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# A revised method for the field collection and storage of fruit ethylene samples using evacuated vials

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ABSTRACT

Ethylene is an important indicator of climacteric fruit maturity. Measuring ethylene production may be delayed because of the distance between the sample collection site and the gas chromatograph. Consequently, fruit maturity may be overestimated and harvested prior to optimal ripeness. In this study, 12-mL evacuated Exetainer® vials were used to hold ethylene samples from a static respiration system containing harvested fruit. The collection and transfer system was tested for accuracy of sample transfer to vials and for storability with evacuated vials containing samples held for up to 28 days at room temperature with no loss of sample integrity. Ethylene production differed between day of harvest (day 0) and the following day (day 1) of several stone fruit selections ('Autumn Bright' nectarine, 'August Flame' peach and 'Golden May' apricot) when testing this system. The use of evacuated vials as a storage tool for at harvest collection of ethylene samples, allowing delayed laboratory analysis, was shown to be a viable option for researchers with field sites at great distance to analysis equipment.

## 1. Introduction

Climacteric fruit undergo elevated ethylene production and respiration throughout the ripening phase. This has led to the use of ethylene as an indicator of fruit maturity (Watkins et al., 1989). The ability to accurately determine fruit maturity has effects far beyond predicting the correct harvest time in the orchard, as fruit maturity impacts postharvest storage and the distribution potential of the fruit (Crisosto et al., 1995; Kader and Mitchell, 1989). Furthermore, ethylene has been implicated to regulate some pre-cursors in volatile production in peach (Ortiz et al., 2010) and apple (Xiaotang et al., 2016) fruit and post-storage softening in pear fruit (Chiriboga et al., 2013), both of which are important for consumer acceptance. Unfortunately, ethylene production accelerates once the fruit is abscised from the tree, even in fruit at different stages of ripeness (Reid, 1985). Consequently, delaying the analysis of ethylene production beyond the time of harvest may lead to inaccuracies in determining fruit maturity and understanding of the timing of the climacteric onset and the impact on fruit quality during postharvest handling and distribution.

Measuring ethylene production of fruit is often determined by capturing and quantifying emitted ethylene within sealed, gas tight chambers during a set amount of time (Pre-Aymard et al., 2003; Tsantili et al., 2010). Delays between harvesting fruit and returning to the laboratory for ethylene analysis can substancially increase the time interval from harvest to measurement. This explained some inconsistencies in our samples of fruit ethylene production when tested on the day of harvest, compared to those tested more than a day later. A way to overcome this would be to analyse the ethylene production immediately after fruit picking. However, this is usually difficult with orchards often several hours drive from laboratory equipment that will adequately analyse very low concentrations, usually observed before the climacteric onset. There are portable ethylene analysers, but when tested, these lacked the sensitivity to measure very low concentrations, especially for small volume chambers.

Watkins et al. (1989) applied this same rationale using evacuated blood sampling vials to collect accumulated ethylene from in-field sealed chambers containing fruit for later analysis in the laboratory. They suggested it would be a more efficient and accurate method for sampling and calculating ethylene production of fruit harvested distant locations. The method was deemed successful; however, there was some interference in the analysis due to the elution of unknown peaks when analysed by gas chromatography, even from vials containing only an ethylene standard. These peaks were determined to be a result of the vial sterilisation process. This restricted the number and quality of

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samples that could be analysed. Poropak Q columns, as suggested by Watkins et al. (1989) alleviated some of this interference, but not all.

A possible solution to the interference peaks, are vials now used extensively for the analysis of carbon dioxide and nitrogen gases in soil. The vials are gas tight and evacuated before use, therefore, only gases emitted from the samples are analysed (Drury et al., 2007). This will allow for an accurate indication of the ethylene production by the fruit at that time. Precise determination of the physiological age of the fruit using the ethylene production rate can then be correlated with other rapid, non-destructive measurements of fruit maturity such as the Index of Absorbance Difference ($I_{AD}$) (DA meter 53,500 T.R. Turoni, Forli, Italy). Validation and correlation of actual physiological maturity with refractormetry, spectrometry or other non-destructive techniques enables fruit maturity to be measured rapidly, efficiently and effectively.

The aim of this work was to determine if there are differences between delayed or non-delayed testing for ethylene production and to test the accuracy and efficacy of using evacuated vials to hold ethylene samples transferred from static respiration chambers, used to measure respiratory gas production of fruit, for laboratory for later analysis using a gas chromatograph (GC). Several laboratory and field experiments were undertaken to: a) test a model to estimate ethylene concentration of samples transferred to the vials; b) examine the integrity of the sample held in the vials during storage; c) determine the accuracy of the method by comparing ethylene levels of samples transferred to the vials with samples collected directly from static respiration chambers; d) compare ethylene samples collected and transferred to the vials in the field with the ethylene production rate of the same fruit after transport to the laboratory.

## 2. Materials and methods

### 2.1. Gas chromatograph

Samples were injected into a GC (Shimadzu 14B, Shimadzu Corporation, Japan) fitted with a flame ionisation detector and a 2 m × 1/8″ stainless steel mesh packed column (Porapak™ PS 100/110, Sigma-Aldrich Pty. Ltd., Sydney, Australia) and set at an oven temperature of 50 °C, a detector temperature of 200 °C and a flow rate of 40 mL/min $N_2$. Results were compared to a known standard gas mix with a concentration of 2.2 µL ethylene/L in nitrogen (Coregas Ltd.,Yennora NSW, Australia).

### 2.2. Evacuated vials

The vials used were gas tight evacuated, 12-mL vials with a two layer rubber and silicone seal (Exetainer®, LABCO, UK). To ensure consistency in re-usability, all vials were re-evacuated on site prior to use for sample collection according to Drury et al. (2007).

#### 2.2.1. Ethylene concentration model

The following model to estimate ethylene concentration was tested:

$$C = Csa * \left[1 + \left(1 - \frac{Va}{Vy}\right)\right] \quad (1)$$

$$if\ Va \geq Vy\ then = Vy \quad (2)$$

where $C$ was the concentration of ethylene in the sample, $Csa$ was the concentration of ethylene measured by the GC, $Va$ was the volume of sample in the vial according to Eq. (2) and $Vy$ was the total volume of the system (vial + syringe). In the experiments reported here, $Vy$ = 13-mL (vial volume = 12-mL, syringe volume = 1 ml).

To test the accuracy and precision of the model, 10 vials were filled with 14-mL (Va > Vy) of the 2.2 µL ethylene/L standard. Precisely 1-mL of gas was progressively extracted five times from each vial using a syringe (Terumo, Laguna, Phillipines; Fig. 1b) and measured in the GC.

### 2.3. Sample integrity and stability

To test for possible leakage due to increased differential pressure, six replicate vials were filled by syringe (Fig. 1b) with 12-mL ($Va < Vy$), 13-mL ($Va = Vy$), or 14-mL ($Va > Vy$) of the 2.2 µL ethylene/L standard and stored in the dark at room temperature (18–21 °C) for up to 28 days until analysis. Precisely 1-mL of gas was extracted two times from each replicate vial, further increasing the difference between $Va$ and $Vy$, on days 0, 1, 2, 3, 7, 14, 21 and 28 and measured in the GC. The vials were labelled for replication, testing day and injection sequence number as determined by statistical randomisation (144 in total). Each vial was used at one time only and discarded after sampling. Control sample vials using 13-mL input volume were made on each day of evaluation as a comparison.

### 2.4. Chamber to vial transfer accuracy

Airtight, 750-mL glass chambers were used to test whether dilution of the sample occurred during the transfer to vials. A range of volumes (0, 1.5, 15, and 150 mL) of the 2.2 µL ethylene/L standard were injected by syringe into each chamber. Volumes were replicated five times. Prior to the ethylene standard being injected into a given chamber, the equivalent volume of internal gaseous content was removed, by syringe, from the already sealed chamber achieving the theoretical ethylene concentration inside of the chambers of 0.0, 0.004, 0.044 and 0.440 µL ethylene/L. A sample of room air was taken at the time of sealing the chambers to determine ambient ethylene concentration. After adding the ethylene standard to the chambers, the headspace was stirred by inserting the needle of a large, 25-mL syringe through the chamber septum and pumping three times. After pumping, 1-mL of gas was extracted from the chambers and immediately measured by the GC. At the same time, 14-mL of gas was collected from the chambers and injected into the evacuated sample vials. Then 1-mL of gas was extracted from the sample vial and measured by the GC.

### 2.5. Comparison of immediate and delayed ethylene sampling

'Autumn Bright' nectarine, 'August Flame' peach and 'Golden May' apricot fruit were harvested (day 0) and immediately numbered and taken to a shaded area, where fruit were sealed individually into respiration chambers of known volume. The fruit was held for up to three hours allowing the accumulation of respiratory gases within the chamber (Pre-Aymard et al., 2003; Tsantili et al., 2010). The internal chamber atmosphere was then transferred to the vials by syringe, using the protocol described by Frisina and Stefanelli (2016). Fruit were then transported to the laboratory where after 24-h the same fruit were re-assessed (day 1) for ethylene production using the same static respiration chamber collection system described above (Pre-Aymard et al., 2003; Tsantili et al., 2010); Frisina and Stefanelli (2016). Both sets of evacuated vial samples (day 0 and day 1) were analysed by the GC and then compared.

### 2.6. Statistical analyses

All experiments consisted of a two factor, completely randomised design with at least five replicates. A vial was an experimental unit and sequence of testing vials (input and replicate combination) was completely randomised. Statistical analysis was conducted using analysis of variance (ANOVA) accounting for the experimental design. In-field immediate and laboratory delayed ethylene data was compared using a paired, double tailed t-test. All statistical analyses were undertaken in Genstat 17.1 (VSN International Limited, Oxford, UK).

## 3. Results and discussion

The result in Table 1 shows a progressive dilution of the original