42	4
MISS-	BP AS NO	37	98	41	50	78
CLASSIFIED	NO AS BP	15	36	62	58	96

[1]N $mg.g^{-1}$ FW < 60 for non-pitted fruit (NO), > 60 for bitter pit fruit (BP)

[2](K+Mg):Ca < 30 for NO, >30 for BP

[3] K:Ca < 30 for NO, >35 for BP

[4]Ca $mg.g^{-1}$ FW > 5 for NO, <2 for BP

TABEL V

Average fruit mineral values and mineral ranges found in bitter pit and non-pitted 'Golden Delicious' apples compared to the guidelines published by Terblanche et al. (1980)

Variables: Mineral concentration or Ratio	Average values of variables for classes BP and NO using all data		Individual fruit conc. ranges within each class for all data		Optimum concentration or standard ratio for 'Golden Delicious' apples Terblanche *et al.*(1980)
	*NO	BP	NO	BP	NO
N $mg.g^{-1}$ FW	36	41	12-81	17-128	40-60
P $mg.g^{-1}$ FW	8	9	2-23	3-20	9-10
K $mg.g^{-1}$ FW	89	100	25-230	52-222	95-105
Ca $mg.g^{-1}$ FW	3.4	2.6	1-8	1-14	4.5-5.0
Mg $mg.g^{-1}$ FW	4.5	4.8	3.5-6	4-6	<5
N:Ca	12	19	2-40	2-63	9-14
(K+Mg):Ca	30	46.6	9-106	8-125	<30
P:Ca	2.2	4.1	0.5-0.9	0.5-15.3	1.8-2.2

* NO = non-pitted fruit; BP = pitted fruit

TABEL VI

Results for bitter pit classification according to individual fruit mineral analysis and the 'DISCRIM' variables on randomly selected samples of 25 fruit

		Set 1		Set 2		Set 3		Set 4		Set 5	
	Class	NO	BP	NO	BP	NO	BP	NO	BP	NO	BP
no. fruit	NO	6	4	13	2	10	0	8	1	21	1
% fruit	NO	60	40	87	13	100	0	89	11	95	5
no. fruit	BP	4	11	2	8	1	14	6	10	0	3
% fruit	BP	27	73	20	80	7	93	38	63	0	100

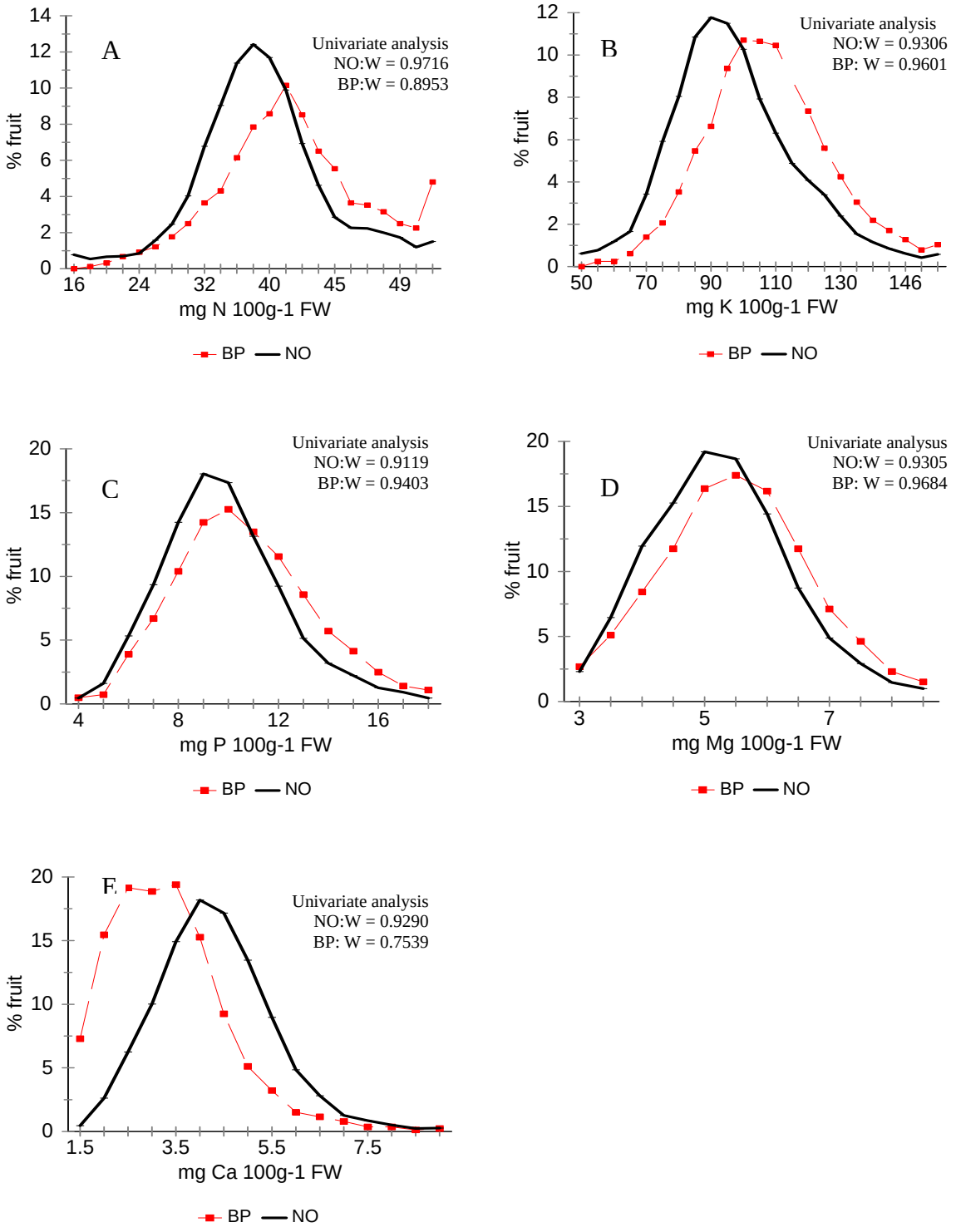

FIG. 1

Univariate analysis distributions (moving averages) of mineral concentrations (mg.100g^{-1} FW) of pitted (BP) and non-pitted (NO) classes of 'Golden Delicious' apples after storage (W = normal; $P < 0.0001$).

Effect of bearing position on two-year-old wood of 'Golden Delicious' and 'Royal Gala' apples on nutrient content and dry weight.

Summary

Fruit set and fruit dry weight was higher on terminal spur positions of two-year-old shoots for 'Golden Delicious', but not for 'Royal Gala' apples. This is contrary to results on pears where the basal position on a shoot showed higher fruit set and dry weight (Reynolds *et al*., 2005). The distal shoot pieces on the longer shoots may have inhibited bud growth (Cook *et al*., 1998; Cook & Bellstedt, 2001) and is possibly related to the export of auxin by the distal shoot piece, as described by Bangerth (1989). The improved set could have been influenced by leaves (re-growth) enhancing cytokinin delivery to the terminal position due to transpiration (Cook & Bellstedt, 2001). Fruit set on a half bearing unit (headed in winter) was comparable to set on terminal positions on long shoots in one season, but not in the other. Calcium allocation could not be contributed to either bearing position or dry weight accumulation in either cultivar.

Introduction

Various parameters influence the mineral content of fruit, including the position of fruit in the tree, sink-source relationship and fruit size (Schumacher *et al*., 1980; Volz *et al*., 1994; Minchin *et al*., 1997). Volz *et al.* (1994) reported a dilution of Ca concentration with an increase in fruit size. Bigger fruit are normally found on spurs on two-year-old wood and terminals of one-year-old shoots, than on lateral bearing positions on one-year-old long shoots or spurs older than three years (Volz *et al.*, 1994). Increases in fruit size can also be manipulated by hand thinning and is often followed by a decrease in Ca content of the

remaining fruit (Schröder & Link, 2002). Differences in Ca content occur between cultivars, due to differences in bearing habits and fruit sizes. In 'Granny Smith' and 'Royal Gala' for example, the highest Ca content is found in terminal fruit on one-year-old shoots that are larger than fruit on spurs on two-year-old wood, where as in 'Fuji', the highest Ca content is found in fruit from on spurs on two-year-old wood (Volz *et al.*, 1994). Jones & Samuelson (1983) suggested that differences in the Ca content of terminal and lateral fruit could also be due to the natural occurrence of higher Ca concentrations and bigger fruit at the base than tip of a branch of 2- and 3-year old wood.

Soon after anthesis, spur leaves positively affect the Ca uptake of fruit (Jones & Samuelson, 1983; Volz *et al.*, 1996). Fruit on spurs with bourse shoots have a higher rate of Ca content increase than fruit without bourse shoots (smaller total leaf area), whereas terminal fruit with high leaf areas contain more Ca than fruit from spurs. Although an increase in total leaf area will benefit fruit Ca, the efficacy of the leaves are clearly determined by leaf number and type. In general, it is found that the Ca content of fruit increases curvilinear with an increase in total leaf area, irrespective of position (Ferree & Palmer, 1982; Jones & Samuelson, 1983).

Leaf water potential tends to be strongly related to differences in leaf exposure and this is related to fruit position. Xylem sap flow and Ca influx may therefore be higher in fruit in terminal bearing positions with more growth, than fruit on spurs, with a lower transpiration rate (Lang & Volz, 1993). Fruit bourn terminally on shoots often produce more neighbouring leaves than fruit on two-year old spurs, which increase its sink capacity.

According to Volz *et al.* (1994), differences in fruit growth between fruit on spurs on two-year-old wood and fruit on one-year-old wood are not due to differences in spur leaf area, but to bearing position. Yet, the Ca content of fruit is influenced negatively by early removal of the spur leaves, irrespective of fruit size. The removal of bourse shoots result in a decrease

in Ca for fruit from spurs on two-year old wood and fruit bourn terminally on one-year-old shoots than fruit bourn in lateral positions on one-year-old shoots. Partial defoliation of spurs at bloom reduces Ca accumulation significantly by nine weeks after full bloom in the fruit on these spurs (Ferree & Palmer, 1982; Jones & Samuelson, 1983; Lang & Volz, 1993), indicating the localised effect of spur and bourse shoots on fruit Ca concentration. The importance of the presence of young spur leaves for Ca accumulation in fruit on spurs confirmed earlier work by Ferree & Palmer (1982) and Jones & Samuelson (1983). These results also indicated the importance of spur leaves as sink in drawing Ca via the transpiration stream from the roots to the fruit. An additional effect of the spur defoliation is a decrease in average fruit size as well as the spur weight. Rom & Ferree (1983) confirmed the positive effect of a large leaf area per spur on bigger fruit and higher yields. Ford (1979) also found a reduction in growth and thus sink strength during the critical first weeks after full bloom following a reduction in vegetative growth in apple trees resulting in a lower final Ca content.

Mean dry weight of 'Packham's Triumph' pears is higher on shorter and thicker bearing units, compared to longer and thinner units (Reynolds *et al.*, 2005). In this paper, the effect of bearing position and re-growth on Ca concentration and dry weight accumulation of apples on two-year-old wood will be discussed.

Materials and Methods

Two-year-old spurred units between 20 to 40 cm, and similar in diameter (6-8mm), were selected on mature, fruit-bearing trees during winter in a commercial orchard in the Vyeboom area. Five treatments with ten replicates were allocated randomly on various 'Golden Delicious' (2003/4 and 2004/5) and 'Royal Gala' (2003/4) trees on M793 rootstock, planted in 1998 at a spacing of 4 x 1.5 m. Fruit were allowed to develop on two lateral spurs

in either a basal position or terminal position on the two-year-old units (Plate 1), while all other bearing positions were removed. In ten of these units, all re-growth was removed except some spur leaves (no-growth), whereas in the other ten, the re-growth was retained (re-growth) (Plate 2). A fifth treatment consisted of a halved two-year-old unit with basal fruit and re-growth (half unit) (Plate 1). This treatment was headed at the end of August. At anthesis (October), the two clusters per bearing unit were identified and tagged according to treatment. The king flower and excess laterals were removed. Only two lateral flowers per cluster were hand pollinated twice with viable, compatible pollen from 'Royal Gala' (2003/4) and 'Braeburn' (2004/5) for 'Golden Delicious', and 'Granny Smith' (2003/4) for 'Royal Gala', using a small paint brush. This was followed by pruning excess leaves and shoot growth where applicable according to the treatments, regularly during the season, leaving a few spur (4-5) leaves on the bearing spurs. Unforeseen commercial hand thinning was performed and small fruit (28 < mm diameter 42 at dafb) were also removed. Just before commercial harvest, all bearing units were harvested on the same day for data recording in our laboratory. The following data were recorded: fruit number per unit, number of seed per fruit, dry weight (DW) and mineral composition of bourse shoots, leaves and one-year-old shoots (re-growth), bearing unit (wood) and fruit, as well as fruit fresh weight (FW). Mineral analysis was done at a commercial laboratory, Bemlab (Pty) Ltd., Strand, South Africa.

Statistical Analysis

Analyses of variance were performed on the data using the General Linear Models Procedure of Statistical Analysis System (SAS) programme (SAS Institute Inc, 1999-2001).

Results and discussion

Significant differences in fruit set were observed between treatments for ‘Golden Delicious’ during both seasons (Tables 1), but not for ‘Royal Gala’ (Table 2). In the two ‘Golden Delicious’ trials, fruit set was the lowest on the basal bearing position of long units. In contrast, Reynolds *et al*. (2005) found the highest fruit set for ‘Packham’s Triumph’ on basal positions. However, in their experiment, the short shoots were pruned with the result that no shoot growth occurred distal to the fruit. In our case, in the treatment where the shoots were not pruned, a shoot piece with or without re-growth, occurred distal to the fruit. It appears therefore, that in the case of ‘Golden Delicious’, the shoot piece may have inhibited fruit set in the long units with basal fruit. This conclusion is supported by the results achieved in the first season (2003/04) with the half-unit treatment, where ‘Golden Delicious’ shoots were cut in half, but this was not found in the following season. Inhibition of bud growth by distal shoot pieces is well documented (Cook *et al*., 1998; Cook & Bellstedt, 2001) and is possibly related to the export of auxin by the distal shoot piece (Bangerth, 1989). Fruit set was higher with this treatment (half units) than in treatments with long units and basal fruit, which is in agreement with the results of Reynolds *et al*. (2005). The results of the long units with fruit in the terminal position and re-growth, were comparable with the half cut units, but also set more fruit than long units with terminal positions and no-growth. Whether the improved set is due to leaves (re-growth) enhancing cytokinin delivery to the terminal position due to transpiration, should be considered as a possible explanation (Cook & Bellstedt, 2001). However, the positional affects were not observed with ‘Royal Gala’, indicating the difference in plant strategies at cultivar level and season that affected fruit set.

Terminal fruit set was better both seasons and comparable to set on the half units in one season, but not in the other. Due to the terminal position, the shoot piece distal to the fruit

was small and the improved set can possibly be explained on the same basis as was advanced for basal fruit. Fruit number primarily determined the total fruit DW per position, for both 'Golden Delicious' (both seasons) and 'Royal Gala'. Both fruit number and size contributed to the total fruit DW per position. No consistent trend could be established.

'Royal Gala' differed from 'Golden Delicious' in that set was not affected by position to the same degree as for 'Golden Delicious'. As with 'Golden Delicious', no consistent trend in fruit size could be related to fruit bearing position. Factors affecting fruit set and size in relation to fruit position, differs greatly between cultivars (Volz *et al*., 1994) and is in agreement with results for different pear cultivars (Reynolds, 2004).

Only for 'Golden Delicious', statistical differences were found between treatments regarding fruit Ca concentration (Table 1). These were not consistent between seasons or bearing positions. During 2003/04, a higher Ca concentration was evident in the two treatments with no-growth (0.35 mg $Ca.g^{-1}$ DW) than the two treatments with long units and re-growth (0.28 mg $Ca.g^{-1}$ DW), which is in agreement with results reported by Ford (1979). The average fruit size of the no-growth treatments was smaller (104 g FW) than the fruit size of the treatments with re-growth (110 g FW), and the lower the Ca concentration could have been partly due to less dilution in the smaller fruit (Schumacher *et al*.,1980; Schröder & Link, 2002).

During the consecutive season, the higher Ca concentration was found in fruit in terminal bearing positions (0.42 mg $Ca.g^{-1}$ DW) compared to the basal bearing position (0.28 mg $Ca.g^{-1}$ DW), contradicting results reported by Jones & Samuelson (1983). No association was noticed with fruit size, but the terminal bearing positions had a higher number of fruit (2.8) than the basal bearing positions (1.25) that might have acted as a stronger sink for Ca allocation early in the season (Table 1). The Ca concentration in the 'Royal Gala' trial was not affected by position or re-growth (Table 2).

Conclusion

'Royal Gala' differed from 'Golden Delicious' in that fruit set was not affected by position to the same degree. With 'Golden Delicious' higher fruit set was consistently found in the terminal bearing position, with or without re-growth. This could have been due to inhibition of basal bud growth by distal shoot pieces (Cook *et al*., 1998; Cook & Bellstedt, 2001) via the export of auxin by the distal shoot piece (Bangerth, 1989). Alternatively, the improved set in terminal buds could be due to re-growth enhancing cytokinin delivery to the terminal position (Cook & Bellstedt, 2001). No consistent trends for Ca accumulation of fruit in the different treatments were evident for either cultivar.

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Table 1. Effect of bearing position and re-growth on Ca content and dry weight of 'Golden Delicious' apples from five different treatments.

Season	Treatment	Total Fruit DW	Avg. Fruit FW	Number of fruit/unit	mg Ca/1g DW Fruit
2003/04:	Half shoot, Dormant cut	43.5 ac*	93.3 b	2.5 a	0.32 ac
	Basal; No re-growth	32.1 bc	107.6 ab	1.6 b	0.34 ac
	Terminal; No re-growth	36.1 ac	100.9 ab	1.9 bc	0.36 a
	Basal; Re-growth	32.0 bc	109.8 ab	1.5 b	0.27 bc
	Terminal; Re-growth	47.2 a	111.0 a	2.2 ac	0.29 ac
	Significance level:	*0.0530*	*0.2108*	*0.0105*	*0.2320*
2004/05:	Half shoot, Dormant cut	26.2 bc	76.3	1.7 bc	0.42 ac
	Basal; No re-growth	23.6 bc	85.3	1.3 bc	0.27 bc
	Terminal; No re-growth	66.2 a	99.0	3.3 a	0.39 ac
	Basal; Re-growth	17.0 bc	75.5	1.2 b	0.29 ac
	Terminal; Re-growth	38.3 ac	77.5	2.4 ac	0.45 a
	Significance level:	*0.0341*	*0.4192*	*0.0132*	*0.0999*

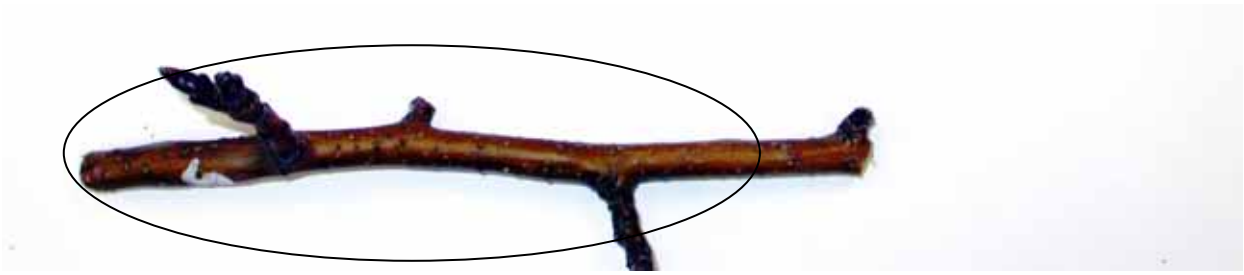
* Means separation in columns using LSD = 5 %.

Table 2. Effect of bearing position and re-growth on Ca content and dry weight of ‘Royal Gala’ apples from five different treatments (2003/04).

Treatment	Total Fruit DW	Avg. Fruit FW	Number of fruit/unit	mg Ca/1g DW Fruit
Half shoot, Dormant cut	24.6	95.1	1.5	0.40
Basal; No re-growth	27.5	96.6	1.5	0.36
Terminal; No re-growth	24.1	84.7	1.3	0.39
Basal; Re-growth	20.7	87.5	1.2	0.36
Terminal; Re-growth	26.0	82.8	1.6	0.41
Significance level:	*0.6803*	*0.3411*	*0.5026*	*0.8201*

* Means separation in columns using LSD = 5 %.

Plate 1

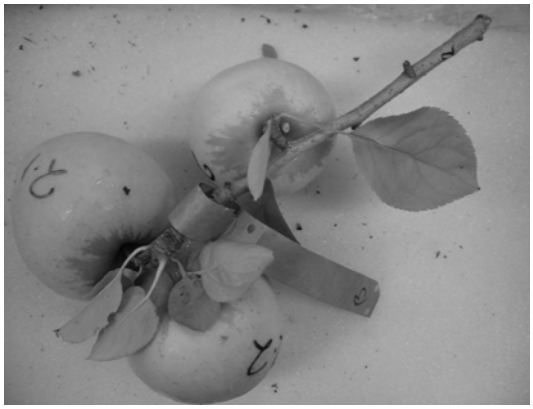


Half bearing unit with dormant cut

Fruit at basal end of bearing unit

Fruit at terminal end of bearing unit

Plate 2



Shoot with a) no re-growth except spur leaves, b) with re-growth additional to spur leaves