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Evaluation of Relationship between Moisture Loss in Grapes and Chlorophyll Fluorescence Measured as F_0 (F-α) Reading

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ABSTRACT

Chlorophyll fluorescence was studied as a rapid technique to detect weight loss of table grape cultivars "Thompson and Flame seedless" under air storage conditions (20°C) and in a 0ºC cold room. Grape clumps (*ca*. 1kg) were divided into 12 groups (six for each cultivar) and initial fresh weight, soluble solid content, titratable acidity, pH and color values were recorded. Three groups were placed inside ventilated baskets with a HarvestWatch sensor facing down on the grapes and placed in a 20ºC room in front of a forced air fan. These samples were used to generate continuous recording of F-α. The other groups were handled in a similar manner and were used to generate weight loss. The control treatments were held in a 0ºC cold room and constantly measured by a HarvestWatch sensor. F-α (F_0) ratio curve for Thompson generally declined over times, and the rate of reduction was maximal between days 1 and 6 which is equal to *ca.* 20 percent in weight loss. The response for Flame grapes was almost the same as for the Thompson cultivar. There were good relationships between F-α values and weight loss values for both cultivars. From these relationships it appears that, for both cultivars, at about 20% weight loss (equal to 0.8 weight loss ratio), the F-α value stopped its decline. The other fruit quality such as SSC, TA, pH and color value indicated that the drying treatment affected these responses, compared with the fruit in the control treatment. Our results indicated that chlorophyll fluorescence techniques can detect weight loss in grapes after harvest, and thus has a potential as a rapid and non-destructive method for monitoring fruit weight loss and senescence in grape during storage.

Keywords: Chlorophyll fluorescence, Postharvest, *Vitis vinifera* L., Weight loss.

INTRODUCTION

Fresh fruit, vegetables and ornamentals are mostly composed of water which is fundamentally important in all life process (Wills *et al.,* 2002). The table grape (*Vitis vinifera* L.) a non-climacteric fruit is subject to serious water loss following harvest, which can result in stem drying and browning, berry shatter, and even wilting and shrivelling of berries (Salunkhe and Desaie, 1984)). High consumer acceptance is attained for fruit with high SSC and berry appearance. Berry firmness is also an important factor for consumer acceptance as is the lack of defects such as decay, cracked berries, stem browning, shrivelling and dried berries. On the other hand, in the grape industry, fruit quality can be improved after harvest with a controlled drying of the fruit to about 80 percent of its initial weight. The current method of monitoring weight loss is by regular weighing of the fruit. There is considerable interest in adopting a less labour-intensive, non-destructive, accurate measurement of the

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weight loss.

In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant physiological studies. No investigation into the photosynthetic or stress performance of plants under field conditions seems complete without some fluorescence data (Maxwell and Johnson, 2000). Chlorophyll fluorescence techniques are often used to detect environmental, chemical, and biological stress in plant tissue (Lichtenhaler, 1988). Measurements used a sustained decrease in dark-adapted F_v/F_m and an increase in F_0 to indicate the occurrence of environmental damage in response to high or low temperatures, excess Photon Flux Density (PFD) and water stress (Gamon and Pearcy, 1989; Ogren and Sjostrom, 1990; Sjostron, 1990; Epron *et al*., 1992; Groom and Baker, 1992; Epron *et al*., 1992). Despite improvements in technology and the evolution of modulated systems, these observations remain valid and changes in F_v/F_m and F_0 are still accepted and widely used as a reliable diagnostic of environmental damages (He *et al*., 1996; Valladares and Pearey, 1997).

Recently, these techniques have been used in postharvest studies of chilling injury in banana and mango (Smillie *et al.,* 1987), in cucumbers (van Kooten *et al.,* 1992) and in green peppers (Lurie *et al.,* 1994). Application of chlorophyll fluorescence techniques (F-based system) in postharvest physiology has been well documented (DeEll *et al.,* 1999). One of the most interesting postharvest applications is the effect of low O_2 and /or high CO_2 storage atmospheres on chlorophyll fluorescence in stored products. The HarvestWatch system has proven to be very effective at determining low oxygen tolerance limits in fruits and vegetables prior to the development of off-flavours and physiological disorders which are associated with low-O_2 injury (DeEll and Prange, 1995; Prange *et al.,* 1997; Prange *et al.,* 2002; DeLong *et al.,* 2004). Chlorophyll fluorescence (Fv) also was found to correlate positively with scald development in "Stuvdeespur" apples during storage at 0°C in ambient air (DeEll, 1996).

Chlorophyll fluorescence techniques have been found to be useful in predicting the shelf-life or keeping quality of cucumber, based on initial color (Schouten *et al*., 1997). Chlorophyll fluorescence techniques have been used to assess early postharvest changes in vegetables, such as broccoli (Toivonen, 1992).

Many of researches have applied this technique to fruits and vegetables stored in control atmosphere storage to detect stress due to either high or low O_2/CO_2 in the store. However, there is no information at this time for using this technique for water loss during storage in horticultural crops. The aim of this work was to study chlorophyll fluorescence as a physiological indicator of fruit senescence in grapes, to assess fruit weight loss in a non-destructive manner.

MATERIALS AND METHODS

Thompson and Flame seedless table grapes were obtained from the superstore. All the fruits were firm, disease-free and of marketable quality. Initial fresh weight, soluble solid content, titratable acidity and pH were measured. The berries were chopped into small pieces, and extracted with an electric juice extractor. The juice from each sample was pooled, filtered through cheese-cloth and used for chemical analysis. pH was evaluated immediately with a glass electrode pH meter. Total soluble solids (TSS) were calculated from refractive indexes using a hand refractometer requiring a drop of undiluted juice (Model K-0032, Cosmo, Japan). Titrable acidity was also calculated from the titrated volume of standard 0.1 N NaOH to pH 8.2 and expressed as mg of tartaric acid (TA) per 100 cc of juice (AOAC, 1980).

Color was measured with a Minolta Chromameter CR-200, Japan, calibrated with a white standard (Y= 94.3, x= 0.3142 and y= 0.3211). Measurements were done on each single individual berry (20 berries) using extra samples. Two measurements were taken at opposite points around the equator of the fruit and recorded as an average of 2

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different points in each fruit. Data were expressed as a* (green-red), b* (blue-yellow), L* (dark-light as lightness), C (Chroma) and H° (hue angle).

Grape clusters (1 kg) were divided into 12 groups (6 of each cultivar). Three groups per cultivar were placed inside ventilated baskets, each basket with a sensor on the inside of the lid and facing down on the grapes. A new chlorophyll fluorescence (F_0) sensor system called HarvestWatch using fluorescence interactive response monitor (FIRM) sensors was developed and used (Atlantic Inc., Halifax, NS., Canada), which measures F_0 at low irradiance. This system is non-pulsed amplitude modulated proprietary technology, which produces a theoretical estimate of F_0 at zero irradiance for which we have coined a new fluorescence term, F-α. The six ventilated containers were then placed inside 3 stacked bushel containers (each bushel container holding two baskets, one of each cultivar) and placed in a 20ºC room with forced air fan. These samples were used to generate continuous measurements of F-α reading. The F-α values were measured in absolute terms (raw F-α) but they were then converted to F-α ratio values which are calculated by dividing each F-α by the initial value. The other 6 groups were handled in similar manner but they did not have HarvestWatch sensors. These three bushel containers were placed in front of the 3 HarvestWatch bushel containers and were used to generate weight loss measurement. Weight loss was determined by placing these six baskets on an electronic balance every day. Similarly to F-α, the weight measurement values were converted to weight loss ratio values by dividing each measurement by the initial value. As a control treatment (no weight loss), some Thompson and Flame grapes were held in a 0ºC cold room until the termination of the experiment, being measured by a HarvestWatch sensor.

The experimental design was completely randomised with three replications for each treatment. The factors used in the analysis were temperature combined with ventilation (two treatments) and cultivars (Thompson and Flame). The data were analyzed by the analysis of variance procedure using the statistical program Genstat 7.2 and least-square

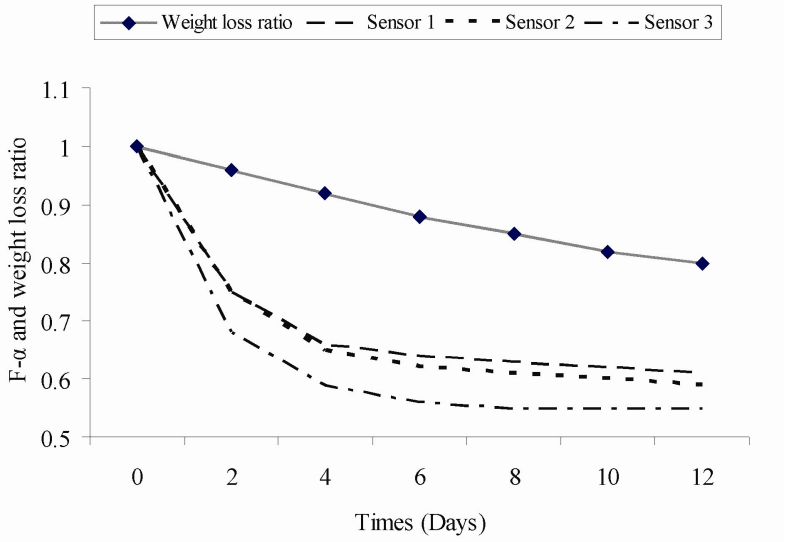

Figure 1. F- α and weight loss (expressed in proportion to the initial value) in Thompson grapes over a period of time. The estimated weight loss value is a mean of three groups of grapes.

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means were computed and differences in mean were tested (LSD) at the $p<0.05$ level. The relationships between chlorophyll fluorescence and weight loss were examined using the Excel program. All chemical analysis was run in duplicate on the same sample and the experiment replicated three times.

RESULTS AND DISCUSSION

The F-α curve for Thompson grapes declined rapidly and then the rate of measurement decrease, slowed over time (Figure 1). Over the same time period, weight loss, estimated from the baskets monitored for weight loss, also declined (Figure 1) but in a more linear fashion.

The response for Flame grapes (Figure 2) was almost the same as for Thompson grapes. The sole difference in Flame grapes was the less rapid loss of moisture initially, compared with Thompson (Figure 1).

The decline in F-α in the drying treatment can be attributed to the moisture loss due to the control treatment (in a 0ºC cold room) resulted in very little change in F-α, compared with the F-α response in the drying treatment (Figure 3).

When the F-α values were regressed against the weight loss values, there was a clear relationship for Thompson and Flame (Figures 4 and 5). In these figures the line is in data point to data point format, a calculated regression line. From these two figures it appears that, for both cultivars, at about 20% weight losses (= 0.8 weight loss ratio), the F-α value stopped its decline. Water stress has been shown to cause a substantial change in the fluorescence induction pattern of leaves (Prange, 1986), similar results have not been found for apples. DeEll *et al*. (1996) found chlorophyll fluorescence did not reflect water stress in apple stored in a standard controlled atmosphere (CA) at 0°C and for 9 months. They reported that storage humidity affected moisture loss and core browning development but did not influence the Fv values, defined as ((Fp-Ft)/Fp) x100 from a non-modulated fluorometer. These results may be inconsistent with our results because of the use of other chlorophyll fluorescence measurements, e.g. Fv. However, Prange and Harrison (Prange and Harrison, 1993) used chlorophyll fluorescence to evaluate the effects of storage

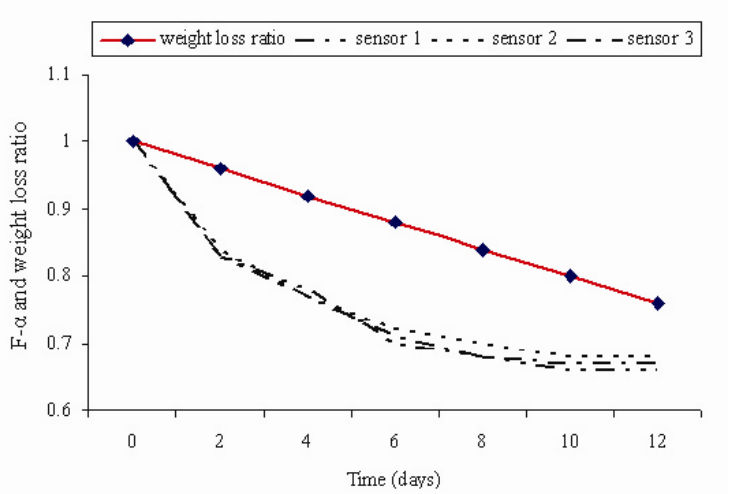

Figure 2. F-α and weight loss (expressed in proportion to the initial value) in Flame grapes over a period of time. The estimated weight loss value is a mean of three groups of grapes.

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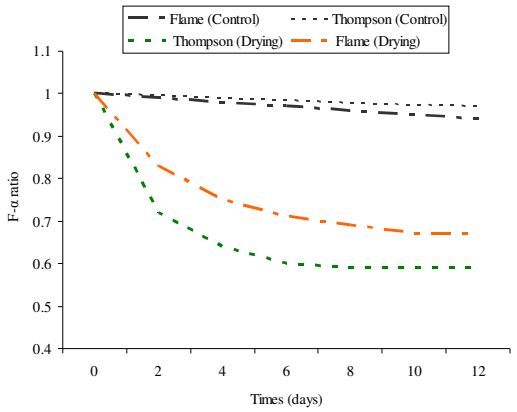

Figure 3. Change over times in control and drying treatments F-α values for Thompson and Flame grapes.

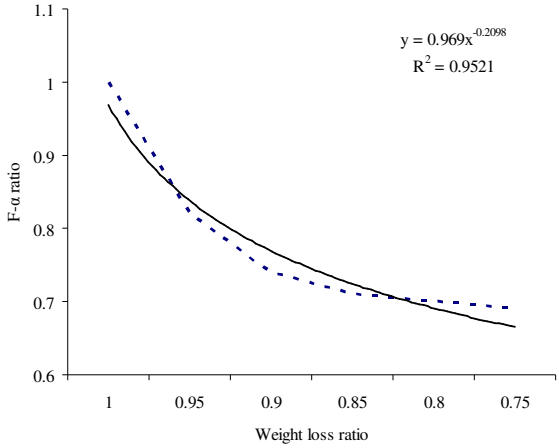

Figure 4. Relationships between weight loss ratio and F-α ratio in Thompson grapes, using data from Figure 1.

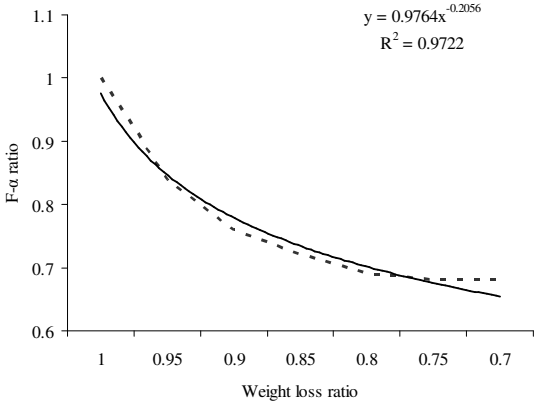

Figure 5. Relationships between weight loss ratio and F-α ratio in Flame, using data from Figure 2.

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humidity on buttercup winter squash. They reported that variable fluorescence appears to be higher in squash stored in CA with a low storage humidity (70-80% RH) than in squash stored in a high humidity (92-95% RH). The ability of F-alpha to detect fruit and vegetables low-O_2 stress also was reported on chlorophyll-containing fruits and vegetables. Prange *et al.* (2003) indicated that, in all fruits (apples, pears, banana, kiwifruit, avocado, and mango) and vegetables (cabbage, green pepper and lettuce), F-alpha was able to indicate the presence of low-O_2 stress.

The measurements of SSC, TA, pH and colour indicated that the choice of drying treatment affected these responses, compared with the fruit in the control treatment (Table 1). Generally, SSC content and titratable acidity was higher in dried fruit and this may have a benefit for the grape industry. However, there were no significant effects in pH between drying and control treatments in both cultivars. Drying (dehydrating of fruit) has been reported that may change some of the internal and external quality parameters of fruits (Will *et al.,* 2002; Bron *et al.,* 2005). Undoubtedly, fluorescence technology remains a powerful technique that some-times provides a useful measure of the crops' performance during storage. Moreover, chlorophyll fluorescence is one of the few physiological parameters that have been shown to be correlated with stress in plants (i.e. water loss).

CONCLUSION

In both grape cultivars, the HarvestWatch F-α measurement appears to be highly correlated with the fruit weight loss. As fruit weight declined from its initial weight, so did the F-α values. The shape of the F-α curve in the drying treatment looks remarkably similar to a water status (water potential) curve as tissue is dehydrated. Thus, it is possible that F-α may be the first

Table 1. Effect of drying on berry SSC, TA, pH and colour in "Thompson and Flame" grapes. The control treatment was covered samples in a 0°C cold room.

					Color				
Cultivar	Treatment	SSC[a] (%)	TA Mg 100 ml^{-1}	pH	a*[d]	b*[e]	L[f]	C[g]	H°[h]
Thompson	Initial	18.2	768	3.06	-3.6	12.1	45	12.5	144
	Drying	23.7	1130	3.03	-0.6	2.2	48	2.5	72
	Control	17.3	790	3.16	-0.9	11.1	58	11.4	150
	LSD(p<0.05)	2.3	45	NS	-1.2	4.5	8	4.9	25
Flame	Initial	19.6	516	3.08	10.2	-2.7	33	9.7	343
	Drying	25.5	700	3.1	6.4	-4.2	32	7.7	325
	Control	17.8	544	3.3	7.5	-5.2	48	9.2	324
	LSD (p<0.05)	3.8	58	NS[c]	3.1	NS	11	2.6	19

[a] Soluble solid content
[b] Total acid
[c] Not sign ificant
[d] Redness
[e] Blue-yellow
[f] Lightness
[g] Chroma
[h] Hue angle

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non-destructive measurement technology in plant science that can measure water status in plants.

Water loss from the grape berries was detected after a few hours of treatment, by changes in F-α (F_0) reading. Chlorophyll fluorescence techniques are much more rapid and simpler than measurements of other physical responses to water stress. Thus, the use of chlorophyll fluorescence techniques could provide a reliable mechanism for detecting water loss from grape berries during storage. Such a technique would offer the storage operator an opportunity to market their fruit well in advance of the occurrence of moisture loss stress.

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بررسی رابطه‌ای بین کاهش رطوبت میوه انگور و فلورسانس کلروفیل اندازه‌گیری شده بوسیله شاخص $F_0(F\text{-}\alpha)$

ع. ا. رامین، آر. ک. پرنگ، جی. ام. دلانگ، و پ. آ. هاریسون

چکیده

بازتاب کلروفیل، بعنوان روشی سریع به منظور ردیابی میزان کاهش وزن پس از برداشت بر روی دو رقم انگور به نامهای «تامپسون» و «فلیم» در دمای صفر و ۲۰ درجه سانتیگراد، مورد تحقیق قرار گرفت. به همین منظور خوشه‌های انگور به میزان یک کیلوگرم توزین و در ۱۲ بسته (۶ بسته برای هر رقم)، تقسیم شدند. قبل از شروع تیمار و در پایان، کاهش وزن، میزان مواد جامد قابل حل (SSC)، اسید قابل سنجش (TA)، pH و شاخصهای رنگ سطحی میوه اندازه‌گیری شد. سپس سه گروه از نمونه‌های وزن شده در داخل سبدهای مشبک گذاشته و سنسور دستگاه در بالای خوشه‌ها به فاصله ۲۰cm نصب گردید و سپس در داخل تونل خشک‌کن منتهی به یک بادبزن مکنده در دمای ۲۰°C گذاشته شدند. این نمونه‌ها تقریباً هر کدام به فاصله زمانی یک ساعت به کمک ترازوی الکتریکی توزین شده و عدد خوانده شده ثبت گردید. به موازات آن دستگاه سنسور به‌طور پیوسته میزان F_o (F-α) را ثبت و در صفحه

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مانیتور با گذشت زمان به نمایش گذاشته شد. نمونه‌های دیگر با همین روش در دمای صفر درجه سانتیگراد قرار داده شدند. نتایج نشان دادند که میزان F-α خوانده شده برای رقم تامپسون با گذشت زمان کاهش یافته و سرعت کاهش برای روزهای ۱ تا ۶ حداکثر مصادف با ۲۰ درصد کاهش وزن خوانده شد. واکنش رقم فلیم تقریباً شبیه به رقم تامپسون بود. رابطه‌ای معنادار بین عدد خوانده شده از دستگاه (F-α) با کاهش وزن با گذشت زمان برای هر دو رقم به دست آمد. با توجه به روابط به دست آمده مشخص شد که برای حدود ۲۰ درصد کاهش وزن انگور (برابر با نسبت کاهش وزن ۰/۸)، عدد خوانده شده به وسیله دستگاه تقریباً ثابت باقی ماند. سایر شاخصهای کیفی میوه از قبیل SSC، TA، pH و ارزش رنگ میوه در زمان کاهش وزن میوه و در پایان تغییر یافت (در مقایسه با نمونه اولیه). در پایان با توجه به نتایج به دست آمده می‌توان کاهش وزن میوه انگور را در طی مدت انبار از روی اعداد خوانده شده از دستگاه با گذشت زمان ثبت و پایان عمر انبار مانی را بدون تخریب میوه مشخص کرد.

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