Hort Innovation

Final Report

Comparing Stonefruit Ripening, Quality and Volatile Composition

Project leader:

Dr. Dario Stefanell

Delivery partner:

Department of Economic Development, Jobs, Transport & Resources

Project code:

SF15001

Project:

Comparing Stonefruit Ripening, Quality and Volatile Composition – SF15001

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the summerfruit research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978 0 7341 4394 5

Published and distributed by: Hort Innovation

Level 8
1 Chifley Square
Sydney NSW 2000

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

Variable quality in summerfruit has been identified as a major impediment to producer profitability and sales in both domestic and export markets. Agriculture Victoria Research (AVR) along with the Royal Melbourne Institute of Technology University (RMIT) undertook to investigate and develop knowledge and tools to assist producers in harvesting peach and nectarine fruit at the optimum physiological maturity, which would allow fruit to continue development and ripening throughout storage and handling systems, resulting in greater customer satisfaction.

Experiments were designed and conducted to monitor and identify fruit physiological maturity in stonefruit cultivars using a non-destructive chlorophyll content index (Index of Absorbance Difference: I_{AD}) correlated with ethylene production. Physicochemical factors, such as soluble solids concentration (SSC, an indicator of sweetness), firmness and titratable acidity, were monitored as well as volatile organic compounds (VOCs) to gain a better understanding of the effects of fruit maturity on aroma during fruit development.

The project established a new technique to collect ethylene and VOCs using evacuated vials. The technique was published in Scientia Horticulturae and was the basis for much of the studies conducted in this project and the associated PhD.

The key message from this work is that the physiological stage of the fruit at harvest is paramount to ontogeny of fruit after harvest, and any fruit harvested without having reached physiological maturity (defined as the onset of ethylene production) is incapable of developing the full sensorial characteristics typical of the cultivar and requested by consumers.

The determination of physiological maturity is key to improving industry outcomes with regards to profitability, and positive experiences in both domestic and export markets. The use of non-destructive tools to determine physiological maturity, such as the DA-meter, are invaluable to assist industry in determining true harvest times. This will improve the number of acceptable fruit in the market place, resulting in higher customer satisfaction, repeat purchase and positive recommendations.

Several videos on what the project was doing as well as explicit details on techniques as they were validated are available on the Horticulture Industry Network (HIN) website. An industry article was published in the Australian Fruit Grower allowed the technical information to reach a much wider audience. Project researchers participated in ‘Road Show’ talks directed to growers in three of Victoria’s main stonefruit growing districts (Renmark, Swan Hill and Cobram) during 2017.

Future work in this area identified by industry must focus on: the effect of temperature preconditioning on fruit quality and VOCs; effects of temperature management and export disinfestation protocols on fruit quality; postharvest cooling and storage on stonefruit quality; and defining fruit quality for exports to Asia.

Keywords

Peach; nectarine; physiological maturity; quality; ethylene; volatile organic compounds; soluble solid concentration; fruit firmness; titratable acidity; fruit development; non-destructive; DA-meter

Introduction

Summerfruit production is worth over $380 million to the Australian horticulture industry, of which Victoria produces 80% (HIA 2017). Australian stonefruit producers are faced with increasing production and distribution costs while competing with lower cost producers in domestic and export markets. Supplying customers with consistently high-quality fruit would differentiate Australian producers in any market place. However, high variation in stonefruit quality in domestic and export markets has been identified as a major impediment to consumer satisfaction and therefore producer profitability. Dissatisfaction with variable fruit quality can lead to customers refusing to purchase stonefruit again for up to 3 weeks, leading to significant profit reductions across the industry (McGlasson et al 2009).

The major components of stonefruit quality that influence consumer acceptance include: sweetness; acidity; firmness; juiciness (HAL SF10021; McGlasson and Golding 2008; Crisosto et al 2006; Crisosto et al 2008; Crisosto and Crisosto 2005) and flavour and aroma (Aubert et al 2003). Fruit produce volatile organic compounds (VOCs) that contribute flavour and aroma. VOCs produced by the fruit have been studied with regards to their relationship to flavour, but their relationship with aroma is poorly understood. Even more poorly understood is the effects of postharvest handling and storage on VOCs produced by stonefruit.

Fruit quality is also driven by the stage of maturity as this can affect the fruits storage and ripening potential (McGlasson et al 2009). Fruit that have not reached physiological maturity, that are under mature, will impact negatively on consumer satisfaction as they will not be able to continue maturing or ripen properly. Consumer preferences are for sweeter and softer fruit (HAL SF10021), therefore fruit needs to be physiologically mature to ripen properly after harvest, enabling fruit to attain expected consumer quality. The use of cold storage usually slows the fruit metabolic processes and allows fruit to be in a similar state to harvest upon placement in the market, so if fruit are not physiologically mature they will enter the market in a state unsatisfactory to the consumer. Storage temperature has been well researched for fruit texture and has been found to affect VOCs associated with flavour such as esters and lactones (Zhang et al 2011); however, understanding the relationship between harvest maturity, storage and ripening with aroma is unclear.

Defining physiological maturity at harvest will benefit decision making for postharvest handling, distribution and marketing. Previously there has been no accurate, field based, measurement of fruit physiological maturity, which has exacerbated the high variability in fruit quality in the market place. Nowadays, portable, non-destructive methods, shown to accurately assist with defining stages of fruit maturity, are becoming more widely accepted. The University of Bologna, Italy, has developed spectrophotometric technology that can index the level of chlorophyll-a in the mesocarp under the skin of the fruit based on difference of absorbance (DA), effectively measuring the greenness of fruit flesh. Using this index (I_{AD}) correlated with ethylene production gives a more accurate indication of fruit maturity. This can then be used to more accurately determine the effects of harvest maturity on storage and ripening as well as allowing more accurate profiling of aromatic VOC development.

The objectives of this project were to identify I_{AD} maturity classes for up to 8 commercial stonefruit cultivars used for export and correlate maturity classes with fruit composition and aroma volatile profiles; develop cultivar-specific storage and ripening factsheets to improve handling processes with particular emphasis on maximising fruit quality and aroma volatile profiles; develop capability and expertise in stonefruit plant physiology by using the data collected toward the attainment of a part time PhD degree; and disseminate industry information and results through the "Food to Asia" initiative.

Methodology

This project used the stonefruit field laboratory (project SF12003) as the fruit source to identify I_{AD} maturity classes and correlate maturity classes with fruit composition and aroma volatile profiles. The stonefruit field laboratory is located at Agriculture Victoria Tatura (36.44° S, 145.27° E; 114 m APSL) in the Goulburn Valley region of Victoria, Australia. The soil is a red-brown earth or Red Sodosol (Isbell 2002) known locally as Shepparton fine sandy loam (Skene and Poutsma 1962). Trees were planted in winter 2013, 2014 and 2015 in north-south orientated rows at 4.5 m row spacing.

The project conducted a review of the literature on volatile organic compounds (VOCs) and the influence of fruit physiological stage and cold storage on fruit quality and VOCs. This review helped in guiding the collection of VOC samples (number of samples to collect and type of traps to use) and in identifying the strategic importance of the segregation of the fruit based on DA-Meter readings. During the process for validating the method for aromatic VOCs collection and analysis, it was determined that ethylene production was absent on the day of harvest for many mid-range maturity fruit, but was present only 24 h later. This led to a redefinition of when the collection of ethylene samples should take place and tightened the input details for maturity class definitions used within the cultivar database. It also led to a series of specific experiments for the determination of the methodology for ethylene sample collection in the field using evacuated vials published in Scientia Horticulturae in 2018.

The following methods were used to achieve the project objectives (see appendix 1 for more detailed description):

- Monitoring during fruit development was performed to determine fruit ethylene production and its correlation with maturity classes and VOCs,. For each cultivar (‘Rose Bright’, ‘August Bright’, ‘Autumn Bright’, ‘September Bright’, ‘August Flame’, ‘Snow Flame 23’, ‘Snow Flame 25’, ‘Ice Princess’, ‘Red Haven’, ‘Snow Fall’, ‘O’Henry’ and ‘September Sun’), up to 120 fruit were randomly harvested at least weekly. Fruit were sorted by I_{AD} value (increment of 0.1) measured by DA meter. Up to 10 unblemished fruit were selected from each I_{AD} grouping, placed into airtight respiration chambers of known volume and sealed to allow the accumulation of gas compounds. After approximately 3 h, ethylene samples were extracted (15 ml) and transferred to a labelled pre-evacuated vial for processing by gas chromatograph (GC) according to Frisina et al (2018). After a total of up to 8 h (depending on the volume of the chambers) VOCs samples were extracted by drawing the entire contents of the chamber across a charcoal trap with air pumps. The aromatic VOCs were later chemically desorbed and processed by GC-MS according to NIOSH method 1501.
- Shelf life experiments were performed to determine the effect of fruit maturity at harvest on VOCs development during commercialization. For each cultivar evaluated (‘Rose Bright’, ‘Autumn Bright’, ‘Snow Flame 25’ and ‘August Flame’), 240 fruit were harvested by I_{AD}, labelled and categorised into 3 maturity classes specific for each cultivar. Classes were based on the level of ethylene production for each cultivar: unripe, onset of ripening or ripe. Fruit were held at ambient temperature (20 °C) for up to 9 days. One batch of 20 fruit were evaluated every 3 days (i.e. at 0, 3, 6, 9 days) totalling 80 fruit per class. For each batch of 20 fruit, 10 were evaluated for ethylene production and aromatic VOCs.
- Storage experiments were performed to improve handling processes with particular emphasis on maximising fruit quality and aroma volatile profiles. For each cultivar evaluated (‘Rose Bright’ and ‘August Flame’), 960 fruit were harvested by I_{AD}, labelled and categorised into 3 cultivar specific maturity classes based on the level of ethylene production as mentioned above. All fruit were treated with a commercially available postharvest fungicide and dried at ambient temperature to avoid fungal diseases during storage. For each maturity class, 80 fruit were placed at ambient temperature to evaluate immediate shelf life (day 0, 3, 6, 9) after harvest. All remaining fruit were placed into cold storage (1 to 2 °C) for up to 28 days. Every 7 days, 60 fruit per maturity class were removed from storage and placed at ambient temperature (20 °C). For each maturity class, a batch of 20 fruit were evaluated on day 0, 3, and 6 after removal from cold storage, of those, 10 fruit were also evaluated for ethylene production and aromatic VOCs.
- On all fruit in the experiments, physicochemical evaluation was performed to better correlate ethylene and VOCs with fruit composition and the standard quality characteristics (soluble solids concentration, firmness, titratable acidity).

The project delivered updates and research findings to industry via presentations, industry articles and publications such as grower magazine articles, fact sheets, newsletters and website content (Horticulture Industry Network, HIN: www.hin.com.au). Numerous industry adoption activities were conducted during the 2015 – 2018 period including workshops and industry (local and regional) roadshow events.

Outputs

The project produced a diverse series of outputs including scientific manuscripts, research presentations, industry magazine articles, industry presentations, fact sheets, newsletters, website content (See Appendix 2 for list and electronic copies).

Cultivar maturity classes database

A cultivar maturity class database was produced during the life of the project. The database was published on the Horticulture Industry Network website to achieve higher industry penetration (http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-maturity-and-fruit-quality/da-meter-iad-maturity-classes-database)

Conference papers (see appendix 2)

One oral paper presented at the 5th symposium on modelling, Model-IT, Wageningen, Netherlands, October 2015.

One abstract submitted to the 5th symposium on modelling, Model-IT, Wageningen, Netherlands, October 2015.

Literature review (see appendix 2)

A draft literature review was written and continuously refined during the life of the experiment. Through the review it was possible to identify initial gaps in research needs. The review is intended to be published, therefore the current version is considered confidential.

Scientific presentations (see appendix 2)

Presentation delivered the AgriBio seminar series on the importance of fruit maturity at harvest.

A seminar delivered at AgriBio from the Italian PhD student from Italy describing his projects and preliminary results.

International PhD defense presentation at University of Bologna, Italy

A presentation was given at the AgriBio "Science Days" conference (Oct 2017)

Presentation delivered at the Tatura innovation forum.

PhD thesis (see appendix 2)

A chapter in an International PhD dissertation at Bologna University, Italy.

Scientific manuscripts (See appendix 2)

Two scientific manuscripts submitted to Scientia Horticulturae, one published and one still under evaluation.

One scientific manuscript published in Acta Horticulturae.

A draft scientific manuscript titled "Ethylene production at harvest is critical to the development of optimal ripening and the production of aroma volatile organic compounds in nectarine 'Rose Bright' after cold storage", to be submitted to Postharvest Biology and Technology Journal.

Industry articles (see appendix 2)

Two articles published in Australian Stonefruit Grower magazine describing experimental results and the protocol on how industry can use evacuated vials for collecting ethylene samples and the direct relationship with the DA meter, respectively. Articles are not available online due to the magazine no longer being published.

Fact sheets (see appendix 2)

Factsheets describing protocols for optimal maturity management were produced for peach and nectarine cultivars investigated in this project. Out of the 12 cultivars under study, it was possible to produce factsheets for 7 cultivars. For the remaining 5 cultivars, additional information is still required to finalize results. Factsheets will be published in the HIN webpage.

Two factsheets describing harvest and storage recommendations for industry for cultivar 'Rose Bright' nectarine and cultivar 'August Flame' peach.

Five factsheets describing optimal harvest recommendations for industry for nectarine cultivars 'Autumn Bright', 'August Bright' and 'September Bright' and for peach cultivars 'Snow Flame 23' and 'Snow Flame 25'.

Industry presentations (see appendix 2)

Two presentations at the Stonefruit Roadshows in Renmark, Swan Hill and Cobram (2016 and 2017), reporting on the various experimental results, and the protocol on how to collect ethylene samples in the field.

Attendance and consultation with the Stonefruit Field Laboratory advisory committee in common with the linked project SF13001.

Progress reports

Six monthly progress reports were submitted to Hort Innovation by the due date and accepted.

Annual progress reports and confirmations of candidature (Milestones 1 and 2) delivered and successfully accepted by RMIT as part of the PhD program.

As part of the Agriculture Victoria's 'Premium Fruit to Asia' (PF2A) 2014/15-2017/18 project extension, progress report on the annual industry adoption plan.

Profitable stonefruit web site

Agriculture Victoria's 'Premium Fruit to Asia' (PF2A) 2014/15-2017/18 project provided extension of 'Stonefruit Research' undertaken by Agriculture Victoria via the Horticulture Industry Networks (HIN) website. The initial PF2A extension focus on marketing, post-harvest management and economic analysis was expanded to include information about the 'Profitable Stonefruit' experiments (SF12003, SF13001 and SF15001), practical video demonstrations of pruning and training practices, of collecting ethylene samples through evacuated vial, on the usage and troubleshooting of the DA-Meter, and links to industry articles. The HIN 'Stonefruit Research' site provides an easily accessible and comprehensive resource for growers. (http://www.hin.com.au/networks/profitable-stonefruit-research).

Results

The literature review highlighted the following major knowledge gaps that helped refine ideas and methodologies at the start of the project:

- There is still no clear understanding of stonefruit maturity, how to define it, or how to measure it adequately. A better understanding of physiological maturity which includes the relationship with metabolic processes, such as ethylene production, is needed. This will enable the determination of the true effect of fruit maturity on postharvest behaviour, and consumer acceptability.
- There is a need to focus on individual fruit. The majority of published literature undertook studies with bulked samples. Given the volume of work already done on the variation of stonefruit quality, using individual fruit to define the maturity without interference from other fruit would be highly beneficial.

DA meter calibrations:

Through the course of this project, ethylene was used as the key measure of fruit maturity and was correlated with a non-destructive spectrophotometer (DA meter). This allowed for an easy, repeatable indication of fruit physiological stage. Appendix 3 shows I_{AD} values corresponding to fruit physiological stage for cultivars tested during the project.

It was discovered that without any ethylene production at harvest, fruit could not progress to complete ripening. Fruit harvested with no ethylene production were consistently poor quality even after only a few days from harvest.

The measurement of Absorbance Difference Index (I_{AD}) using the DA meter indicated that cultivars behaved similarly between seasons. The monitoring of fruit maturity across 12 cultivars of peach or nectarine showed that the harvest windows changed each season and may be as much as two weeks different from the previous season for a given cultivar.

Monitoring showed that traditional harvest indicators of SSC, firmness or date were inadequate to ensure physiological maturity at harvest. SSC and firmness did not change appreciably near the cross over point from no ethylene production (immature) to physiologically ready, as indicated by the onset of ethylene production. This suggests these parameters are not good indicators of fruit maturity in peaches or nectarines. This was across all cultivars tested. In contrast, the DA meter was very useful in relating the fruit maturity to ethylene production and therefore stage of physiological maturity.

Some cultivars only had data from one season due to limitations in the orchard. Limitations included poor production volume, disease pressure from insect or fungal infestations, and multiple clones of the one cultivar (e.g. O'Henry). Clones have now been identified based on flowering and fruiting times. This has meant this cultivar has remained poorly defined for physiological development stage.

Cultivar specific optimum storage information:

Shelf life experiments suggest fruit physiological maturity (onset of ethylene) is important for fruit response to harvest and shelf life conditions. Both peaches and nectarines harvested with no ethylene production (immature) did not produce any ethylene or significant aromatic VOCs during the 9 days held at 18-21 °C. This was regardless of fruit type (peach or nectarine) or cultivar.

Storage experiments were conducted on the early season, yellow flesh nectarine, 'Rose Bright', and the mid to late season, yellow flesh peach, 'August Flame'. The key results from these experiments indicated there was a crucial need for ethylene production at harvest. Fruit harvested with 'no ethylene' and placed into cold storage was highly affected by this process. Fruit showed poor recovery in ethylene and volatile production upon removal from cold storage and this followed through into shelf life conditions. It often took a minimum of 6 days re-warming for the non-ethylene fruit to produce any discernible amounts of ethylene; fruit were not metabolically ready to be harvested. Furthermore, immature fruit with no ethylene production at harvest consistently had disorders associated with poor quality including rubbery flesh, mealy flesh, and poor juice expression.

'Rose Bright' nectarines could be stored for up to 4 weeks if they were harvested with a DA value of less than 0.8 and greater than 0.3. At DA values higher than 0.8, the fruit did not ripen adequately. At DA values below 0.3 the fruit was prone to over softening while contending with the supply chain requirements. Fruit with no ethylene production at harvest had nil or lower detectable concentrations of the key aroma groups that are associated with peachy/floral aroma. With any level of ethylene production, these issues were alleviated. 'Onset' fruit were able to maintain their quality and acceptability, after returning to warm temperature for 3 days, even after 4 weeks of cold storage. 'Climacteric' fruit had the highest ethylene and key aroma VOCs concentrations; however, these declined as the fruit progressed to senescence.

From the data available so far, 'August Flame' peach could be stored for a maximum of 2 weeks if they are harvested with a DA value of less than 1.3 and greater than 0.9. At DA levels higher than 1.3 the fruit will not ripen adequately, becoming rubbery, mealy and juiceless. At DA values below 0.9 the fruit will be prone to over softening and decay. Storage for any longer than 2 weeks may result in fruit showing indications of flesh disorders including, flesh browning, rubbery flesh, mealy flesh and poor juice expression.

Outcomes

The project identified the importance of ethylene production on the day of harvest to ensure adequate fruit ripening, without which the fruit will only soften but not ripen. Cultivar specific I_{AD} values (measured by the DA meter) corresponding to fruit physiological maturity class (based on measures of ethylene production) were determined. Growers can access the maturity class I_{AD} values from the HIN to enable more accurate harvest timing. Unripe fruit in the market place increases poor repeat purchase. Identification of the optimal harvest time will improve the profitability and sustainability of the Australian stone fruit industry. In addition, it will help reducing wastage due to a better understanding of post-harvest storage behaviour of stone fruits and better logistic management.

The project established a new technique to collect ethylene using evacuated vials. Consistently checking the production of ethylene with other non-destructive measures (e.g. DA-Meter) will improve the level of consistency of acceptable fruit in the market place.

Shelf life and storage experiments suggest fruit physiological maturity (on-set of ethylene) is important for fruit response to harvest and shelf life conditions. Both peaches and nectarines harvested with no ethylene production (immature) did not produce any ethylene or significant aromatic VOCs. This was regardless of fruit type (peach or nectarine) or cultivar, implying that consumer satisfaction will not be achieved by fruit harvested outside of the optimal physiological stage. The combination of optimal harvest I_{AD} values, post-harvest factsheets and improved quality will increase the export for the cultivars under study by at least 5% within the predicted ten years from the conclusion of the project.

Industry has already started to adopt some of the above concepts and information into their production and export protocols. It is expected that the rate of adoption will keep increasing with time and reach the anticipated 20% within ten years from the conclusion of the project. With increased adoption, both domestic sales and international market competitiveness will also increase within the anticipated ten years from the conclusion of the project.

Monitoring and evaluation

During the project, industry has been made aware of any information they could use to make better decisions for their outturn. The strategies for adoption of project information included collaboration with Summerfruit Australia and DEDJTR's Biosecurity and Agriculture Services horticulture team to establish an extension program within the Victorian Government's Food to Asia program (Appendix 2).

The PhD program and relevant work was continually monitored by milestones set down between DEDJTR and RMIT (Appendix 2).

Feedback from industry through the Stonefruit Field Laboratory advisory committee meetings, direct contact and road show presentations to growers has been valuable in understanding how industry would use the information in their businesses (Roadshow feedback see Appendix 4).

Data is being shared and all peach and nectarine fruit data will be made available to the SummerFruit Australia industry via industry and scientific presentations and publications. In addition, Agriculture Victoria's 'Premium Fruit to Asia' (PF2A) 2014/15-2017/18 project provided extension of 'Stonefruit Research' undertaken by Agriculture Victoria via the Horticulture Industry Networks (HIN) website. These systems are being adopted by industry, as demonstrated by Project leader Dr Dario Stefanelli being asked to supervise the scientific aspect of an industry project from Integrity Fruit Ltd (part of the Victorian Horticulture Innovation Funds). The industry project used the vial protocol developed during the course of our investigations, with the aim to create procedures to identify optimal harvest time for pome and stonefruit.

Project visibility was increased internationally by participating to international conferences and visiting international research institutes. As a result of a visit to Bologna University undertaken in September 2015, a PhD student (A. Ceccarelli) visited Australia for 9 months to perform experiments measuring aroma volatiles during storage as well as during fruit ripening. As a result of a visit to the Spanish government research institute in Lleida (IRTA) undertaken in September 2016, a PhD student (L. Torregrosa) visited Australia for 5 months to study the effects of postharvest insect disinfestation on fruit quality traits. The student used fruit harvested from the stonefruit field laboratory based on the I_{AD} maturity classes identified during the project. These studies will add value to existing projects.

Industry have shown a great interest in the research the project undertook, especially with relation to defining fruit maturity through ethylene production. This is shown by the uptake of monitoring fruit development by industry using the techniques validated in this project to increase their collective knowledge base and continue enabling the industry in best harvest practices.

The adoption of ethylene production testing has also increased adoption of non-destructive instrumentation, including the DA-meter, as shown by the increased number of instruments acquired in Australia. This enables the concept of continuous monitoring of fruit on the tree and throughout the handling chain. To be able to fully apply the monitoring of the whole value chain concept, new sensors in addition to the DA-Meter, need to be developed. To promote this concept the Victorian Government approved an industry project for the development of new sensors to be utilized along the value chain (part of the Victorian Horticulture Innovation Funds). It is believed that this would increase acceptable fruit and customer satisfaction.

Recommendations

This project supports the stonefruit industry to become more globally competitive by improving fruit quality management. The ability to harvest fruit according to physiological fruit maturity will have an impact on postharvest storage and distribution and flow on to better results in the market place.

The key message from this project is that the physiological stage of the fruit at harvest is paramount to ontogeny of fruit after harvest, and any fruit harvested without having initiated physiological maturity, onset of ethylene production on the day of harvest, is incapable of developing the full sensorial characteristics typical of the cultivar and requested by consumers.

Stonefruit should be monitored for physiological maturity prior to harvest with non-destructive sensor technology to identify and sometimes predict the optimal harvest period. The determination of the optimal period should be done by correlating non-destructive sensing (e.g. DA-Meter) with ethylene production to identify physiological maturity. This fruit can be segregated in maturity classes with similar ethylene production behaviour.

When using DA technology to segregate fruit in maturity classes, different cultivars have different I_{AD} values for each class. Maturity classes for some cultivars peach and nectarine have been published on the HIN website.

Non-destructive technology to segregate fruit based on maturity indexes should be used along the entire horticulture value chain to enable better and faster decisions and to better deliver optimal quality fruit to consumers.

Intact fruit aroma is a fundamental component in consumer appreciation and satisfaction. Understanding how components of the value chain (maturity at harvest, pre-cooling, length of storage, temperature of storage, disinfestation treatments, ripening delayer treatments) act in modifying the volatile mix typical of the cultivar is important to deliver higher quality fruit to consumers and should be further investigated.

Refereed scientific publications

Journal article

Frisina, C., Stefanelli, D., Giri, K., Tomkins, B., 2018. A revised method for the field collection and storage of fruit ethylene samples using evacuated vials. *Scientia Horticulturae*, **236**, 123–126

Stefanelli, D., Lopresti, J., Hale, G., Jaeger, J., Frisina, C., Jones, R. and Tomkins, B., 2017. Modelling peach and nectarine ripening during storage using the I_{AD} maturity index. *Acta Horticolturae,* **1154**, 17–24. https://doi.org/10.17660/ActaHortic.2017.1154.3

Ceccarelli, A., Frisina C., Allen D., Farnet B., Donati I., Buriani G., Cellini A., Costa G., Spinelli F. and Stefanelli D. Maturity stage at harvest and length of cold storage influence on flavour development in peach fruit. Submitted to *Scientia Horticulturae.*

Ceccarelli, A., 2018. Aroma of peaches and nectarines: interaction between maturity at harvest, postharvest conditions and fresh-cut processing. *PhD dissertation* **Ch4**, 41–66, Universita' di Bologna, April 2018.

References

Aubert, C., Günata, Z., Ambid, C., Baumes, R., 2003. Changes in physicochemical characteristics and volatile constituents of yellow- and white-fleshed nectarines during maturation and artificial ripening. *Journal of Agricultural and Food Chemistry*, **51(10)**, 3083–91 doi:10.1021/jf026153i.

Crisosto, G.M., Day, K.R., Crisosto, C.H., 2008. Using “in store” consumer tests to understand fresh fruit consumer acceptance*. Acta Horticulturae*, **804**, 51–53.

Crisosto CH, Crisosto G, and Nery F. (2006) Understanding tree fruit quality based on consumer acceptance. *Acta Horticulturae*, **712**, 183–190.

Crisosto C, and Crisosto G. (2005) Relationship between soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L) Batsch) cultivars. *Postharvest Biology and Technology*, **38**, 239–246.

Frisina C, Stefanelli D, Giri K, Tomkins B. (2018) A revised method for the field collection and storage of fruit ethylene samples using evacuated vials. *Scientia Horticulturae*, **236**, 1236–126.

Horticulture Innovation Australia Ltd. (HIA). (2017). Summerfruit Industry Strategic Investment Plan 2017–2021. Horticulture Innovation Australia Ltd.

Isbell, R., 2002. The Australian Soil Classification, Revised edn (Collingwood: CSIRO Publishing).

McGlasson B, Golding J and Holford P. (2009) Improving the dessert quality of stone fruits. Postharvest Technologies for Horticultural Crops, Research Signpost 9788130803562.

McGlasson, W.B., Golding, J.B., 2008. Providing consumers with consistently high quality stonefruit. *Acta Horticulturae*, **804**, 45–50.

Skene, J.K.M., Poutsma, T.J., 1962. Soils and land use in part of the Goulburn Valley, Victoria comprising the Rodney, Tongala-Stanhope, North Shepparton and South Shepparton Irrigation Area. Technical Bulletin No. 14 (Victoria, Australia: Department of Agriculture).

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

Acknowledgements

The technical support and assistance of Dr Mark O'Connell, Paul Morrison, Jim Selman, Dave Haberfield, Janine Jeager, Alessandro Ceccarelli, Jane Rollinson, Laia Torregrosa Sauret and Nur Ashikin Binti Hazmi is gratefully acknowledged.

Appendices

Appendix 1. Project activities and publications

Activity/venue	Title/specifics	Attachment
Scientific paper	Frisina C, Stefanelli D, Giri K, Tomkins B. (2018) A revised method for the field collection and storage of fruit ethylene samples using evacuated vials. Scientia Horticulturae 236:123-126	A revised method for the field collecti
Scientific paper	Stefanelli, D., Lopresti, J., Hale, G., Jaeger, J., Frisina, C., Jones, R. and Tomkins, B., 2017. Modelling peach and nectarine ripening during storage using the I_{AD} maturity index. *Acta Horticolturae* **1154**, 17-24. https://doi.org/10.17660/ActaHortic.2017.1154.3	Published on Acta 1154-2017.pdf
Scientific paper	Ceccarelli, A., Frisina C., Allen D., Farnet B., Donati I., Buriani G., Cellini A., Costa G., Spinelli F. and Stefanelli D. Maturity stage at harvest and length of cold storage influence on flavour development in peach fruit. Submitted to *Scientia Horticulturae.*	Under journal evaluation
Draft Literature review	Frisina C., Stefanelli D. Comparing peach and nectarine maturity, quality and volatile composition.	
Chapter in International PhD dissertation, Bologna, Italy 2018	Chapter 4: Maturity stage at harvest and length of cold storage influence on flavour development in peach fruit	Ceccarelli_PhDdissertation_Ch4_Bologn
Industry article published in Australian Stonefruit Grower. August 2016	Frisina C and Stefanelli D. (2017) The DA-meter, from theory to practice.	Evacuated vials protocol_Au-Stonefr
Industry article published in Australian Stonefruit Grower. May 2015	Stefanelli D. (2015). Determination of stonefruit maturity, quality and volatile composition.	ASG - MAY 2015 Issue .pdf
Stonefruit Research Roadshow at Renmark, Swan Hill and Cobram, 2016	Ethylene Sampling Protocols to calibrate for fruit maturity using the DA meter. http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-grower-events	Stefanelli_workshoptour_2016.pdf
Stonefruit Research Roadshow at Renmark, Swan Hill and Cobram, 29-30-31 August 2017	Picking fruit for optimal flavour and storage impacts on fruit quality	Stonefruit Roadshow Aug2017
PhD milestone 1 (Feb. 2016)	Confirmation of Candidature: Masters Applied Chemistry (MR229)	
PhD milestone 2 (Feb. 2017)	Confirmation of Candidature: Doctorate Applied Chemistry (DR229)	
Oral presentation at the 5th symposium on modelling, Model-IT, Wageningen, Netherlands, October 2015	Modelling peach and nectarine ripening during storage using the I_{AD} maturity index.	Stefanelli_Modelling ripening during st

Abstract submitted to the 5th symposium on modelling, Model-IT, Wageningen, Netherlands, October 2015	Modelling peach and nectarine ripening during storage using the I_{AD} maturity index.	Postahrvest IAD_Published abstr
Oral presentation at AgriBio seminar series June 2016	Scientific seminar titled 'Is the physiological maturity at harvest influencing peach flavor after cold storage?'.	Alessandro seminar Agribio June3-2016.
Oral presentation at AgriBio science day, 2017	Invited presentation titled 'The aromatic volatiles of summer fruit: Harvest maturity impacts on fruit aroma during storage and marketing'.	Christine Frisina AgB conf Oct 2017.p
International PhD defense, Bologna, Italy, 2018	Scientific seminar titled 'Aroma of peach and nectarines: Interaction between maturity at harvest, postharvest condition and fresh cut processing'.	PhD presentation_compr
Oral Presentation at DEDJTR innovation Forum in Tatura May 2016	Invited presentation titled 'Pre and post-harvest management of fruit maturity and quality for market access'.	Pre and post harvest quality_Tat2
Oral presentation at AgriBio seminar series August 2015	Scientific seminar titled 'Capitalising on the importance of fruit maturity through innovative technology'.	AgriBio seminar .pptx.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest Fact Sheet for cv 'Autumn Bright'	Harvest Fact Sheet for AutB.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest Fact Sheet for cv 'August Bright'	Harvest Fact Sheet for AugB.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest Fact Sheet for cv 'September Bright'	Harvest Fact Sheet for SB.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest Fact Sheet for cv 'Snow Flame 23'	Harvest Fact Sheet for SF23.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest Fact Sheet for cv 'Snow Flame 25'	Harvest Fact Sheet for SF25.pdf
Fact Sheet draft, to be uploaded on HIN webpage	Harvest and storage Fact Sheet for cv 'August Flame'	Harvest and Storage Fact Sheet f

Fact Sheet draft, to be uploaded on HIN webpage	Harvest and storageFact Sheet for cv 'Rose Bright'	PDF Harvest and Storage Fact Sheet f
Agriculture Services (Horticulture Extension)	Annual Industry Technology Adoption Plan – Milestone 11	PDF CMI 105224 Milestone 11 Report

Appendix 2. I_{AD} values for cultivars tested during the project life in relation to the production of ethylene as an indicator of fruit physiological development stage.

Cultivar	No ethlyene production: immature	Onset ethylene production: Commercial ripe	Climacteric ethylene production: full ripe
Snow Flame 23: 2016-17	2.0-0.8	0.8-0.3	0.3-0
: 2017-18	2.0-0.8	0.8-0.3	0.3-0
Snow Flame 25: 2015-16	2.0-1.0	1.0-0.7	0.7-0
: 2017-18	2.0-1.2	1.2-0.7	0.7-0
Ice Princess [2]: 2017-18	2.0-1.5	1.5-0.3	0.3-0
O'Henry [1 2]: 2015-16	2.0-0.9	0.9-0.7	0.7-0
Early: 2016-17	2.0-1.0	1.0-0.6	0.6-0
Early: 2017-18	2.0-1.0	1.0-0.6	0.6-0
Late: 2016-17	2.0-1.2	1.2-0.9	0.9-0
Late: 2017-18	2.0-1.2	1.2-0.5	0.5-0
August Flame: 2015-16	2.0-1.3	1.3-0.8	0.8-0
: 2017-18	2.0-1.4	1.4-0.6	0.6-0
Red Haven [2]: 2017-18	2.0-1.1	1.1-0.8	0.8-0
September Sun [2]: 2016-17	2.0-1.1	1.1-0.8	0.8-0
Rose Bright:2015-16	2.0-1.0	1.0-0.5	0.5-0
: 2017-18	2.0-1.2	1.2-0.4	0.4-0
Autumn Bright: 2015-16	2.0-1.4	1.4-0.9	0.9-0
: 2017-18	2.0-1.4	1.4-0.7	0.7-0
August Bright [2]: 2016-17	2.0-0.8	0.8-0.5	0.5-0
: 2017-18	2.0-0.5	0.5-0.2	0.2-0
September Bright: 2016-17	2.0-1.2	1.2-0.8	0.8-0
: 2017-18	2.0-1.1	1.1-0.8	0.8-0

[1] The variability is due to the different clones mixed within the culitvar

[2] Confidential: portions of this are to be confirmed in coming seasons; not to be reproduced.

Appendix 3. Industry feedback from regional roadshows (2016 and 2017)

Cobram Roadshow Evaluation_2016.pdf

Renmark Roadshow Evaluation_2016.pdf

Swan Hill Roadshow Evaluation_2016.pdf

Renmark Stonefruit Roadshow Evaluatic

Swan Hill Stonefruit Roadsho

Cobram Stonefruit Roadshow Evaluatic

Scientia Horticulturae 236 (2018) 123–126

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

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

Christine Frisina[a,b,*], Dario Stefanelli[b], Khageswor Giri[c], Bruce Tomkins[b]

[a] RMIT, School of Science, 124 LaTrobe Street, Melbourne, VIC 3000, Australia
[b] Agriculture Victoria, AgrioBio Centre, 5 Ring Road, Bundoora, VIC 3083, Australia
[c] Agriculture Victoria, 32 Lincoln Square North, Carlton, VIC 3053, Australia

ARTICLE INFO

Keywords:
Climacteric fruit
Delayed analysis
Gas systems
Static collection chambers
Sample
Volume
Gas chromatography

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

* Corresponding author at: Agriculture Victoria, AgriBio Centre, 5 Ring Road, Bundoora, VIC 3083, Australia.
E-mail address: Christine.frisina@ecodev.vic.gov.au (C. Frisina).

https://doi.org/10.1016/j.scienta.2018.03.045
Received 27 October 2017; Received in revised form 18 March 2018; Accepted 20 March 2018
Available online 27 March 2018
0304-4238/

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 reassessed (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

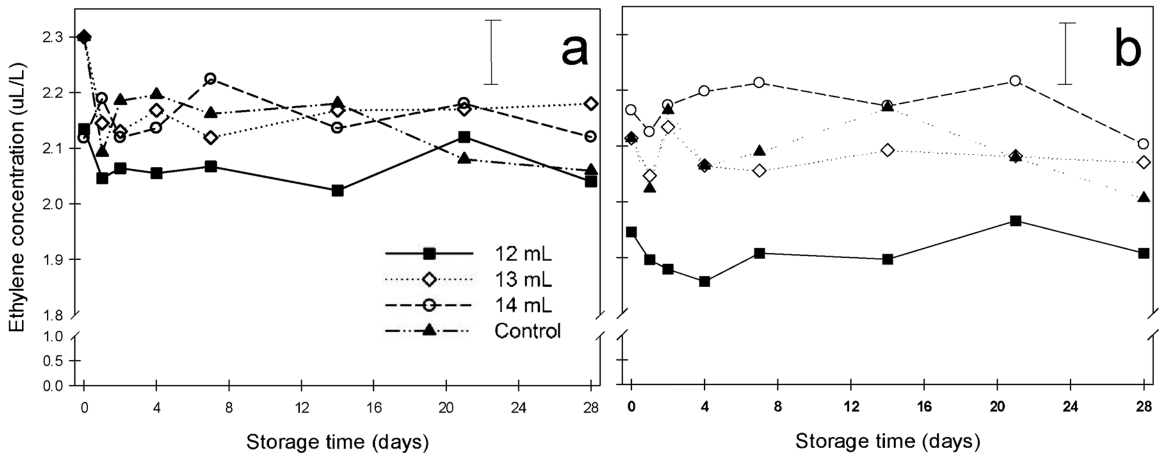


Fig. 1. Ethylene concentrations (μL/L) of (a) first extraction, and (b) second extraction from 12-mL evacuated vials containing a measured input of 12, 13 or 14-mL known 2.2 μL ethylene /L standard and stored for up to 28 days at room temperature. Control vials were filled with 13-mL of ethylene standard on the day of testing. Error bar represents the LSD*(P = 0.05)* when comparing treatments of the same storage time.

Table 1
Ethylene concentration of 12-mL vials measured and calculated using the model according to Eqs. (1) and (2). All vials were filled with 14-mL of 2.2 μL ethylene/L standard and each vial was sampled 5 times.

			Ethylene Concentration (μL/L)				
Extraction	Va[a]	Vy[b]	Measured	Calculated	SD[c]	Fprob	CV (%)[d]
1	14	13	2.25	2.25	0.0695	0.00483	3.099
2	13	13	2.25	2.25	0.0768	0.00590	3.395
3	12	13	2.10	2.26	0.0478	0.00228	2.283
4	11	13	1.98	2.29	0.0704	0.00496	3.568
5	10	13	1.78	2.19	0.0803	0.00645	4.538

[a] Volume of the sample in the vial.
[b] Volume of the system (vial plus syringe).
[c] Standard deviation.
[d] Coefficient of Variation.

concentration of the sample. When we filled the 12-mL evacuated vial with 14-mL of sample a positive pressure of approximately 1.17 atm was created inside the vial. At the second extraction from the vial, the pressure was reduced to 1.0 atm. By the third extraction, the pressure was reduced to approximately 0.92 atm, thus lowering the concentration of the sample in the vial by a factor of 0.92. The subsequent fourth and fifth extractions of 1-ml lowered the concentration of the sample in the vial by a factor of 0.85 and 0.77, respectively. Increasing the input volume addresses the integrity of the original concentration of the sample due to this dilution when taking a measurement. Drury (Drury et al., 2007) noted there is a pressure change due to the increased volume, which occurs when the syringe is inserted into the vial when the sample is extracted. This change increases the volume of the system, which affects the concentration of the sample. Using a positive pressure maintains the volume of the system allowing for a more accurate representation of the sample. With this in mind Eqs. (1) and (2), representing a model to account for the reduction of the system, were successfully evaluated.

Fig. 1a and b shows the consistency in analysed ethylene concentrations for each input volume for samples collected and stored for up to 28 days in vials at ambient temperature. As Watkins et al. (1989) suggested the length of storage time had no significant effect on the concentration of the samples at analysis. There was a significant effect of input volume on the measured ethylene concentration which was related to the observations previously described and was observed for both the first and second extractions from each vial. When the model Eqs. (1) and (2) were used to re-calculate the ethylene concentrations the differences between the input volumes were negated. This indicates that using any of the input volumes tested would be adequate to collect and store samples for up to 28 days at ambient temperature as long as the model was used to take into account the reduction in concentration caused by sampling.

All vials for the chamber to vial transfer accuracy part of the study were filled using a 14-mL input volume. No significant differences were found between the direct injection from chambers and passing samples through the vials (Table 2). The very low concentration of 0.004 μL ethylene/L was at the limit of instrumental detection. This shows the use of these vials is a suitable replacement when direct analysis is unavailable.

In addition to the ability to store ethylene samples as originally suggested by Watkins et al. (1989), we wanted to show the significant effect of testing for ethylene on the day of harvest compared to delaying collection. Fruit were harvested and ethylene production rate was measured in chambers immediately after harvest (growers shed; day 0) as well as 24 h after harvest (laboratory; day 1). Fig. 2 shows the difference in ethylene concentration between these two time frames for the same fruit of three types of stone fruit, 'Autumn Bright' nectarine, 'August Flame' peach and 'Golden May' apricot. Ethylene concentration was significantly lower in samples collected from fruit on day 0 harvest than in the same fruit evaluated in the laboratory after 24 h. This implies that fruit maturity was more advanced after 24 h. For some fruit there was an increase in ethylene concentration of greater than 2 ppm. Enabling the accurate analysis of ethylene production rate by sampling immediately after harvest will result in obtaining real time ethylene production rate data. This will be especially important when using ethylene production rate for comparison with other harvest maturity indicators. Precision in these measurements would be even more important when considering ethylene is an activating factor for some physiological pathways (Ortiz et al., 2010; Barry and Giovannoni, 2007) and is important in predicting subsequent fruit behaviour during

Table 2
Ethylene concentrations (μL/L) observed when sampled directly from the 0.75-L testing chambers and from sample vials collected from the same chambers.

Injected ethylene standard	0-mL	1.5-mL	15-mL	150-mL
Calculated concentration: prior to sampling (μL/L)	0	0.004	0.044	0.440
Analysed concentrations:				
Jar	nd	nd	0.034	0.365
Vial	nd	nd	0.052	0.363
LSD (*P = 0.05*)	0.047			

(nd = nil detected).

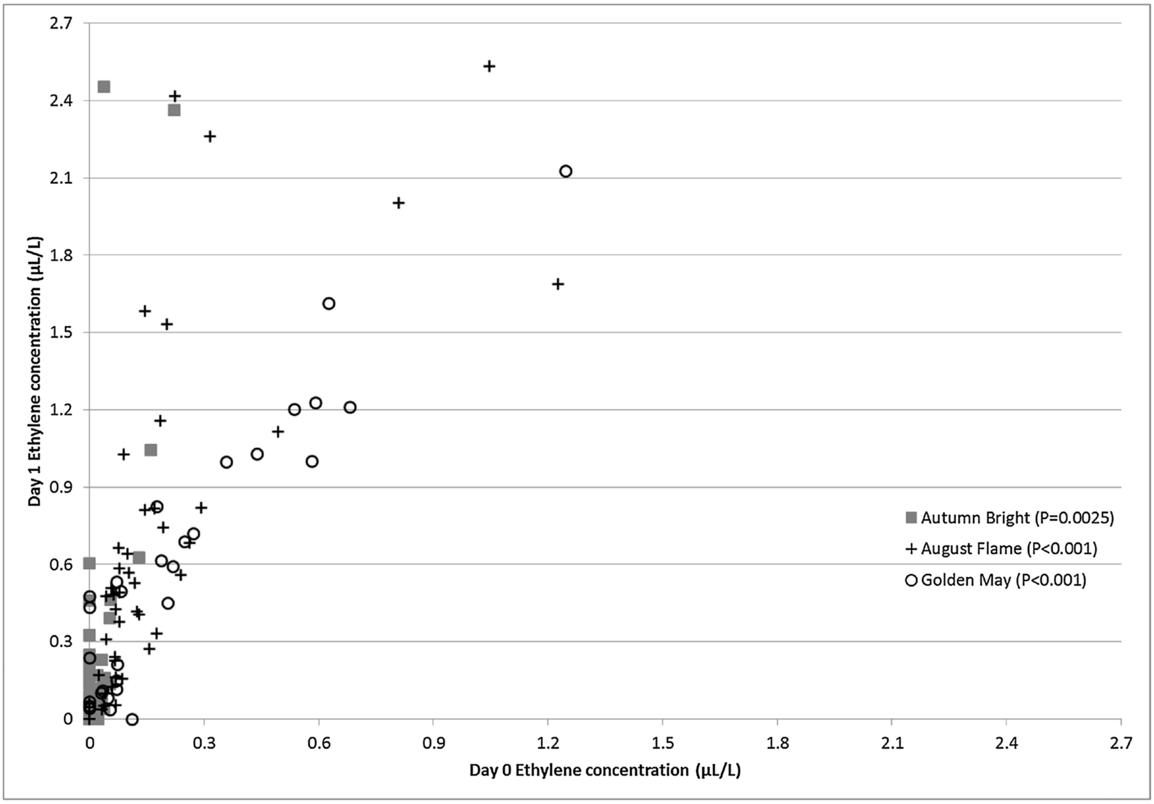


Fig. 2. Ethylene concentration (µL/L) from individual fruit of Autumn Bright nectarine, August Flame peach and Golden May apricot immediately after harvest (day 0) and after 24 h (day 1). The same fruit were used in both tests.

handling, storage and distribution (Kader and Mitchell, 1989).

4. Conclusion

The model in Eq. (1) was successfully tested in these experiments. There was no dilution or loss of sample integrity when using vials for temporary storage of gas samples. In addition, samples transferred to vials could be stored for up to 28 days at ambient temperature with no measureable loss in sample concentration. Delaying ethylene collection from fruit after harvest was shown to result in significantly increased values; therefore it is suggested that when immediate sample analysis is impossible an evacuated vial gas collecting system should be used. From our experiments it is also suggested when using the evacuated vials to increase the sample volume (i.e. Va) by at least two times the volume extracted by the syringe for GC analysis, allowing multiple samples to be taken from the vial. In the event that this is not possible, or too many samples have already been taken from a vial, using the model (Eqs. (1) and (2)) will correct the results.

This method and model can be used for any static gas collection system requiring storage of vial samples for at least 28 days independently of chamber volume, evacuated container volume, sample volume collected, injection volume and product type.

Acknowledgements

The experiment was part of the project SF15001 "Comparing Stonefruit ripening, quality and volatile composition" funded by Horticulture Innovation Australia Limited using the Summerfruit levy and funds from the Australian Government with co-investment from the Department of Economic Development, Jobs, Transport and Resources.

References

Barry, C.S., Giovannoni, J.J., 2007. Ethylene and fruit ripening. J. Plant Growth Regul. 26 476-159.

Chiriboga, M.-A., Saladie, M., Bordonaba, J.G., Recasens, I., Garcia-Mas, J., Larrigaudiere, C., 2013. Effect of cold storage and 1-MCP treatment on ethylene perception, signalling and synthesis: influence on the development of the evergreen behaviour in 'Conference' pears. Postharvest Biol. Tech 86 221-220.

Crisosto, C.H., Mitchell, F.G., Johnson, M., 1995. Factors in fresh market stone fruit quality. Postharvest News Info. 6 (2), 17N–21N.

Drury, C.F., Myrold, D.D., Beauchamp, E.G., Reynolds, W.D., 2007. Chapter 37: Denitrification techniques of soils. In: Carter, M.E., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis, 2nd Ed. CRC Press Print ISBN: 978-0-8493-3586-0, eBook ISBN: 978-1-4200-0527-1.

Frisina, C., Stefanelli, D., 2016. The DA-Meter, from Theory to Practice. Austral. Stonefruit Grower. August 2016. pp. 36–40.

Kader, A.A., Mitchell, F.G., 1989. Maturity and quality. In: LaRue, J.H., Johnson, R.S. (Eds.), Peaches, Plums and Nectarines: Growing and Handling for Fresh Market. Univ. Ca. Dept. Agr. and Natural Resources Publication No. 3331.

Ortiz, A., Graell, J., Lopez, M.L., Echeverria, G., Lara, I., 2010. Volatile ester-synthesising capacity in 'Tardibelle' peach fruit in response to controlled atmosphere and 1-MCP treatment. Food Chem. 123, 698–704.

Pre-Aymard, C., Weksler, A., Lurie, S., 2003. Responses of 'Anna', a rapidly ripening summer apple, to 1-methylcyclopropene. Postharvest Biol. Technol. 27, 163–170.

Reid, M.S., 1985. Ethylene and abscission. HortScience 20 (1), 45–50.

Tsantili, E., Shin, Y., Nock, J., Watkins, C.B., 2010. Antioxidant concentrations during chilling injury development in peaches. Postharvest Biol. Technol. 57, 27–34.

Watkins, C.B., Bowen, J.H., Walker, V.J., 1989. Assessment of ethylene production by apple cultivars in relation to commercial harvest dates. N. Z. J. Crop Hort. Sci. 17 (4), 327–331.

Xiaotang, Y., Jun, S., Forney, C., Leslie, C.-P., Sherry, F., Wismer, P., Zhang, Zhaoqi, 2016. Ethylene and 1-MCP regulate major biosynthetic pathways in apple fruit. Food Chem. 194, 325–336.

ACKNOWLEDGEMENTS

This research was carried out as part of IWT project 100885 supported by the Agency for Innovation by Science and Technology in Flanders (IWT) and Logistieke en Administratieve Veilingassociatie (LAVA), Belgium.

Literature cited

Bobelyn, E., Serban, A.-S., Nicu, M., Lammertyn, J., Nicolai, B.M., and Saeys, W. (2010). Postharvest quality of apple predicted by NIR-spectroscopy: study of the effect of biological variability on spectra and model performance. Postharvest Biol. Technol. *55* (*3*), 133–143 http://dx.doi.org/10.1016/j.postharvbio.2009.09.006.

Cai, W., Li, Y., and Shao, X. (2008). A variable selection method based on uninformative variable elimination for multivariate calibration of near-infrared spectra. Chemom. Intell. Lab. Syst. *90* (*2*), 188–194 http://dx.doi.org/10.1016/j.chemolab.2007.10.001.

Enninghorst, A., and Lippert, F. (2003). Postharvest changes in carbohydrate content of lamb' s lettuce (*Valerianella locusta*). Acta Hortic. *604*, 553–558 http://dx.doi.org/10.17660/ActaHortic.2003.604.65.

Geladi, P., MacDougall, D., and Martens, H. (1985). Linearization and scatter-correction for NIR reflectance spectra of meat. Appl. Spectrosc. *39* (*3*), 491–500 http://dx.doi.org/10.1366/0003702854248656.

Lucasius, C.B., Beckers, M.L.M., and Kateman, G. (1994). Genetic algorithms in wavelength selection: a comparative study. Anal. Chim. Acta *286* (*2*), 135–153 http://dx.doi.org/10.1016/0003-2670(94)80155-X.

Martens, H., Høy, M., Wise, B.M., Bro, R., and Brockhoff, P.B. (2003). Pre-whitening of data by covariance-weighted pre-processing. J. Chemometr. *17* (*3*), 153–165 http://dx.doi.org/10.1002/cem.780.

Mehmood, T., Liland, K.H., Snipen, L., and Sæbø, S. (2012). A review of variable selection methods in partial least squares regression. Chemom. Intell. Lab. Syst. *118*, 62–69 http://dx.doi.org/10.1016/j.chemolab.2012.07.010.

Naes, T., Isaksson, T., Fearn, T., and Davies, T. (2002). A User Friendly Guide to Multivariate Calibration and Classification (Charlton, UK: NIR Publications), pp.352.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., and Lammertyn, J. (2007). Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol. Technol. *46* (*2*), 99–118 http://dx.doi.org/10.1016/j.postharvbio.2007.06.024.

Nicolaï, B.M., Verlinden, B.E., Desmet, M., Saevels, S., Saeys, W., Theron, K., Cubeddu, R., Pifferi, A., and Torricelli, A. (2008). Time-resolved and continuous wave NIR reflectance spectroscopy to predict soluble solids content and firmness of pear. Postharvest Biol. Technol. *47* (*1*), 68–74 http://dx.doi.org/10.1016/j.postharvbio.2007.06.001.

Rico, D., Martín-Diana, B., Barat, J.M., and Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci. Technol. *18* (*7*), 373–386 http://dx.doi.org/10.1016/j.tifs.2007.03.011.

Wold, S., Sjöström, M., and Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. Chemom. Intell. Lab. Syst. *58* (*2*), 109–130 http://dx.doi.org/10.1016/S0169-7439(01)00155-1.

Xiaobo, Z., Jiewen, Z., Povey, M.J.W., Holmes, M., and Hanpin, M. (2010). Variables selection methods in near-infrared spectroscopy. Anal. Chim. Acta *667* (*1-2*), 14–32 http://dx.doi.org/10.1016/j.aca.2010.03.048. PubMed

Zeaiter, M., Roger, J.-M., Bellon-Maurel, V., and Rutledge, D.N. (2004). Robustness of models developed by multivariate calibration. Part I: the assessment of robustness. Trends Analyt. Chem. *23* (*2*), 157–170 http://dx.doi.org/10.1016/S0165-9936(04)00307-3.

Modelling peach and nectarine ripening during storage using the I_{AD} maturity index

D. Stefanelli[a], J. Lopresti, G. Hale, J. Jaeger, C. Frisina, R. Jones and B. Tomkins

Department of Economic Development, Jobs, Transport & Resources, Bundoora, Victoria, Australia.

Abstract

Consumer dissatisfaction due to poor and variable eating quality of peaches and nectarines can severely limit sales growth in domestic and export markets. The effect of fruit maturity at harvest on subsequent ripening behaviour and eating quality during storage is extremely important, but not fully understood. A recent technology, the index of absorbance difference (I_{AD}), provides a non-destructive index of fruit physiological maturity and enables accurate monitoring of fruit ripening behaviour before and after harvest. *Prunus persica* L. 'Summer Flare 34' and 'Summer Bright' nectarine and 'September Sun' peach were segregated at harvest into classes based on ethylene production rate and then monitored during storage. 'Summer Bright' and 'September Sun' were monitored with a DA-meter and the I_{AD} was measured twice weekly for up to 42 days during storage at both 0 and 7°C, whilst the effect of ambient temperature on ripening was determined by monitoring 'Summer Flare 34' at 3-day intervals for up to 10 days at 18°C. Changes in the I_{AD} ripening index were best described by a logistic sigmoidal curve. Storage temperature, cultivar and maturity class at harvest affected the magnitude of single curve parameters but not the type of relationship between ripening and storage time. A series of sigmoidal curves were identified and differences in the *y*-intercept (i.e., I_{AD} value at harvest) were mainly explained by cultivar and maturity stage at harvest, while differences in the slope and inflection point were mostly due to storage temperature. Adjusted correlation coefficients were cultivar dependent and decreased with storage temperature, with weaker correlations found for fruit at 0°C, due to a lower rate of ripening during storage. In this study, a common postharvest ripening trend was identified among the peach and nectarine cultivars studied with a logistic sigmoidal curve best describing the change in I_{AD} during storage.

Keywords: fruit quality, fruit maturity, non-destructive monitoring, index of absorbance difference, storage period

INTRODUCTION

Commercial stone-fruit value chains are characterised by a series of operations that affect fruit ripening and quality from grower to consumer. Harvest maturity is a key factor in determining the sensory quality of fruit and its ability to withstand handling, storage and distribution (Echeverría et al., 2015). The challenge is to harvest fruit at a physiological maturity that provides sufficient ripening potential for good eating quality and that is still adequate for storage and transport to domestic and export markets (Infante et al., 2008). Harvesting at optimum physiological maturity to suit the handling chain requires fruit ripening to an acceptable level on the tree and the development of typical fruit quality characteristics that maximize consumer satisfaction (Lavilla et al., 2002; Infante, 2012). The ability to predict the ripening behaviour of stone fruit along the value chain is crucial to optimising storage, transport, postharvest handling and marketing conditions to provide fruit that meets consumer expectations (Shinya et al., 2013).

A recently developed technology measures difference of absorbance in chlorophyll-*a*

[a]E-mail: Dario.Stefanelli@ecodev.vic.gov.au

Acta Hortic. 1154. ISHS 2017. DOI 10.17660/ActaHortic.2017.1154.3
Proc. V Int. Symp. on Applications of Modelling as an Innovative Technology in the Horticultural Supply Chain
Ed.: P. Tijskens

in fruit mesocarp tissue non-destructively. Known as the index of absorbance difference (I_{AD}), it can be correlated to fruit ethylene production and thus to the physiological maturity of fruit (Ziosi et al., 2008). Using I_{AD}, it is possible to monitor fruit ripening non-destructively both pre- and postharvest (Costa et al., 2009). Consumer demand for high-quality fruit with excellent flavour and high nutritional and nutraceutical value highlights the need to monitor the effect of postharvest handling and storage on fruit quality attributes (Echeverría et al., 2015). Several authors have reported the usefulness of the I_{AD} in identifying and predicting optimal harvest time to maximise ripening potential and consumer liking. In this work. we used the I_{AD} to measure the rate of peach and nectarine ripening at different storage temperatures with the aim of identifying a common pattern in fruit I_{AD} decay as a first step in predicting postharvest ripening behaviour.

MATERIALS AND METHODS

Fruit were segregated into maturity classes at harvest by correlating ethylene production with the I_{AD} value according to Ziosi et al. (2008), with the method adapted for Australian climatic conditions according to Bonora et al. (2013) and Hale et al. (2013). Fruit were segregated into three maturity classes based on the amount of ethylene produced: preclimacteric (PC), onset of climacteric (OC) and climacteric (CL). The corresponding fruit I_{AD} was measured on two opposite cheeks with a DA-meter (model 53500, Turoni, Forli, Italy).

Mid-season 'Summer Flare 34' and 'Summer Bright' nectarine and late-season 'September Sun' peach were sourced from commercial orchards in northern Victoria, Australia, during 2010 and 2013. 'Summer Bright' and 'September Sun' fruit were treated with a postharvest fungicide dip (Spin Flo™; active constituent: 500 g L^{-1} Carbendazim) at a rate of 75 mL 100 L^{-1} water prior to storage. Fruit were segregated by maturity class into plastic trays that were placed in unsealed plastic liners (Lifespan®; Bag code L219). Packed fruit was stored at 0 or 7°C and 90±5% relative humidity for up to 42 days. During storage, the I_{AD} index was measured once or twice a week on opposite cheeks of each fruit using the DA-meter. Twenty 'Summer Flare 34' fruit within each maturity class were placed at 18°C for up to 10 days and I_{AD} was measured every 3 days on the opposite sides of each fruit. Four replicates of five fruit for each maturity class were randomised within a storage block at each storage temperature. For each cultivar, the curve of best fit was identified with SigmaPlot 12.5 (Systat Software, San Jose, CA, USA) using the global curve-fitting (GCF) function to determine parallelism within the same cultivar. Global curve-fitting enables simultaneous fitting of an equation to several datasets with the option to specify the behaviour of each equation parameter with respect to the datasets (Smit Consult, 2015). In our analysis, we maintained maturity class and temperature as sub-datasets for each cultivar.

RESULTS AND DISCUSSION

Identification of fruit maturity classes

The range of I_{AD} values describing each maturity class for 'Summer Flare 34' during two fruit seasons were 0.0-0.59 for CL, 0.60-1.20 for OC and 1.21-1.60 for the PC class (Figure 1).

In each season, a similar pattern of ethylene production was observed, with minor differences between seasons, potentially due to a very wet 2010 season that substantially increased the incidence of brown rot in the orchard. Fruit stress response to wounding may have thus affected the rate of ethylene production at similar I_{AD} values from one season to the other. This would also explain the small difference in class identification between 2010 measurements and those reported by Bonora et al. (2013). The maturity class separation for 'Summer Bright' and 'September Sun' used to segregate fruit in this work were reported by Lopresti et al. (2016). Our study verified the cultivar-specificity of I_{AD} correlation with ethylene production found by Shinya et al. (2013) and Bonora et al. (2014). It also demonstrated the usefulness of the index in identifying maturity classes at harvest with similar ethylene production patterns (Lleó et al., 2011; Bonora et al., 2013), and their implications for consumer acceptance (Echeverría et al., 2015).

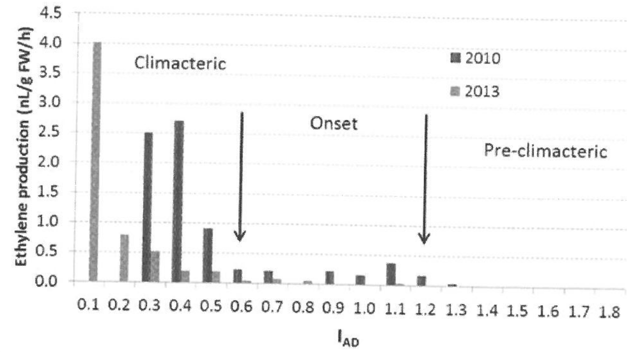


Figure 1. Relationship between ethylene emission at 20°C and index of absorbance (I_{AD}) at harvest for 'Summer Flare 34' nectarine in 2010 and 2013.

Curve fitting and identification

Of the various regressions fitted with the GCF function, the best curve describing I_{AD} decay during postharvest storage was sigmoidal following a logistic model with three parameters (equation 1), confirming the work of Lurie et al. (2013) and as shown in Figures 2-4 and in Table 1.

$$I_{AD} = \frac{a}{\left[1 + \left(\frac{t}{x0}\right)^{b}\right]} \quad (1)$$

In this logistic model, a is the intercept representing the average I_{AD} value of the fruit in the specific maturity class, t is the storage length in days (in the case of $t = 0$, the intercept a was used); x_0 is the inflection point of the curve, and b is the slope of the curve. By using the GCF function, a parameter can be localized to have a separate value for each data set, or a parameter can be shared to have the same value for all data sets. In our analysis, the best correlations were obtained by sharing the curve slope b between temperature and maturity class within the same cultivar, creating a commonality in the I_{AD} decay rate among datasets (Table 1).

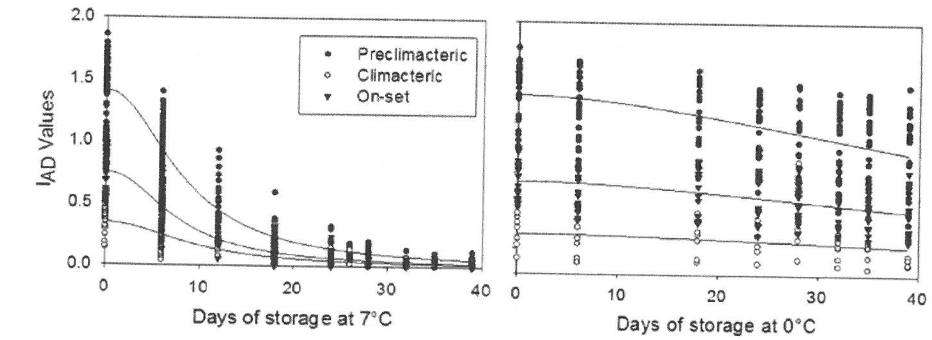


Figure 2. Relationship between index of absorbance (I_{AD}) and storage period at 7°C (left) and 0°C (right) for 'September Sun' peach classified by maturity class at harvest (●, preclimacteric; ▼,onset climacteric; ○, climacteric). Sigmoidal logistic curves were fitted using the global curve-fitting option in SigmaPlot 12.5 with the slope parameter "b" shared between maturity classes.

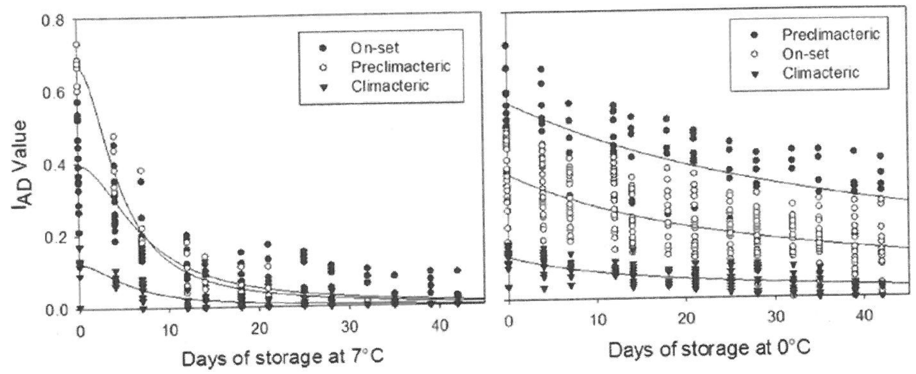


Figure 3. Relationship between index of absorbance (I_{AD}) and storage period at 7°C (left: ○ preclimacteric, ● onset climacteric, ▼ climacteric) and 0°C (right: ● preclimacteric, ○ onset climacteric, ▼ climacteric) for 'Summer Bright' nectarine classified by maturity class at harvest. Sigmoidal logistic curves were fitted using the global curve fitting option in SigmaPlot 12.5 with the slope parameter "b" shared between maturity classes.

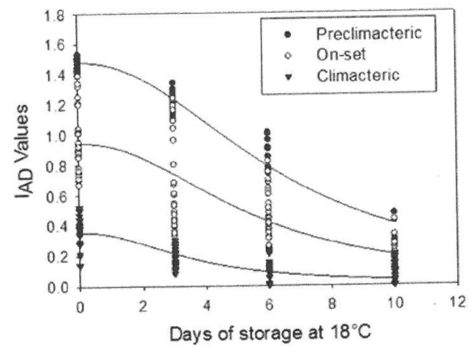


Figure 4. Relationship between index of absorbance (I_{AD}) and storage period at 18°C for 'Summer Flare 34' nectarine classified by maturity class at harvest (● preclimacteric, ○ onset climacteric, ▼ climacteric). Sigmoidal logistic curves were fitted using the global curve fitting option in SigmaPlot 12.5 with the slope parameter "b" shared between maturity classes.

Table 1. Adjusted R^2 and parameter values for sigmoidal logistic regression curves fitted to I_{AD} data generated with the global curve-fitting function [f = if(x<=0, a; x>0, $a/(1+\text{abs}(x/x_0)^b)$] in SigmaPlot 12.5 with shared slope parameter "b". Curves were fitted to full dataset after grouping by cultivar, storage temperature and maturity class at harvest.

September Sun peach - overall adjusted R^2=0.88; *P*<0.0001								
Temperature			**0°C**				**7°C**	
Parameter	**b**	**a**	**X_0**	**Adj. R^2**	**b**	**a**	**X_0**	**Adj. R^2**
Preclimacteric	2.055	1.404	56.167	0.26	2.055	1.408	8.263	0.90
On-set	2.055	0.734	52.458	0.27	2.055	0.753	7.645	0.83
Climacteric	2.055	0.322	51.780	0.14	2.055	0.342	9.424	0.60
Summer Bright nectarine - overall adjusted R^2=0.87; *P*<0.0001								
Temperature			**0°C**				**7°C**	
Parameter	**b**	**a**	**X_0**	**Adj. R^2**	**b**	**a**	**X_0**	**Adj. R^2**
Preclimacteric	1.567	0.639	41.163	0.48	1.567	0.671	4.207	0.95
On-set	1.567	0.399	24.818	0.52	1.567	0.405	6.689	0.81
Climacteric	1.567	0.137	20.640	0.56	1.567	0.122	5.495	0.77
Summer Flare 34 nectarine - overall adjusted R^2=0.84; *P*<0.0001								
Temperature							**18°C**	
Parameter					**b**	**a**	**X_0**	**Adj. R^2**
Preclimacteric					2.158	1.478	6.359	0.94
On-set					2.158	0.943	5.412	0.62
Climacteric					2.158	0.356	3.577	0.60

Figures 2-4 show that the rate of decay of the I_{AD} value during storage was cultivar-specific, confirming preharvest results from multiple studies, as well as results from postharvest studies by Eccher Zerbini et al. (2011) and Lurie et al. (2013). Figures 2 and 3 also show that the I_{AD} rate of decay is temperature-dependent, with clearly different logistic curves describing ripening behaviour during storage at 0 and 7°C. Storage temperature strongly affects the value of the curve inflection point, with x_0 values increasing with decreasing storage temperatures. The strength of correlation between ripening and storage time was also affected by storage temperature, as demonstrated by a lower adjusted correlation coefficient (R^2) at 0°C compared with that at 7°C in both 'September Sun' and 'Summer Bright' (Table 1). Eccher Zerbini et al. (2011) found a similar effect of storage temperature on the degree of correlation between ripening and storage period in work to predict changes in nectarine firmness during storage at 0 and 4°C.

As an advance on the work of Lurie et al. (2013), who used a single maturity class at harvest, our study found an important effect of maturity class on the shape and degree of fit of the different curves identified, as demonstrated by differences in adjusted correlation coefficients (Table 1; Figures 2-4). The importance of maturity class at harvest on peach and nectarine ripening behaviour may be explained by differences in enzymatic activity among maturity classes, as found by Ziosi et al. (2008) and Eccher Zerbini et al. (2011). An interaction between temperature and maturity class was also found with increasing harvest maturity at both 7 and 18°C, resulting in a declining R^2 value in all cultivars, whilst no such decline in R^2 was observed in fruit stored at 0°C (Table 1). This interaction between temperature and maturity class is also demonstrated in Figure 5 by plotting predicted versus measured I_{AD} values for 'September Sun' fruit. The relationship was constructed using all data for the cultivar and calculating the predicted I_{AD} value from the identified regression curves fitted to the data for each maturity class at 0 and 7°C. The strong correlation between measured and predicted I_{AD} values (R^2=0.86) demonstrates that a sigmoidal logistic curve best describes the I_{AD} decay of 'September Sun' during storage. Similar results were found for 'Summer Flare 34' and 'Summer Bright' nectarine (data not shown).

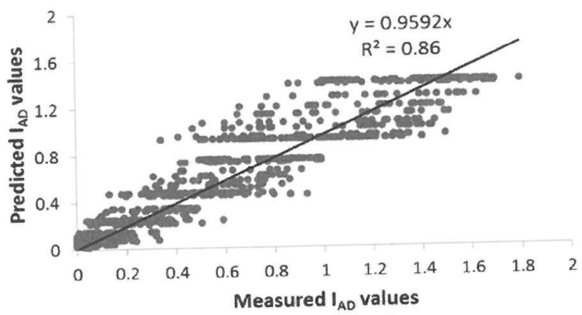


Figure 5. Relationship between measured and predicted I_{AD} values for 'September Sun' peach. Predicted values were calculated by segregating all data into their respective storage temperature and maturity class and applying the identified regression equation.

This result suggests that any regression model describing peach and nectarine ripening behaviour during storage needs to account for the effects of storage temperature and maturity class at harvest. When these effects were factored into regression models, the overall adjusted R^2 was greater than 0.80 in all cultivars (Table 1). Our work has demonstrated the usefulness of the I_{AD} as an index for monitoring fruit ripening behaviour during postharvest storage. The I_{AD} could become the measurement of choice on which to base real-time decisions related to harvest timing, storage period and fruit ripening protocols, as was highlighted by Stefanelli et al. (2016).

CONCLUSIONS

In this study, a common postharvest ripening trend was identified among peach and nectarine cultivars, with a logistic sigmoidal curve best describing the decay in I_{AD} during storage. Storage temperature, cultivar and maturity class at harvest had important effects on single curve parameters, but not on the type of curve. A series of sigmoidal curves were identified, where most of the variability in the *y*-intercept was explained by cultivar and fruit maturity class at harvest. Differences in slope and inflection points between curves were mainly due to effects of storage temperature. The magnitude of adjusted R^2 values was cultivar-dependent and decreased with decreasing storage temperature, with the weakest correlation between I_{AD} and storage period found in fruit stored at 0°C.

ACKNOWLEDGEMENTS

This research is sponsored by Horticulture Innovation Australia (HIA) and DEDJTR Victoria as part of the "Profitable Stone fruit" and "Maximize Stone fruit Production Efficiency" projects from the "Food and Fiber" and "Fruit to Asia" initiatives with full support of the Victoria summer fruit industry.

Literature cited

Bonora, E., Stefanelli, D., and Costa, G. (2013). Nectarine fruit ripening and quality assessed using the index of absorbance difference (I_{AD}). Int. J. Agron. *2013*, 1–9 http://dx.doi.org/10.1155/2013/242461.

Bonora, E., Noferini, M., Stefanelli, D., and Costa, G. (2014). A new simple modeling approach for the early prediction of harvest date and yield in nectarines. Sci. Hortic. (Amsterdam) *172*, 1–9 http://dx.doi.org/10.1016/j.scienta.2014.03.030.

Costa, G., Noferini, M., Fiori, G., and Torrigiani, P. (2009). Use of Vis/NIR spectroscopy to assess fruit ripening stage and improve management in post-harvest chain. Fresh Produce *1*, 35–41.

Eccher Zerbini, P., Vanoli, M., Lovati, F., Spinelli, L., Torricelli, A., Rizzolo, A., and Lurie, S. (2011). Maturity

assessment at harvest and prediction of softening in a late maturing nectarine cultivar after cold storage. Postharvest Biol. Technol. *62* (*3*), 275–281 http://dx.doi.org/10.1016/j.postharvbio.2011.06.008.

Echeverría, G., Cantín, C.M., Ortiz, A., López, M.L., Lara, I., and Graell, J. (2015). The impact of maturity, storage temperature and storage duration on sensory quality and consumer satisfaction of 'Big Top®' nectarines. Sci. Hortic. (Amsterdam) *190*, 179–186 http://dx.doi.org/10.1016/j.scienta.2015.04.022.

Hale, G., Lopresti, J., Stefanelli, D., Jones, R., and Bonora, L. (2013). Using non-destructive methods to correlate chilling injury in nectarines with fruit maturity. Acta Hortic. *1012*, 83–89 http://dx.doi.org/10.17660/ActaHortic.2013.1012.4.

Infante, R. (2012). Harvest maturity indicators in the stone fruit industry. Stewart Postharvest Rev. *8* (*1*), 1–6 http://dx.doi.org/10.2212/spr.2012.1.3.

Infante, R., Meneses, C., and Predieri, S. (2008). Sensory quality performance of two nectarine flesh typologies exposed to distant market conditions. J. Food Qual. *31* (*4*), 526–535 http://dx.doi.org/10.1111/j.1745-4557.2008.00217.x.

Lavilla, T., Recasens, I., Lopez, M., and Puy, J. (2002). Multivariate analysis of maturity stages, including quality and aroma, in 'Royal Glory' peaches and 'Big Top' nectarines. J. Sci. Food Agric. *82* (*15*), 1842–1849 http://dx.doi.org/10.1002/jsfa.1268.

Lleó, L., Roger, J.M., Herrero-Langreo, A., Diezma-Iglesias, B., and Barreiro, P. (2011). Comparison of multispectral indexes extracted from hyperspectral images for the assessment of fruit ripening. J. Food Eng. *104* (*4*), 612–620 http://dx.doi.org/10.1016/j.jfoodeng.2011.01.028.

Lopresti, J., Hale, G., Stefanelli, D., Frisina, C., and Jaeger, J. (2016). Effect of physiological maturity at harvest and cool storage on chilling injury severity in peach and nectarine. Acta Hortic. *1120*, 157–164 http://dx.doi.org/10.17660/ActaHortic.2016.1120.23.

Lurie, S., Friedman, H., Weksler, A., Dagar, A., and Eccher Zerbini, P. (2013). Maturity assessment at harvest and prediction of softening in an early and late season melting peach. Postharvest Biol. Technol. *76*, 10–16 http://dx.doi.org/10.1016/j.postharvbio.2012.08.007.

Shinya, P., Contador, L., Predieri, S., Rubio, P., and Infante, R. (2013). Peach ripening: segregation at harvest and postharvest flesh softening. Postharvest Biol. Technol. *86*, 472–478 http://dx.doi.org/10.1016/j.postharvbio.2013.07.038.

Smit Consult (2015). http://www.smitconsult.nl/en/products/sigmaplot/.

Stefanelli, D., Lopresti, J., Hale, G., Jones, R., Tomkins, B., O'Connell, M., and Flett, S. (2016). Stone fruit value chain: a systems approach for improved consumer satisfaction. Acta Hortic. *1120*, 527–534 http://dx.doi.org/10.17660/ActaHortic.2016.1120.80.

Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., and Costa, G. (2008). A new index based on *vis* spectroscopy to characterize the progression of ripening in peach fruit. Postharvest Biol. Technol. *49* (*3*), 319–329 http://dx.doi.org/10.1016/j.postharvbio.2008.01.017.

The DA-Meter, from theory to practice

By Christine Frisina and Dario Stefanelli

DA-Meter technology will increase consumer satisfaction as a result of consistent fruit maturity.

The DA-Meter is a new technology that provides a rapid non-destructive method for assessing fruit maturity from orchard to market. Being non-destructive, the DA-Meter can monitor fruit maturity as it develops in the orchard to predict the optimum harvest date. Different maturity classes can be identified to optimize harvest timing to supply local and export markets of choice. In the packhouse, it will be possible to grade fruit according to its shelf life potential. Overall, the DA-Meter technology creates increased consumer satisfaction resulting from the supply of fruit with consistent maturity.

The DA-Meter uses spectroscopy to measure chlorophyll 'a' in the mesocarp (just below the skin) of the fruit through the difference in absorbance between 670 and 720 nm (index of absorbance difference, IAD). The best way to determine the physiological maturity of the fruit is to correlate IAD values with the fruit ethylene production rate. The correlation between the IAD and ethylene is cultivar dependent, but once the correlation is performed correctly, the resulting maturity classes should be independent of factors such as location, agronomical practices and growing season. These factors will influence the date in the growing season at which the fruit will reach a certain value but the relative ethylene production at the specific IAD value will not change.

DR DARIO STEFANELLI is is Team Leader at Fruit Physiology, Agriculture Research Division, Victorian Department of Environment and Primary Industries. He can be contacted on 03 9032 7373 or via email: dario.stefanelli@depi.vic.gov.au

Calibrating the DA-Meter for optimal maturity determination

The main challenge in determining fruit maturity classes is the correlation between IAD value and ethylene production as it requires sophisticated instrumentation, such as a gas chromatograph (GC) to measure ethylene. In general, this equipment is only available in fruit research laboratories. However, the correlation with ethylene production and the identification of the maturity classes is fundamental to the use of the DA-Meter and if not done will reduce the accuracy and effectiveness of the DA-Meter.

To enable owners of DA-Meters access to ethylene analyses, DEDJTR scientists developed a procedure for the collection of ethylene produced by the fruit that can then be sent to a central laboratory for measurement with GC (**see video: Introduction to Ethylene Sampling for Optimal Ripening Prediction with DA Meter and the ethylene sampling protocol below**). The procedure uses pre-evacuated vials for the easy and rapid collection of ethylene samples from fruit. Growers and other handling chain participants can collect the ethylene and ship it to a DEDJTR laboratory for analysis. Central processing of the ethylene samples

ETHYLENE SAMPLING PROTOCOL

The following equipment is required to sample fruit ethylene:

- Fruit pre-measured and labelled for IAD and grouped into 0.1 IAD value increments.
- Respiration chambers: Sealable air tight jars or containers between 500 mL and 1 L capacity depending on availability (see Appendix A)
- Evacuated vials: supplied by DEDJTR Horticultural Production Sciences team.
- 25 cc syringe: with 25G needle.
- Timer/clock.
- Scale to record fruit weight.
- Recording sheets

ABOVE: Equipment needed to perform the procedure of sampling fruit ethylene. A example of a glass chamber of approximately 1L volume.
LEFT: Example of plastic chamber of approximately 1L volume.

Download
Ethylene collection recording sheet

See Video
How to sample ethylene from stonefruit

makes the process less costly and allows for systematic identification of maturity classes for each cultivar. Measurements of IAD with the DA-Meter must be taken immediately prior to the collection of ethylene samples so that IAD can then be used to identify maturity classes.

For each cultivar tested, ethylene measurement should be conducted at IAD values between 0.2 and 1.7 for stone fruit and between 0.2 and 1.9 for pome fruit. Best results are achieved if ethylene is measured at IAD increments of 0.1. At least 5 individual fruit are required for each ethylene production measurement. This procedure will allow the creation of cultivar specific graphs correlating IAD and ethylene production from which it will be possible to accurately identify maturity classes (Fig. 1).

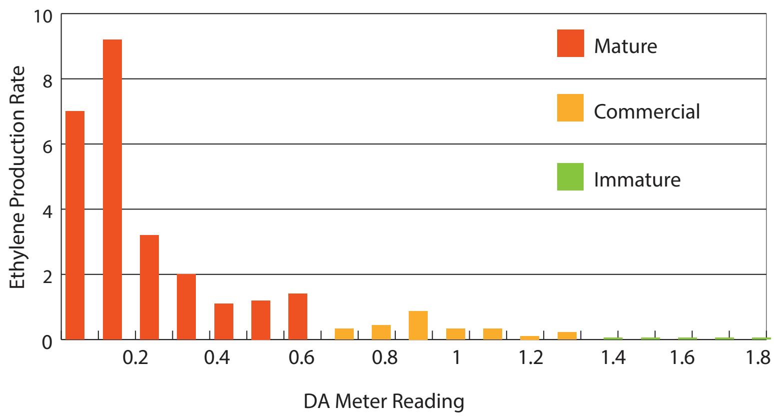


Figure 1. Nectarine Autumn Bright ethylene production and segregation in maturity classes of similar ethylene behaviour correlated to DA values. Maturity classes were: Mature = ripe (climacteric fruit), Commercial = usual maturity at commercial harvest (On-set of climacteric) and Immature = unripe (pre-climacteric fruit).

> "It is possible to sample more than one cultivar at the same time but the samples must be correctly labelled for each cultivar and IAD value."

Fruit selection

IAD monitoring for harvest prediction date should start at least 4 – 5 weeks prior to harvest and be performed at least weekly. It is important to find the IAD values corresponding to pre-climacteric, on-set of climacteric and post-climacteric fruit development. This will ensure the identification of fruit at high IAD values, corresponding to fruit not producing ethylene (Pre-climacteric). Fruit selection should continue as the IAD values decrease with at least 5 fruit for each 0.1 IAD increment (i.e. 0.80 and 0.89 would be placed in the 0.8 group) until fruit between IAD values of 1.7 to 0.2 are measured for ethylene production. This should result in a minimum of 75 fruit sampled for ethylene. It is important that fruit are free from any blemish, wounds, disease, pest damage or bruise as it will affect ethylene production rate. The 5 fruit for each 0.1 IAD increment do not have to be collected on the same day but can be spread across sampling times. It is most important that a minimum of 5 fruit are collected for each 0.1 IAD increment.

It is possible to sample more than one cultivar at the same time but the samples must be correctly labelled for each cultivar and IAD value. Sampling more than one cultivar at a time does not increase the overall time commitment by much but it increases the number of chambers to be used at each single time or it requires multiple samplings with a fixed number of chambers to be able to collect from 5 fruit in each 0.1 IAD group.

Fruit selection can be performed in two ways:

- Harvest minimum of 100 fruit at random into cardboard trays with single fruit liners to separate fruit and minimise bruising; fruit should be collected at shoulder height and from multiple trees, usually no more that 8 – 10 fruit/tree. Measure and write the IAD value on each fruit with a permanent marker. Segregate the fruit in groups within the same 0.1 of IAD increments with a minimum of 5 fruit for each increment from 1.7 to 0.
- Measure fruit with the DA-Meter on the tree. Write the IAD value on the fruit with a permanent marker. Select a minimum of 100 random fruit at shoulder height and from multiple trees, usually no more that 8 – 10 fruit/tree. Harvest the fruit that will be needed to segregate the fruit in groups within the same 0.1 of IAD increments with a minimum of 5 fruit in each group (from 1.7 to 0.2) and place them in cardboard trays with single fruit liners to separate fruit and minimise bruising.

> "The DA meter is an innovative instrument for non-destructively determining a fruit maturity index by measuring the decline in chlorophyll content immediately below the skin during ripening."

Ethylene sampling steps

1. Print or have sample record sheet on computer/tablet open and ready to edit.
2. Label the fruit numerically (e.g. 1 – 20).
3. Record the IAD, the weight of each fruit and the volume of the chamber in which the fruit goes.
4. Place each fruit into its allocated chamber. Seal all the chambers at once and record the time, in hours and minutes. A chamber with no fruit in it should also be sealed at this time to determine if there are any background ethylene contaminations that will affect the results.
5. The chambers should be left for 3 to 4 h in the shade

or inside to allow the accumulation of any ethylene production. It is important that chambers are never exposed to direct sunlight.

6. While this time elapses, label the evacuated vials that match the fruit in the chambers.
7. Include grower/orchard identification, sample type (cultivar), fruit number, chamber label and collection date.
8. After the time has elapsed, sample the chamber using a 25 cc syringe fitted with a 25G needle.
9. Insert the needle through the silicon seal into the chamber as far as it can go.
10. Slowly pump the syringe 3 – 5 times to create air movement inside the chamber stirring the contents.
11. Draw 20 cc into the syringe and remove the syringe from the chamber. Insert the needle into the correspondingly labelled vial. The syringe should release most of its contents automatically as the vial is evacuated.
12. When the syringe plunger stops moving gently push down the plunger forcing the remaining contents into the vial. While holding the plunger down, remove the needle from the vial.
13. Store the vials at room temperature in the dark.
14. Send the vials with a copy of the relevant sample recording sheet to DEDJTR for ethylene analysis.

Example of recording sheet

Fruit	Jar #	Jar Volume (ml)	DA Meter Reading	Fruit Weight (g)	Jar Closing Time (hh:mm)	Ethylene Collection Time (hh:mm)
1	45	1156	0.87	136	1.00pm	4:05 pm
2	12	1150	0.82	141	1.02 pm	4.08 pm

HOW TO BUILD RESPIRATION CHAMBERS

- Size preference is 1L capacity, glass or gas tight plastic; depending on availability chamber volume could be between 500 mL to 1L. Most important is that the chamber opening is large enough to insert and remove large fruit easily.
- Exact volume must be determined (see below).
- Each container must contain at least one hole filled with regular glass, all purpose silicone sealant, neutral cure (see picture 2). Note: Sealant will require a minimum of 24 – 48 h curing time. Do not use any other type of silicon sealant as they contain compounds that will be detected by the instrumentation (GC) used to determine ethylene presence and concentration, and therefore will interfere with the analysis of the samples.
- Label each chamber and the corresponding lid, numerically or other, for ease of recording and re-using the volume information against the individual chamber.

Example of silicone to be used.

DETERMINING THE SAMPLING JAR VOLUME

This is done by weighing the total amount of water required to fill both base and lid of the chamber. This should only need to be done once for each chamber. While the totals will be similar for most of the chosen chambers sometimes there are differences large enough to alter the results so it is important to do this for every chamber used.

EQUIPMENT:

1. a balance or set of scales, accurate to 0.1 g is preferred
2. worksheet

Download
Jar volume recording sheets

See Video:
How to determine the volume of the sampling jar

STEPS:

1. Record the chamber and lid labels.
2. Separate lid from base of chamber.
3. Place base and lid on the balance and record the weight.
4. Remove the lid, pour water into the base until is completely full (to the brim); try to avoid any spillage onto the scales as this will add to weight. Replace the lid upside down on top of the base (still containing water) and fill the lid with water.
5. Record the total weight (base and lid).
6. Repeat from step 1 for all remaining chambers.
7. Record this value for the specific chamber and keep this information for reporting with the sample information each time the chambers are used.

Acknowledgement

The procedure was developed as part of the project SF15001 "Comparing Stonefruit ripening, quality and volatile composition" which is funded by Horticulture Innovation Australia Limited using the summerfruit levy and funds from the Australian Government with co-investment from the Department of Economic Development, Jobs, Transport and Resources.

Additional information

At the time of writing, the cost of the ethylene analysis per cultivar is estimated at $1,250 including supply of syringe, needles and pre-evacuated vials but not the chambers.

The DA-Meter can be sourced through **Summerfruit Australia Limited.**

Growers or organisations interested in the identification of maturity classes should contact Dr Dario Stefanelli (**dario.stefanelli@ecodev.vic.gov.au**) or Mrs Christine Frisina (**christine.frisina@ecodev.vic.gov.au**) at AgriBio building, 5 Ring Road, Bundoora, VIC 3083.

The full downloadable protocol with explanatory videos can be also found on the Horticulture Industry Network website **http://www.hin.com.au/projects/stonefruit-field-laboratory/da-meter/ethylene-sampling-protocols/_nocache** information about beekeeping and pollination. **Subscribe before 3 July 2015 and you will go into the draw to win a range of honey bee and pollination manuals and publications**.

According to PHA's honey bee specialist, **Sam Malfroy**, *BeeAware* provides all of the information that growers need to know about pollination: how it works, use of pesticides, pollination agreements, how to prepare for Varroa mite and how to promote a healthy pollinator ecosystem in a farm or orchard.

"It's also a great place to learn more about honey bees, their biology, and the pests and diseases which affect them, Mr Malfroy said.

A wide variety of crops including almonds, passionfruit, apples and pears, berries, cherries, stonefruit, melons, avocados and some vegetables are known to benefit from pollination by honey bees. Broadacre crops such as faba beans, sunflowers and canola also receive major benefits from honey bee pollination. *BeeAware* explains exactly how growers can gain maximum benefit from these helpful insects and receive valuable yield and quality improvements in the produce that they grow.

"Each crop has a page of its own," added Mr Malfroy, "which details the pollination requirements of the crop as well as giving useful links and fact sheets from Australia and around the world."

Honey bee and pollination books up for grabs

Subscribers who sign up to the *BeeAware* newsletter before 3 July go into the draw to win a copy of the highly sought after RIRDC publications which cover major areas such as honey bee biology, pests and diseases and crop pollination in Australia. "These books are a must-have resource for any beekeeper or grower in Australia," Mr Malfroy said.

The site was developed by a partnership between the Australian Government, the honey bee industry and pollinator-reliant industries through the Pollination Program which is managed by the Rural Industries Research and Development Corporation and Horticulture Innovation Australia. **Visit the *BeeAware* website at** www.beeaware.org.au

---

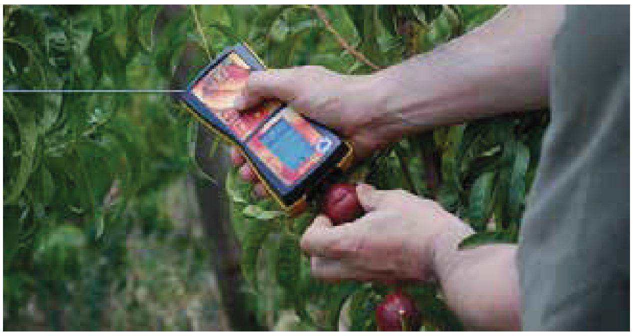
Industry News …

Determination of stonefruit maturity, quality and volatile composition

Comparing Stonefruit Maturity, Quality and Volatile Composition - Project

Variable quality in summerfruit has been identified as a major impediment to producer profitability and sales in both domestic and export markets. The ability to optimize fruit maturity at harvest to meet expectations of the market of choice; to understand how fruit maturity affects storage and the impact on fruit composition and quality, particularly sugar content, firmness and volatiles profile, will greatly improve fruit consistency and quality.

DA-Meter Measuring harvest maturity



Dario Stefanelli

Variable maturity at harvest affects fruit responses to shelf life, cold storage and consequent quality through the handling chain. Fruit composition and flavour volatiles in particular, are a fundamental element of fruit quality and therefore consumer acceptance. The fruit volatile profile is affected by fruit maturity on the tree, during postharvest storage and consequent ripening.

This project aims to generate knowledge of the interaction between harvest maturity measured by the DA-Meter, storage and ripening behaviour and resultant effects on soluble solids content (SCC i.e. brix - fruit sweetness index), firmness and volatiles profile. This project is linked to the stonefruit trial orchard in Tatura and to 'optimal ripening protocols' to which will deliver preliminary data on the varieties used and will add important information for the protocols.

This project will identify maturity classes to optimize harvest timing depending on the market of choice, with particular emphasis to export, for up to 10 nectarine and peach varieties. The project will generate cold storage and ripening protocols. The results will provide knowledge to optimise fruit quality and consistency on domestic and export markets. Consistent, high quality fruit will increase consumer demand and provide a point of difference for Victorian stonefruit increasing both domestic and export markets by at least 5% equating to an increase in export value alone of $15 to $20 million per annum.

Industry News …

Help is now available for beekeepers trying to negotiate access to public land – a vital source of pollen and nectar for the bees that provide essential pollination services to agriculture.

Around 70 per cent of Australian honey production comes from native flowers, many of which are prolific on public lands, but access is increasingly being restricted in state forests and national parks because European honey bees are not considered native to Australia.

A series of fact sheets have been developed to identify the registration, permit and/or licensing requirements for beekeepers seeking access to public lands in each state, as well as the restrictions and criteria for interstate movement of hives and equipment.

The fact sheets will not only help beekeepers better understand the rules and regulations that impact where they can place their hives, but will also allow them to be better informed when seeking permission for the relocation or movement of hives in locations that require government agency approval.

The series of fact sheets and a full report were developed through the Honey Bee and Pollination RD&E Program, which is funded by RIRDC and Horticulture Innovation Australia Limited (HIA).

Beekeeper and spokesman for the Program's Advisory Panel **James Kershaw** says given the level of complexity, beekeepers should take full advantage of the research in order to ensure their operation's sustainability.

"If the bees are not in good health, it's harder to put their pollination services to work, and many horticultural and agricultural products rely on European honey bee pollination," Mr Kershaw said.

To download the fact sheets or for more information about the Honey Bee and Pollination RD&E Program, go to www.rirdc.gov.au/honeybee-pollination.

Source: Rural Diversity in Brief – News from the Rural Industries Research & Development Corporation – 5 May 2015 Issue

RURAL DIVERSITY in brief

Australian Government
Rural Industries Research and Development Corporation

Economic Development, Jobs, Transport and Resources

Fruit Maturity Variability and Correlation with Ethylene

Dario Stefanelli
Christine Frisina

AGRICULTURE VICTORIA

Stonefruit Field Laboratory fruit maturity

Economic Development, Jobs, Transport and Resources

Horticulture Innovation Australia

VICTORIA State Government

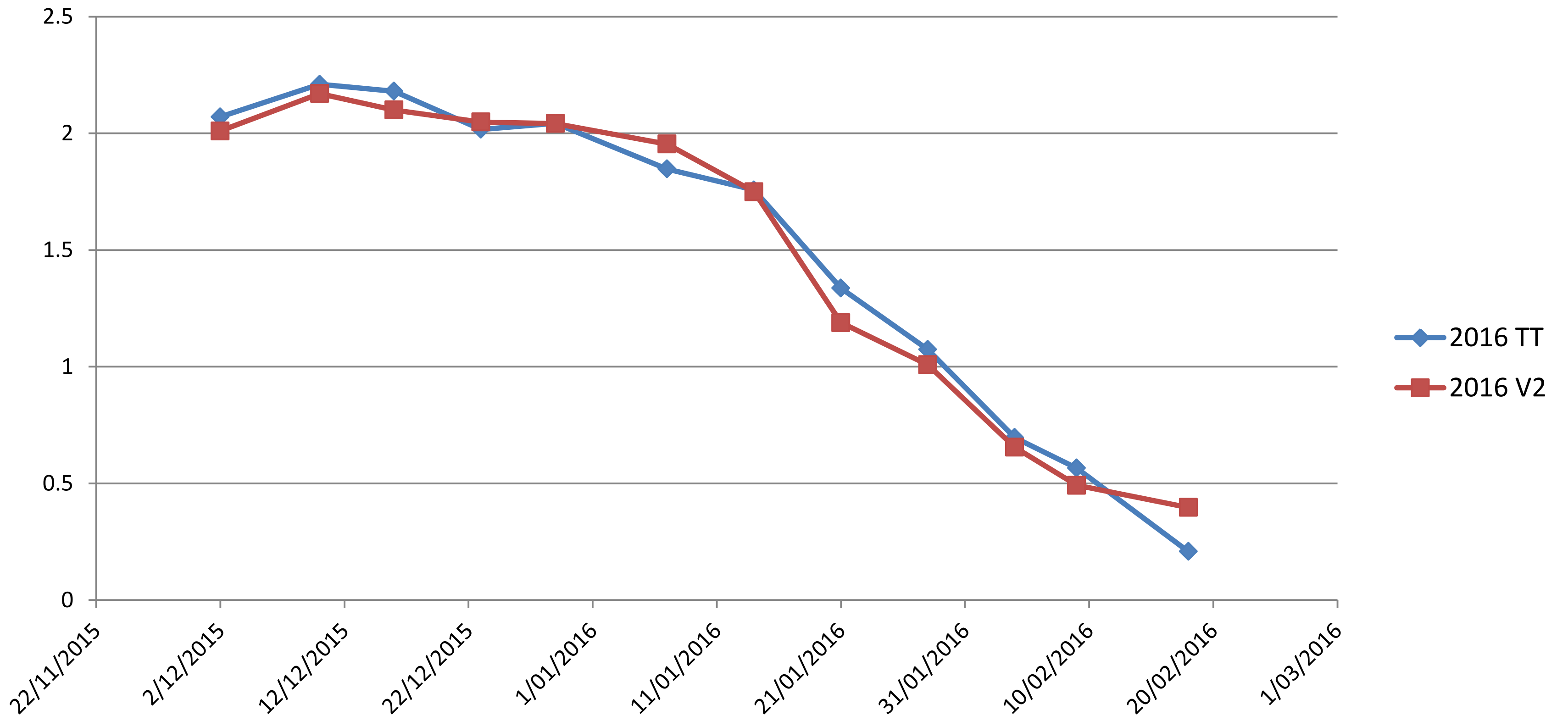
Summerfruit AUSTRALIA

JRE VICTORIA

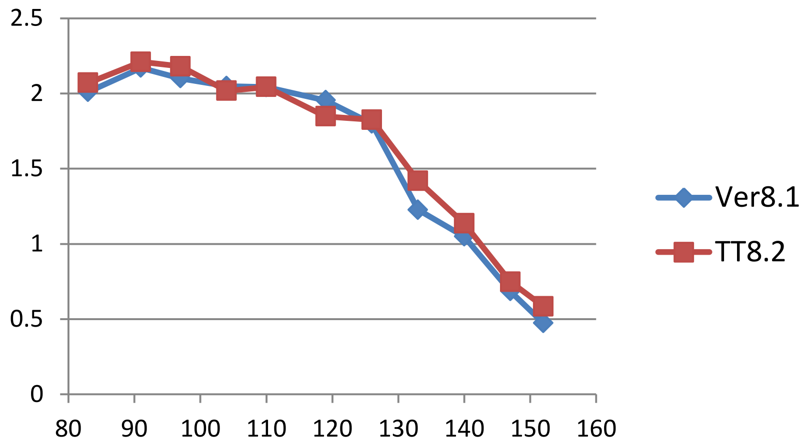
Maturity field monitoring with DA-Meter

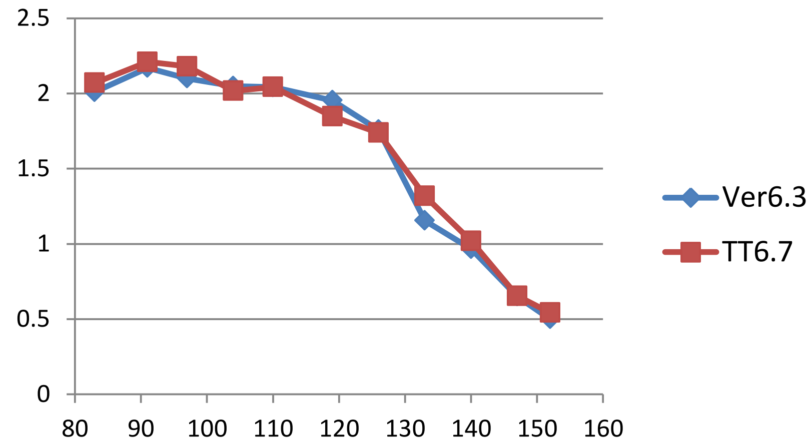
- Started 6-7 weeks prior to harvest
  - Weekly measurements
  - 80-100 fruit random from whole orchard by canopy system
- 4 weeks prior to harvest
  - Separation top, bottom, east and west side of canopy
  - Separation by crop load (high, medium, low)
  - 12 fruit/tree
  - total of 96 fruit each crop load/canopy system/variety

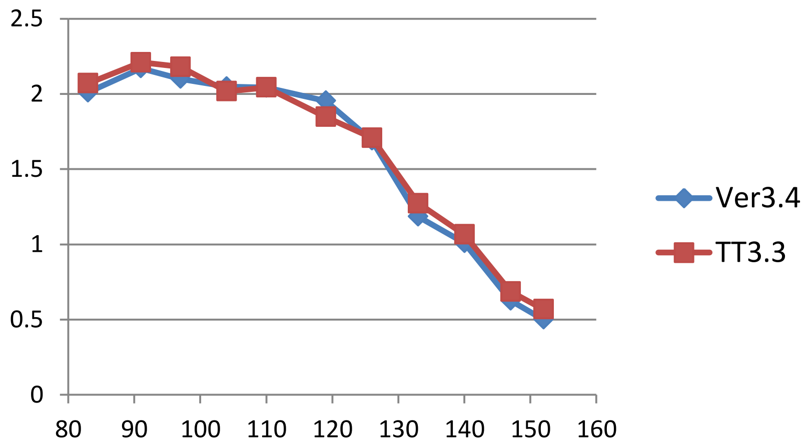
Autumn Bright nectarine I_{AD} field development



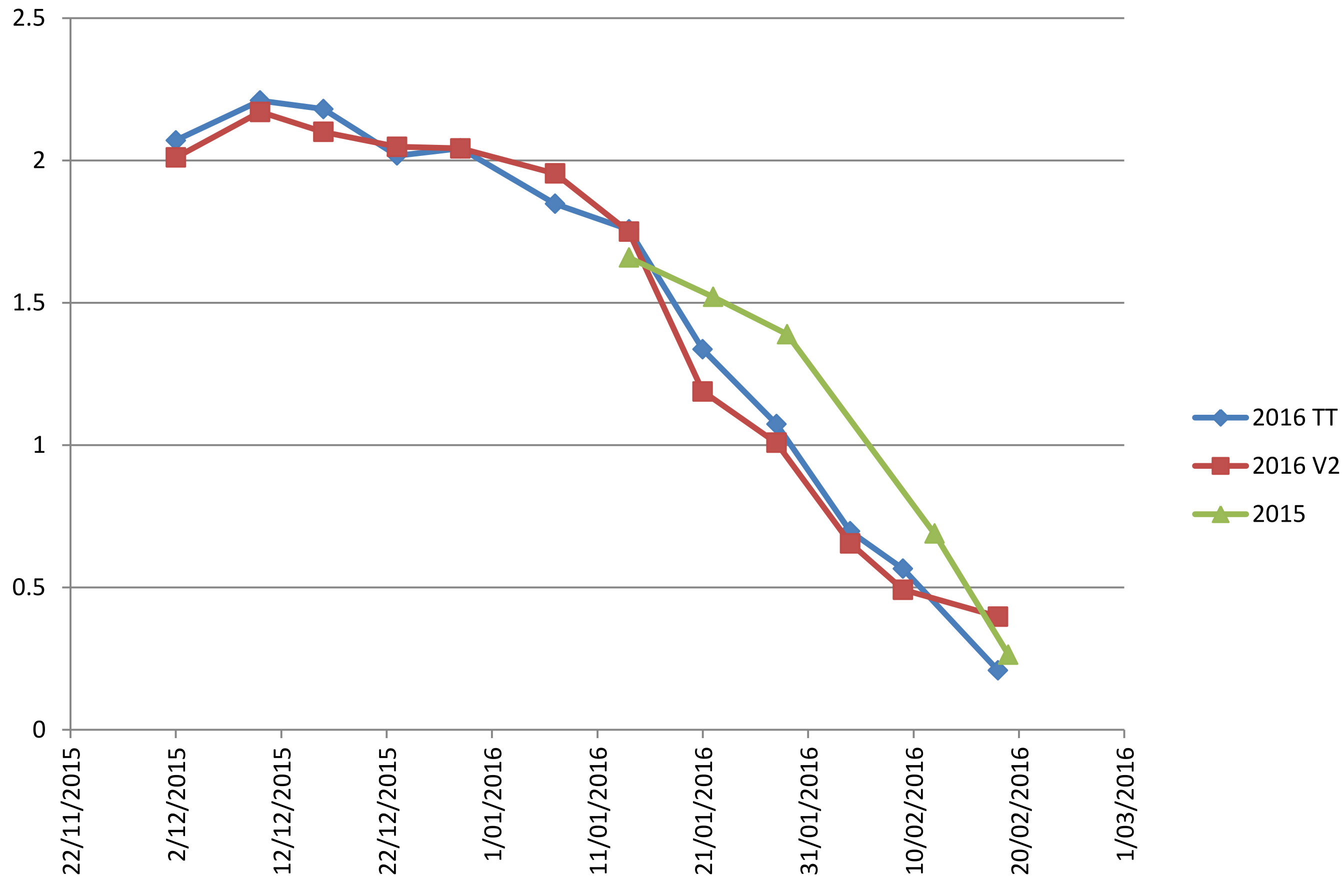
Autumn Bright by crop load in fruit/cm^2



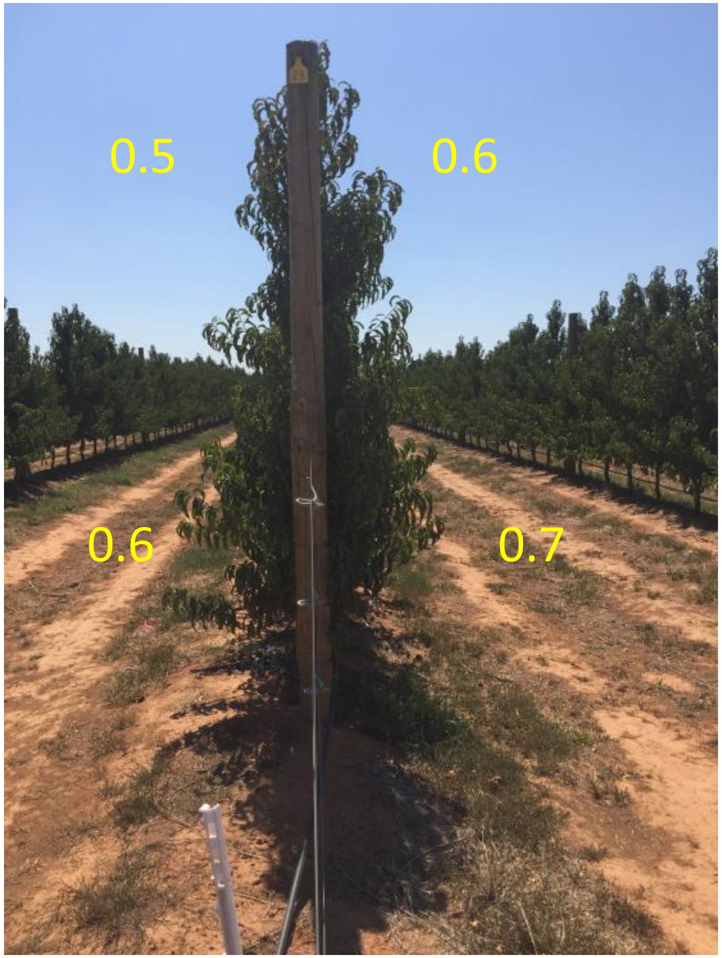


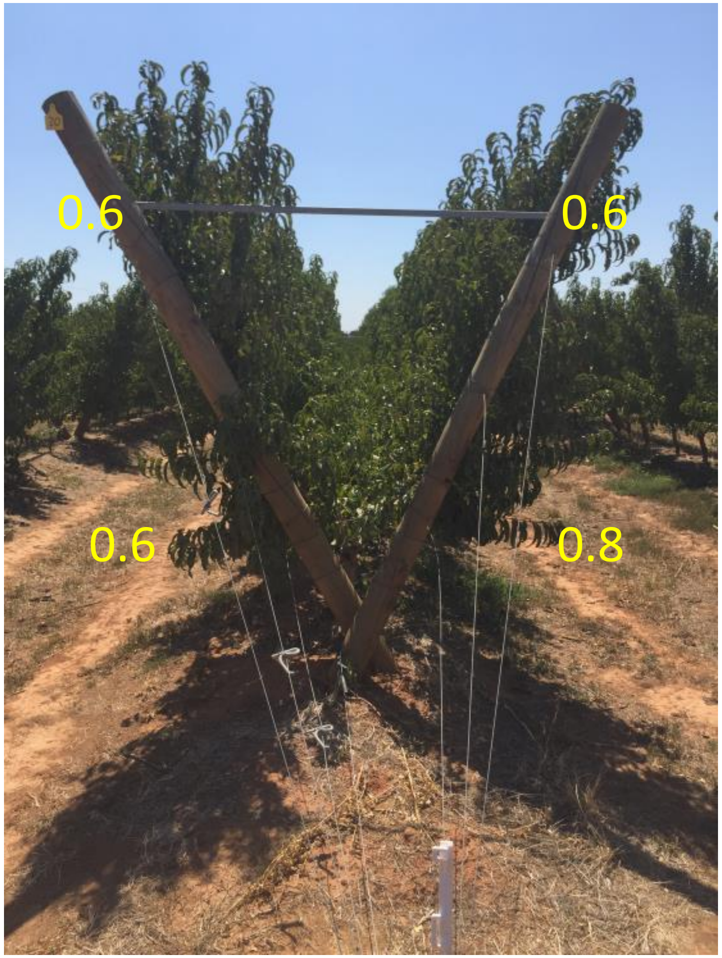


Autumn Bright nectarine I_{AD} field development



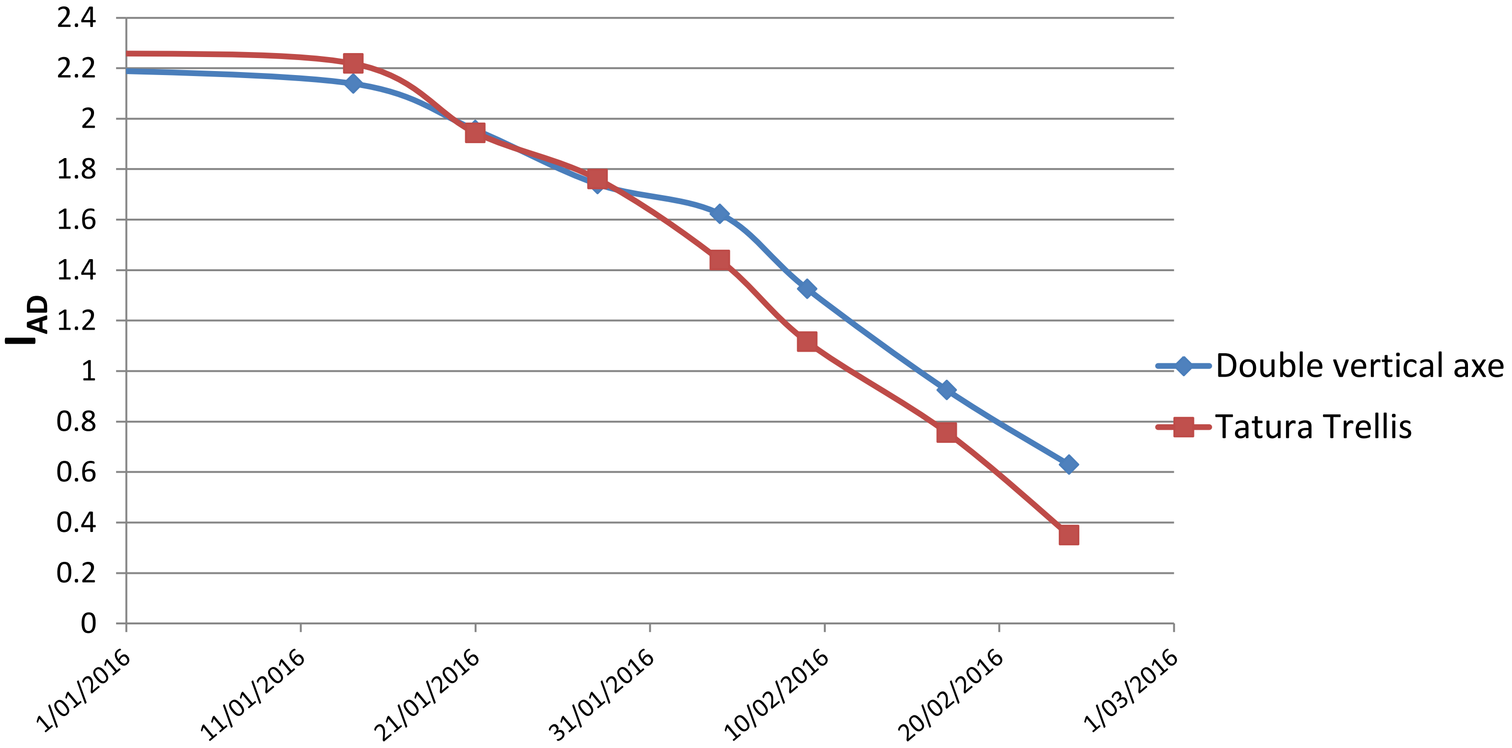
Position X Canopy Experiment



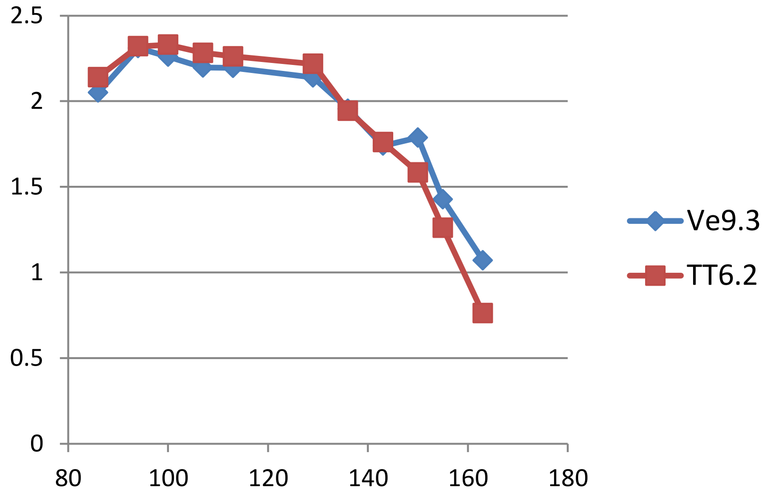


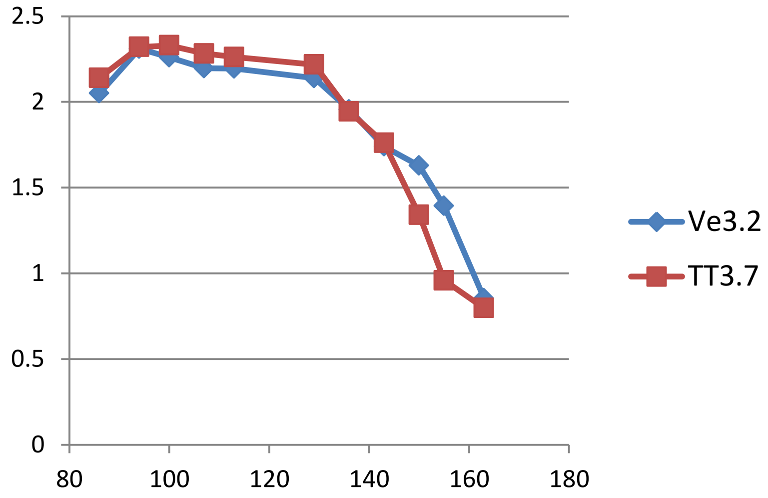
Nectarine 'Autumn Bright'

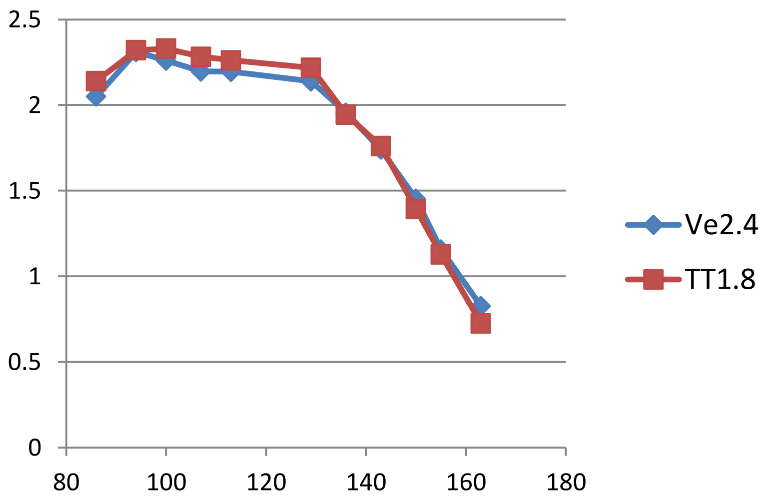
August Flame peach I_{AD} field development



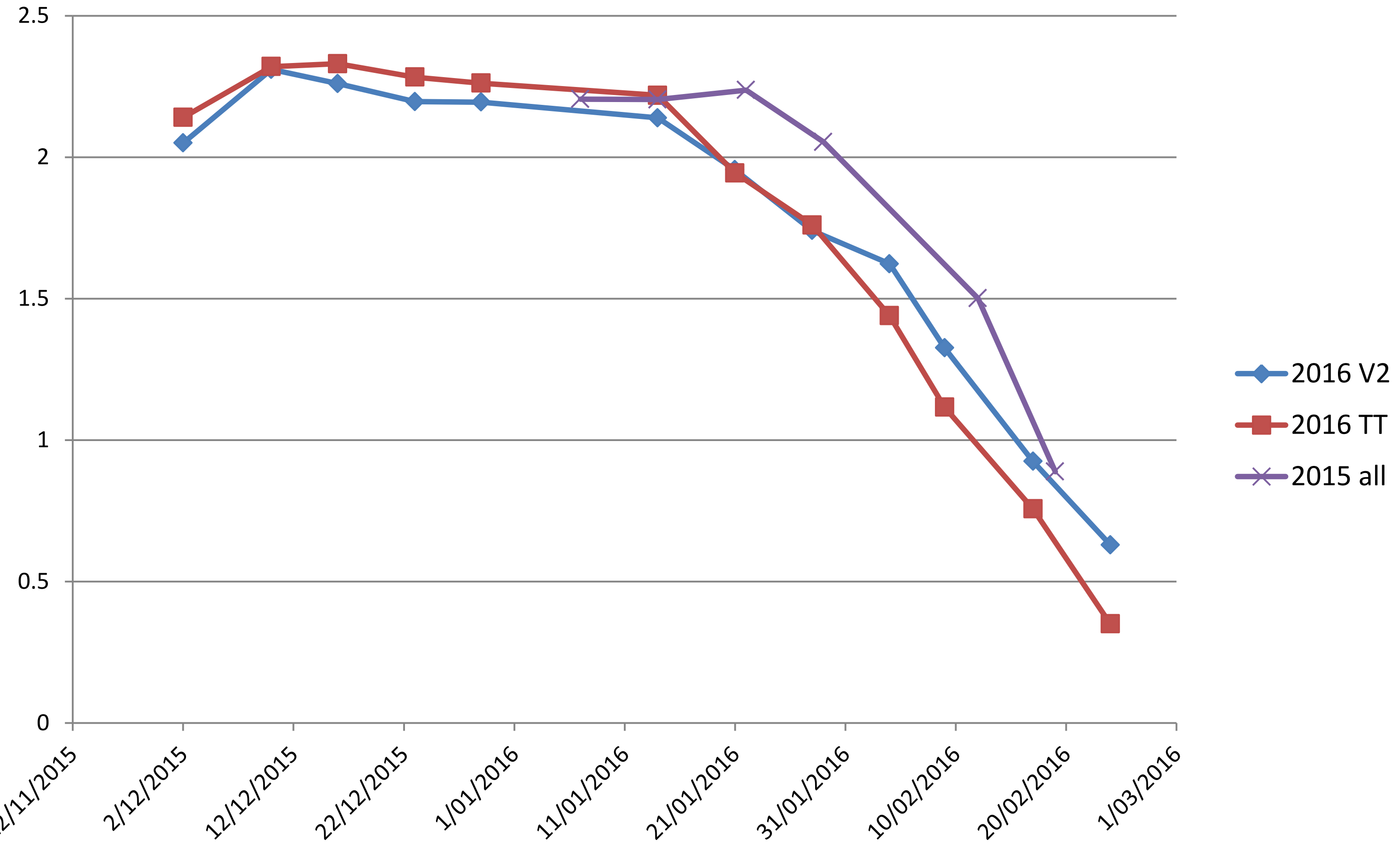
August Flame by crop load in fruit/cm^2







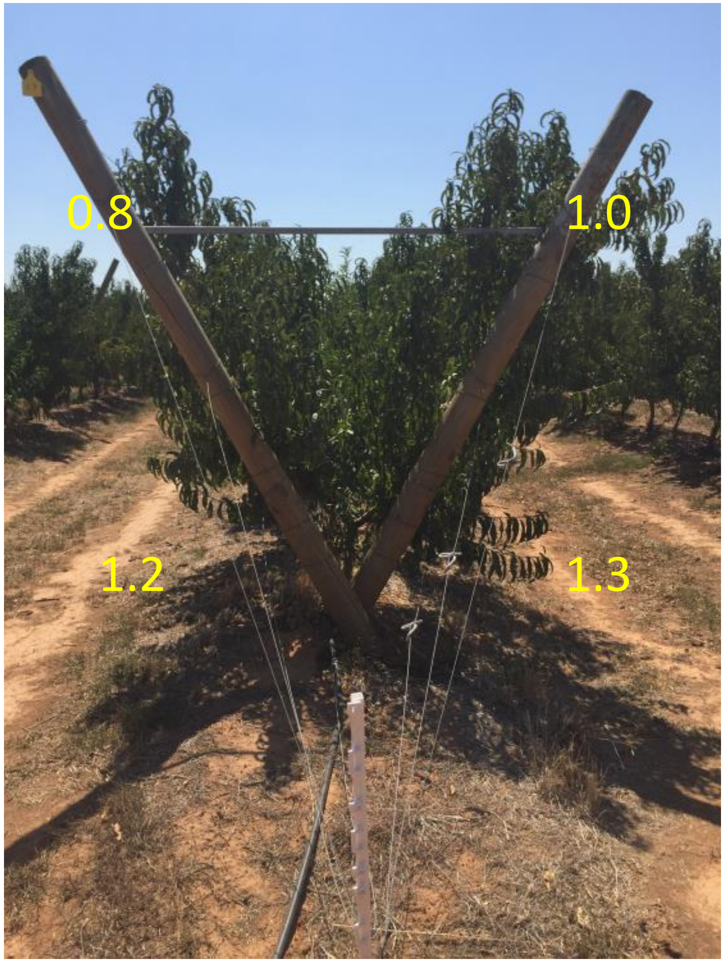
August Flame peach I_{AD} field development



Economic Development,
Jobs, Transport
and Resources

AGRICULTURE VICTORIA

Position x Canopy experiment



Peach ‘August Flame’

Field monitoring summary

- Important to understand and predict optimal harvest
- Variety plays an important role in possible variability:
  - Canopy training system
  - Crop load
  - Canopy position
- More monitoring = more understanding = better planning

I_{AD} in Pre- and Post-Harvest
Optimal functionality with identification of maturity classes

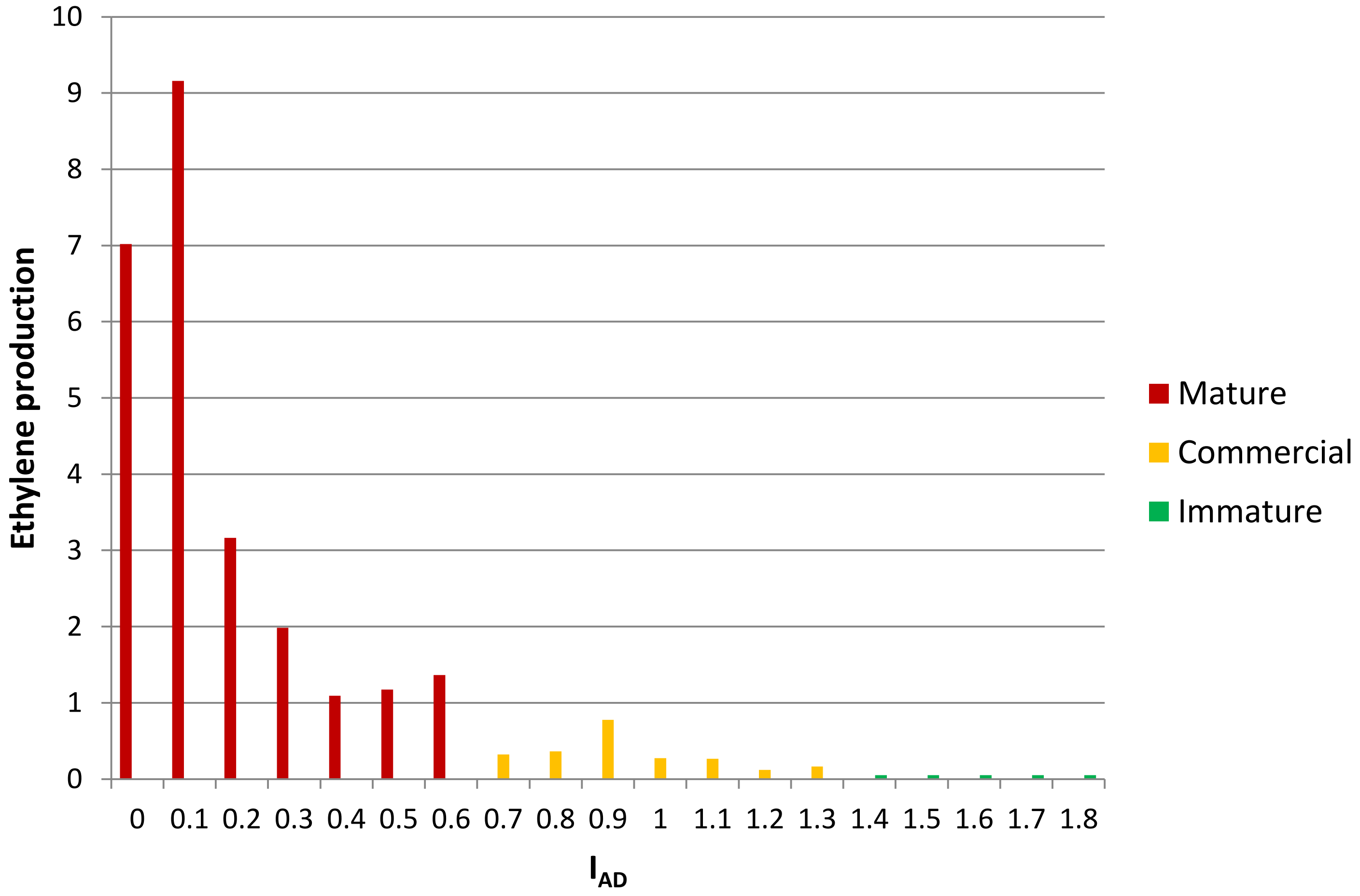
Correlation with ethylene

Maturity classes (optimal harvest – Market Specific)

Variety Specific

Can be done in the field (New protocol)

Autumn Bright I_{AD}-Ethylene correlation



I_{AD}-Ethylene correlation Protocol: Fruit collection

- Measure fruit with the DA-Meter on the tree. Write the I_{AD} value on the fruit with a permanent marker. Select a minimum of 100 random fruit at shoulder height and from multiple trees, usually no more that 8 – 10 fruit/tree. Harvest the fruit that will be needed to segregate the fruit in groups within the same 0.1 of I_{AD} increments (i.e. from 0.80 to 0.89 = 0.8 group) with a minimum of 5 fruit in each group (from 1.7 to 0.2) and place them in cardboard trays with single fruit liners to separate fruit and minimise bruising.

I_{AD}-Ethylene correlation Protocol: Sampling steps

1. Print or have sample record sheet on computer/tablet open and ready to edit.
2. Label the fruit numerically (e.g. 1 – 20).
3. Record the I_{AD}, the weight of each fruit and the volume of the chamber in which the fruit goes.
4. Place each fruit into its allocated chamber.
5. Seal all the chambers at once and record the time, in hours and minutes. A chamber with no fruit in it should also be sealed at this time to determine if there are any background ethylene contaminations that will affect the results.
6. The chambers should be left for 3 to 4 h in the shade or inside to allow the accumulation of any ethylene production. It is important that chambers are never exposed to direct sunlight.
7. While this time elapses, label the evacuated vials that match the fruit in the chambers.
8. Include grower/orchard identification, sample type (cultivar), fruit number, chamber label and collection date.

I_{AD}-Ethylene correlation Protocol: Sampling steps

9. After the time has elapsed, sample the chamber using a 25 cc syringe fitted with a 25G needle.
10. Insert the needle through the silicon seal into the chamber as far as it can go.
11. Slowly pump the syringe 3 – 5 times to create air movement inside the chamber stirring the contents.
12. Draw 20 cc into the syringe and remove the syringe from the chamber. Insert the needle into the correspondingly labelled vial. The syringe should release most of its contents automatically as the vial is evacuated.
13. When the syringe plunger stops moving gently push down the plunger forcing the remaining contents into the vial. While holding the plunger down, remove the needle from the vial.
14. Store the vials at room temperature in the dark.
15. Send the vials with a copy of the relevant sample recording sheet to DEDJTR for ethylene analysis.

Materials required

- Fruit pre-measured and labelled for I_{AD} and grouped into 0.1 I_{AD} value increments.
- Respiration chambers: between 500 mL and 1 L capacity
- Evacuated vials
- 25 cc syringe: with 25G needle.
- Timer/clock.
- Scale to record fruit weight.
- Recording sheets

Vial sampling Recording Sheet

- **Grower:**
- **Cultivar: O'Henry**
- **Date: 16/2/2016**

Fruit #	Jar #	Jar Volume (ml)	DA Meter reading	Fruit Weight (g)	Jar Closing Time (hh:mm)	Ethylene Collection Time (hh:mm)
1	45	1156	0.87	136	1.00pm	4:05 pm
2	12	1127	0.82	141	1.02 pm	4.08 pm

How to build the jars

- Size preference is 1L capacity, glass or gas tight plastic; depending on availability chamber volume could be between 500 mL to 1L. Most important is that the chamber opening is large enough to insert and remove large fruit easily.
- Exact volume must be determined.
- Each container must contain at least one hole filled with regular glass, all purpose silicone sealant, neutral cure. Note: Sealant will require a minimum of 24 – 48 h curing time. Do not use any other type of silicon sealant as they contain compounds that will interfere with the analysis of the samples.
- Label each chamber and the corresponding lid for ease of recording and re-using the volume information against the individual chamber.

Example of silicone

Jar volume determination protocol

1. Record the chamber and lid labels.
2. Separate lid from base of chamber.
3. Place base and lid on the balance and record the weight.
4. Remove the lid, pour water into the base until is completely full (to the brim); try to avoid any spillage onto the scales as this will add to weight. Replace the lid upside down on top of the base (still containing water) and fill the lid with water.
5. Record the total weight (base and lid).
6. Repeat from step 1 for all remaining chambers.
7. Record this value for the specific chamber and keep this information for reporting with the sample information each time the chambers are used.

Jar Volume recording sheet

Jar #	Empty Jar Weight (g)	Jar weight with water (g)	Jar Volume (gg) Jar Volume = Jar weight with water – Empty Jar Weight
45	2000	3150	1150
12	1856	2983	1127

Additional info

- Cost of the ethylene analysis per cultivar is estimated at $1,250 including supply of syringe, needles and pre-evacuated vials but not the chambers.
- The DA-Meter can be sourced at Summerfruit Australia .
- Contact Dr Dario Stefanelli (dario.stefanelli@ecodev.vic.gov.au) or Mrs Christine Frisina (christine.frisina@ecodev.vic.gov.au) at AgriBio building, 5 Ring Road, Bundoora, VIC 3083.
- The full downloadable protocol with explanatory videos can be also found on the Horticulture Industry Network website (http://www.hin.com.au/projects/stonefruit-field-laboratory/da-meter/ethylene-sampling-protocols/_nocache)

Thanks to:

<u>Horticulture Team Tatura:</u>
Dave Haberfield
Mark O'Connell
Jim Selman (casual)

<u>AgriBio:</u>
Dario Stefanelli
Bruce Tomkins
Christine Frisina

AGRICULTURE VICTORIA
Economic Development, Jobs, Transport and Resources

Horticulture Innovation Australia

Summerfruit AUSTRALIA

Economic Development,
Jobs, Transport
and Resources

Picking fruit for optimal flavour and storage

Harvest maturity impacts on fruit aroma during storage and marketing

Christine Frisina &
Dario Stefanelli

AGRICULTURE VICTORIA

Stonefruit Maturity, Quality & Composition

Research to optimize fruit quality for domestic and export market

Problem

High variability fruit quality at harvest : → Consumer dissatisfaction

- Maturity at harvest affects shelf life and storage period
- Cold storage affects fruit quality and composition

Approach

Use the Stonefruit Field Laboratory at Tatura to determine harvest maturity classes to optimise fruit quality and composition during field ripening and after cold storage

Ethylene for maturity classes and VOCs collection based on I_{AD} during fruit development

+

Fruit quality and VOCs as affected by cold storage and shelf life

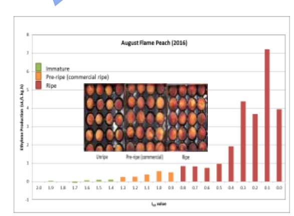
+

Biochemical analysis by GC and GC-MS

Stonefruit growers with knowledge to manage fruit quality:

- ✓ **Optimal harvest maturity for consumer preference**
- ✓ **Maturity class database for grower decision making**
- ✓ **Optimize cold storage and ripening protocols**

Ethylene protocol adopted



integrity FRUIT

Maturity classes adoption

Economic Development, Jobs, Transport and Resources

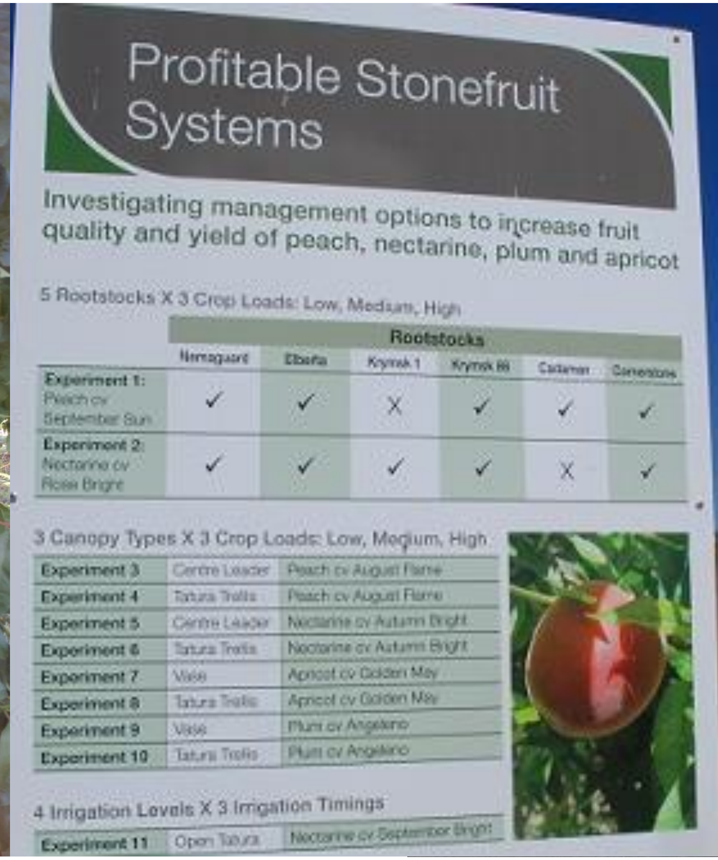
⇒ ***Deliver high quality fruit to domestic and export markets***

⇒ ***Improve marketability and increase profitability***

AGRICULTURE VICTORIA

Stonefruit Maturity, Quality & Composition

Funding and support



Hort Innovation
Strategic levy investment

SUMMERFRUIT FUND

RMIT UNIVERSITY

Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Background

Aromatic Volatile Organic Compounds

Consumer perception of poor quality fruit because doesn't smell peachy/fruity

Why?

What factors may affect fruit aroma?

- Maturity stage at harvest
- Shelf life
- Cold storage

From previous research:
Identified 9 key aroma compounds reported for whole fruit aroma

More than 90% of previous work has been done on bulked samples.

Individual fruit; I_{AD} as maturity index;

Maturity stage at harvest

Fruit were picked and checked for I_{AD} using a DA meter
(most immature on left, more mature on right)

Profiling or ethylene and volatiles:
Fruit were harvested across several days for each cultivar.

All fruit used were also evaluated for flesh firmness and soluble solid concentration.

'September Bright' nectarine (2016-17)

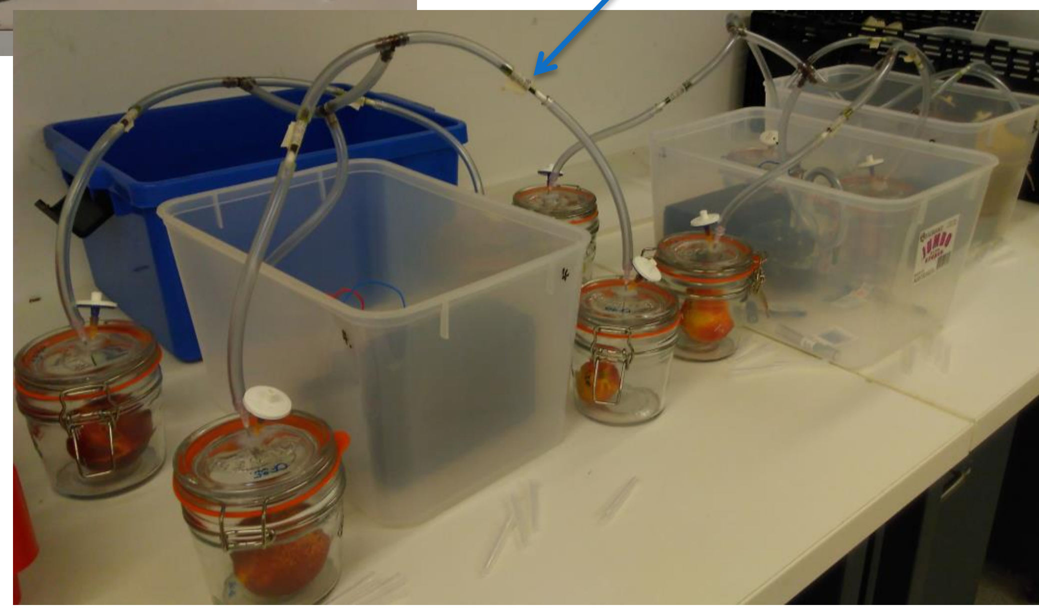
Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Maturity stage at harvest

Evacuated vial

Charcoal trap



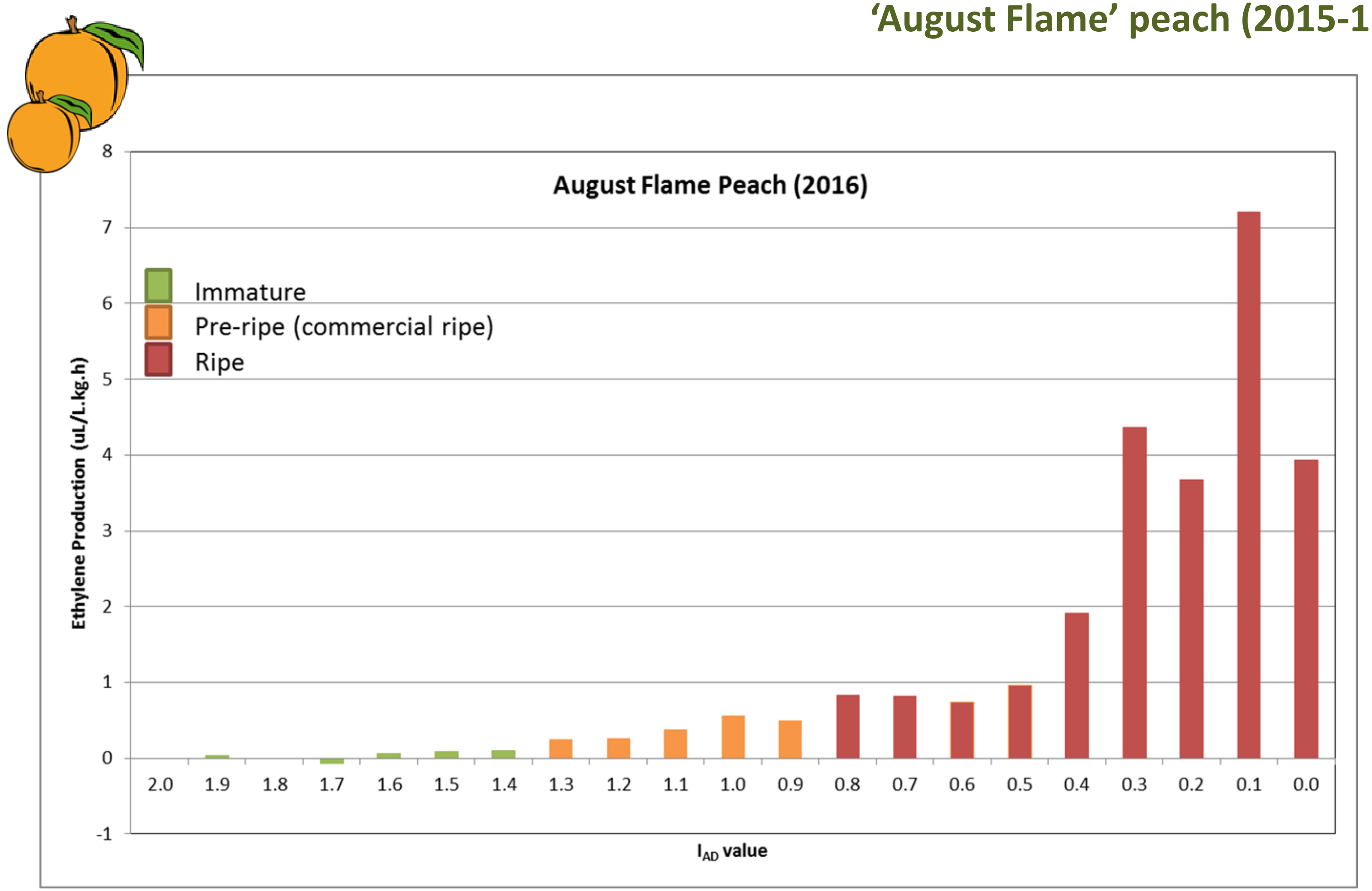
Ethylene and aromatic volatile sampling of individual fruit.

Evacuated vials were used to collect ethylene samples for later measurement by GC.

Charcoal traps were used to collect aromatic volatile samples for later measurement by GC-MS.

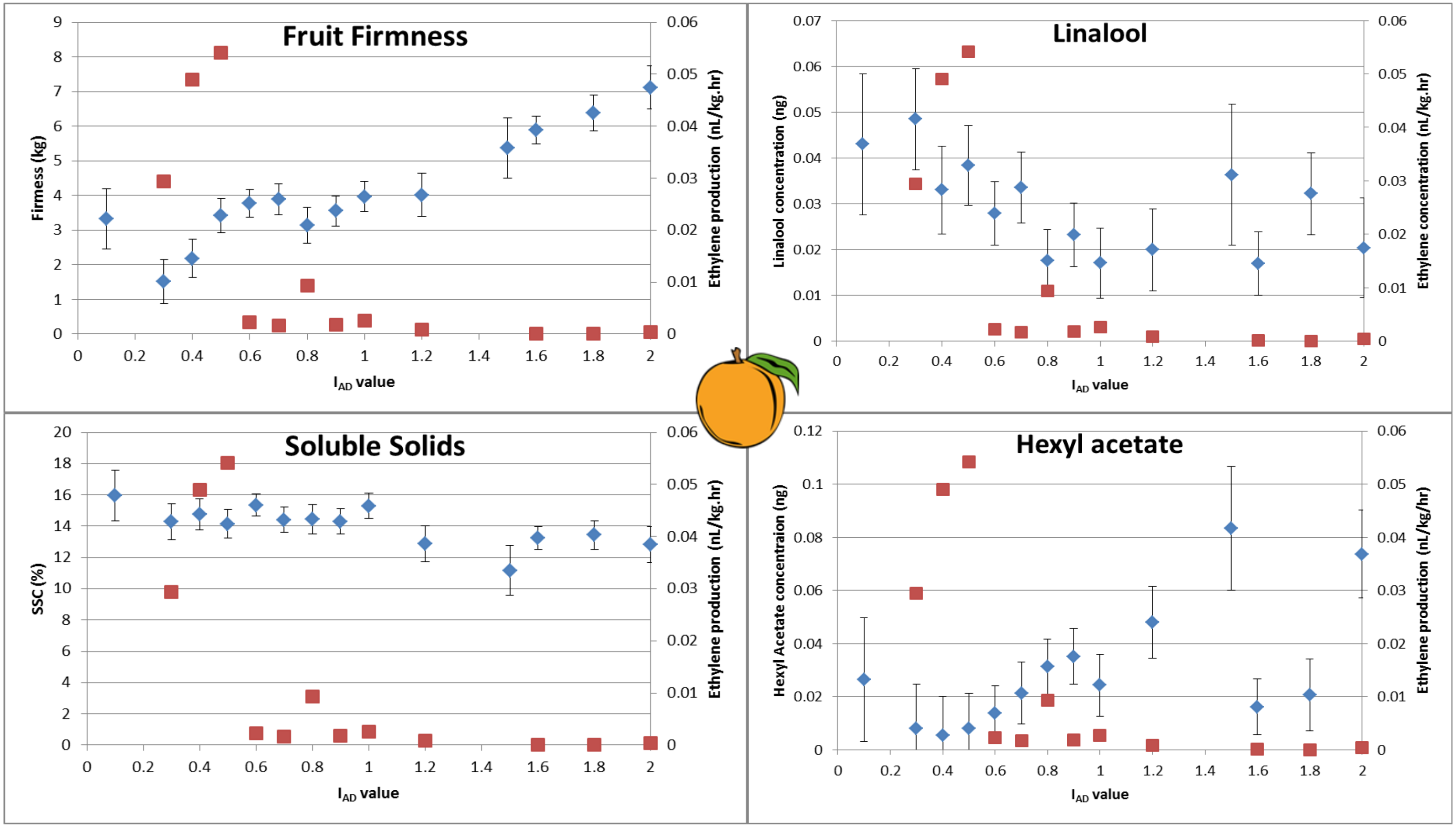
Maturity stage at harvest

'August Flame' peach (2015-16)



Maturity stage at harvest

‘Snow Flame 23’ peach (2016-17)

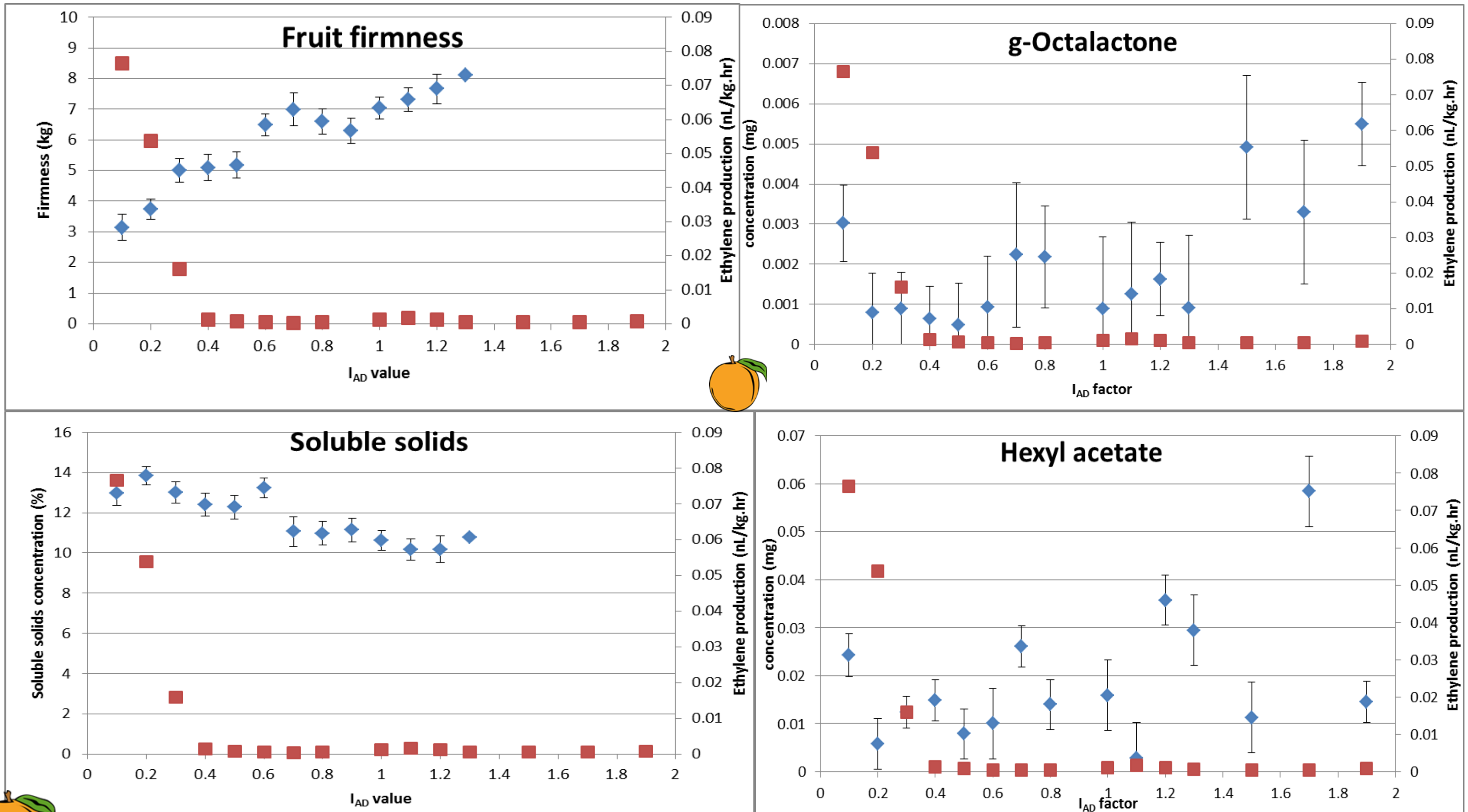


Economic Development,
Jobs, Transport
and Resources

AGRICULTURE VICTORIA

Maturity stage at harvest

‘Rose Bright’ nectarine (2016-17)

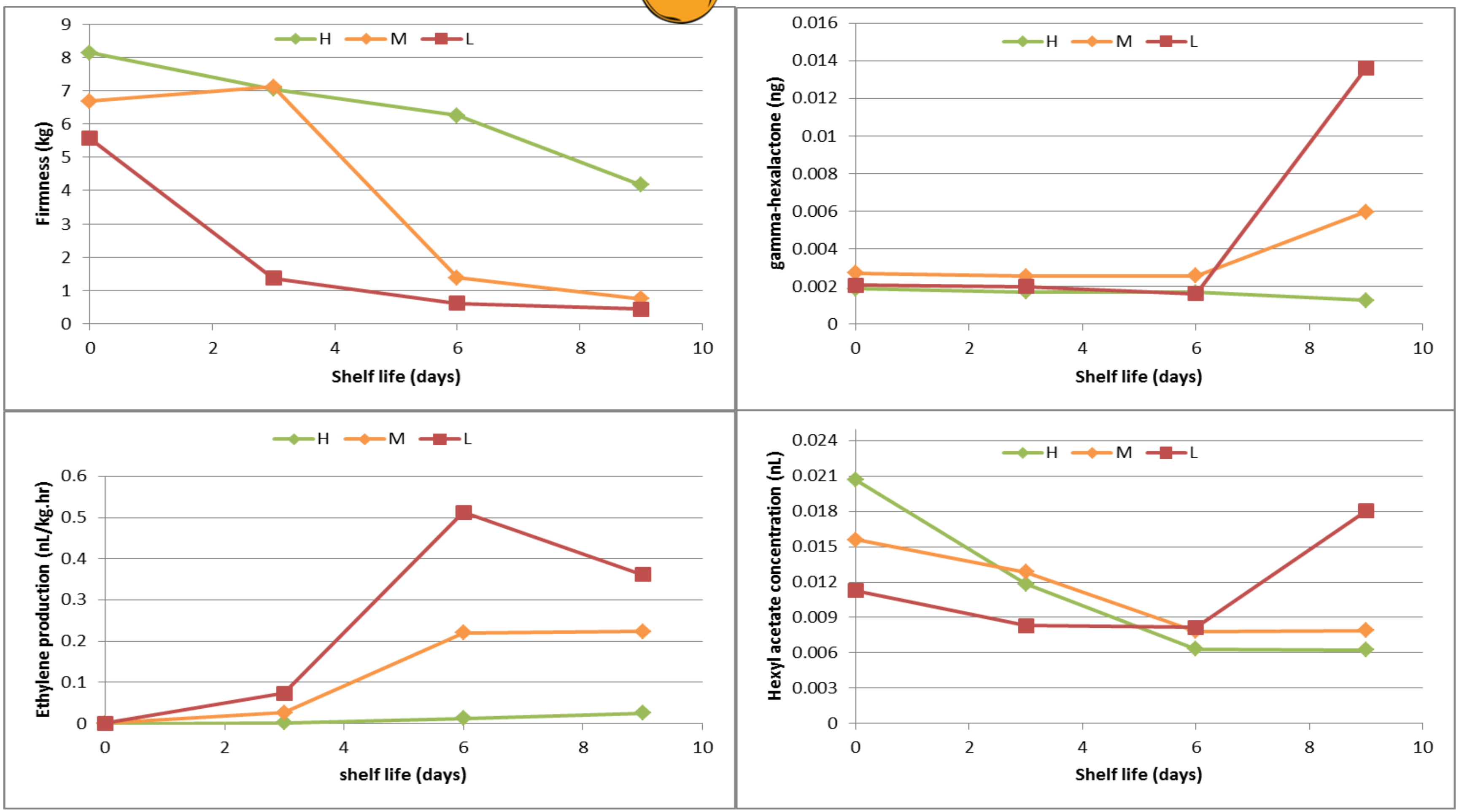


Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

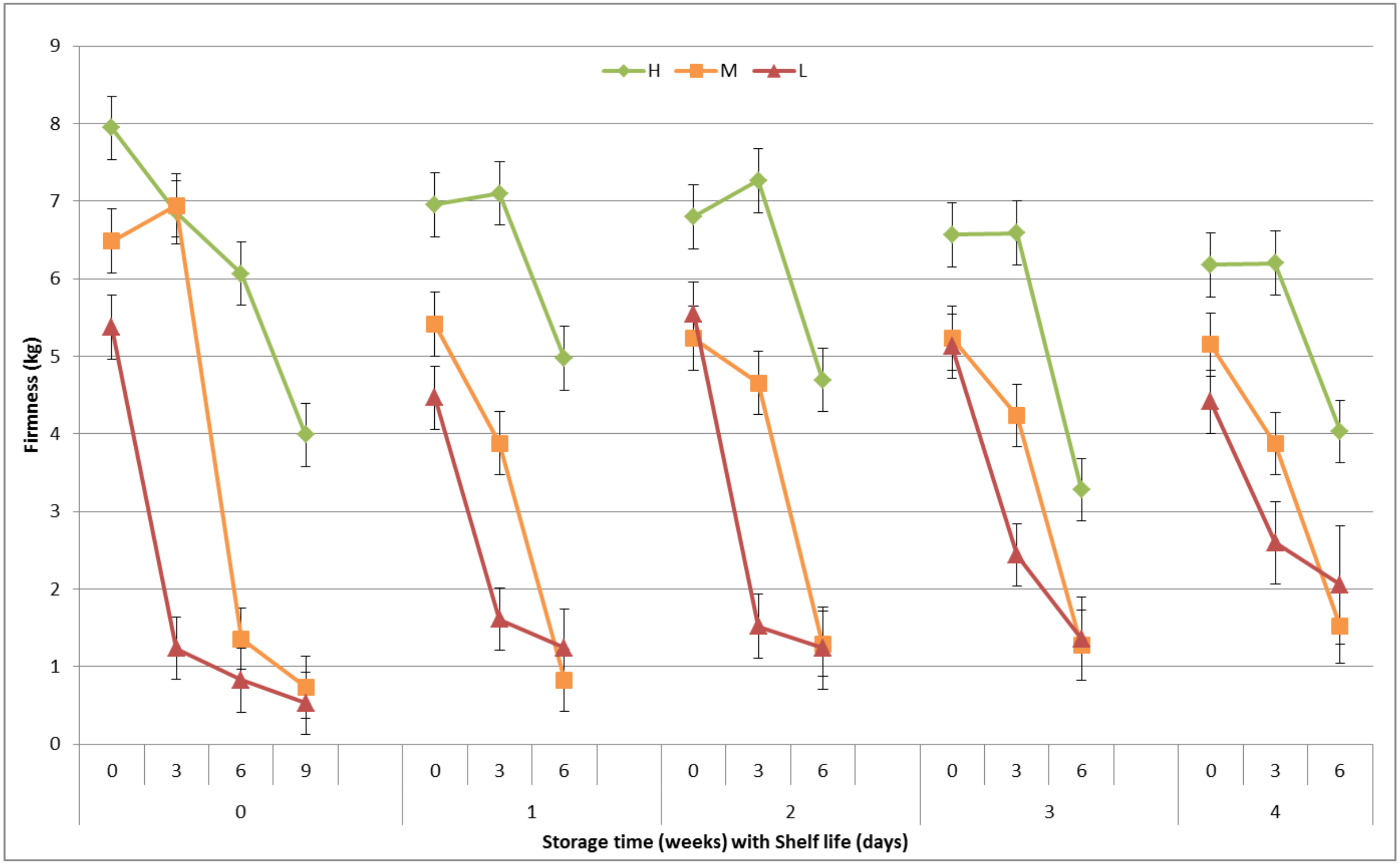
Shelf life

‘Rose Bright’ nectarine (2016-17)



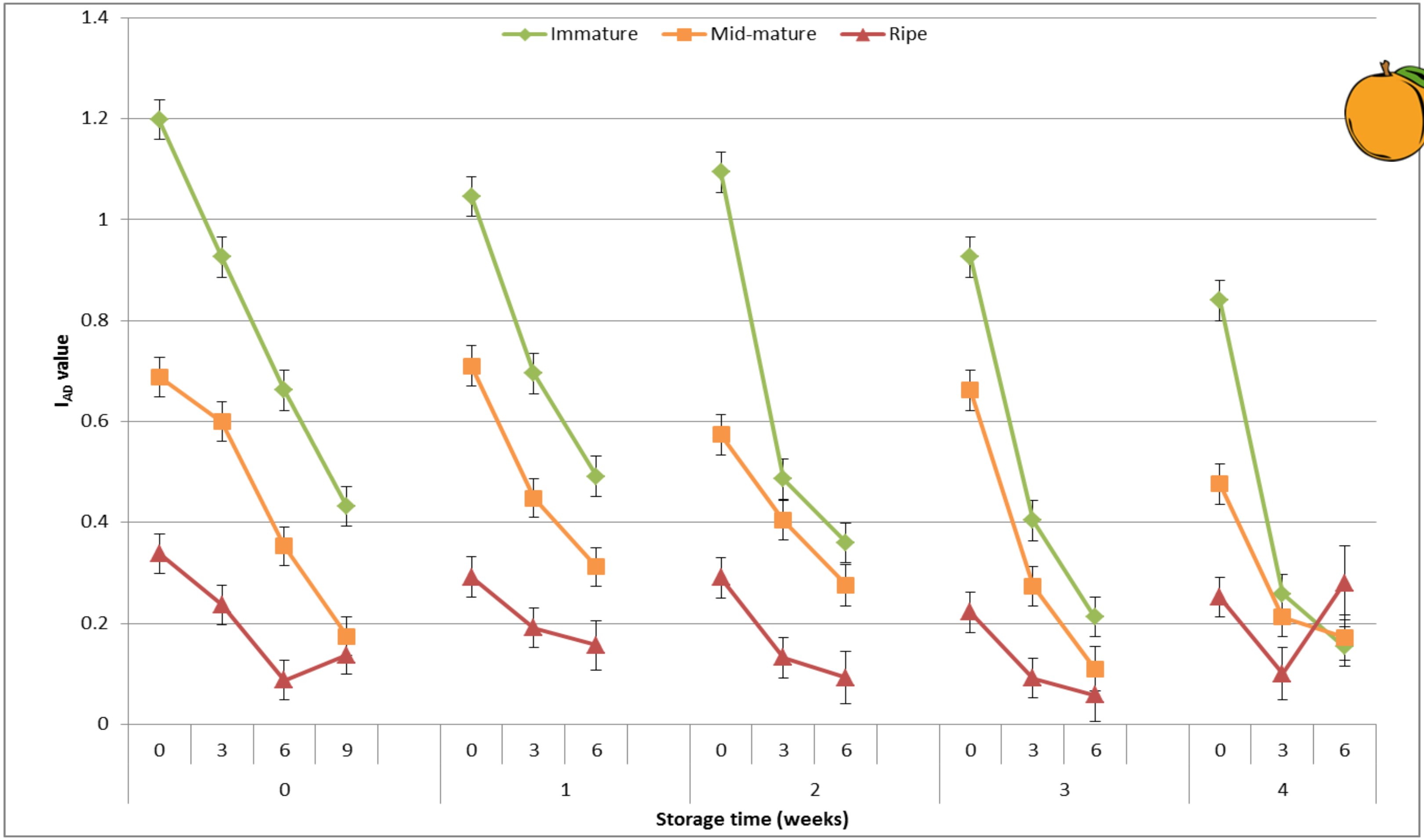
Cold storage

'Rose Bright' nectarine (2016-17)



Cold storage

‘Rose Bright’ nectarine (2016-17)

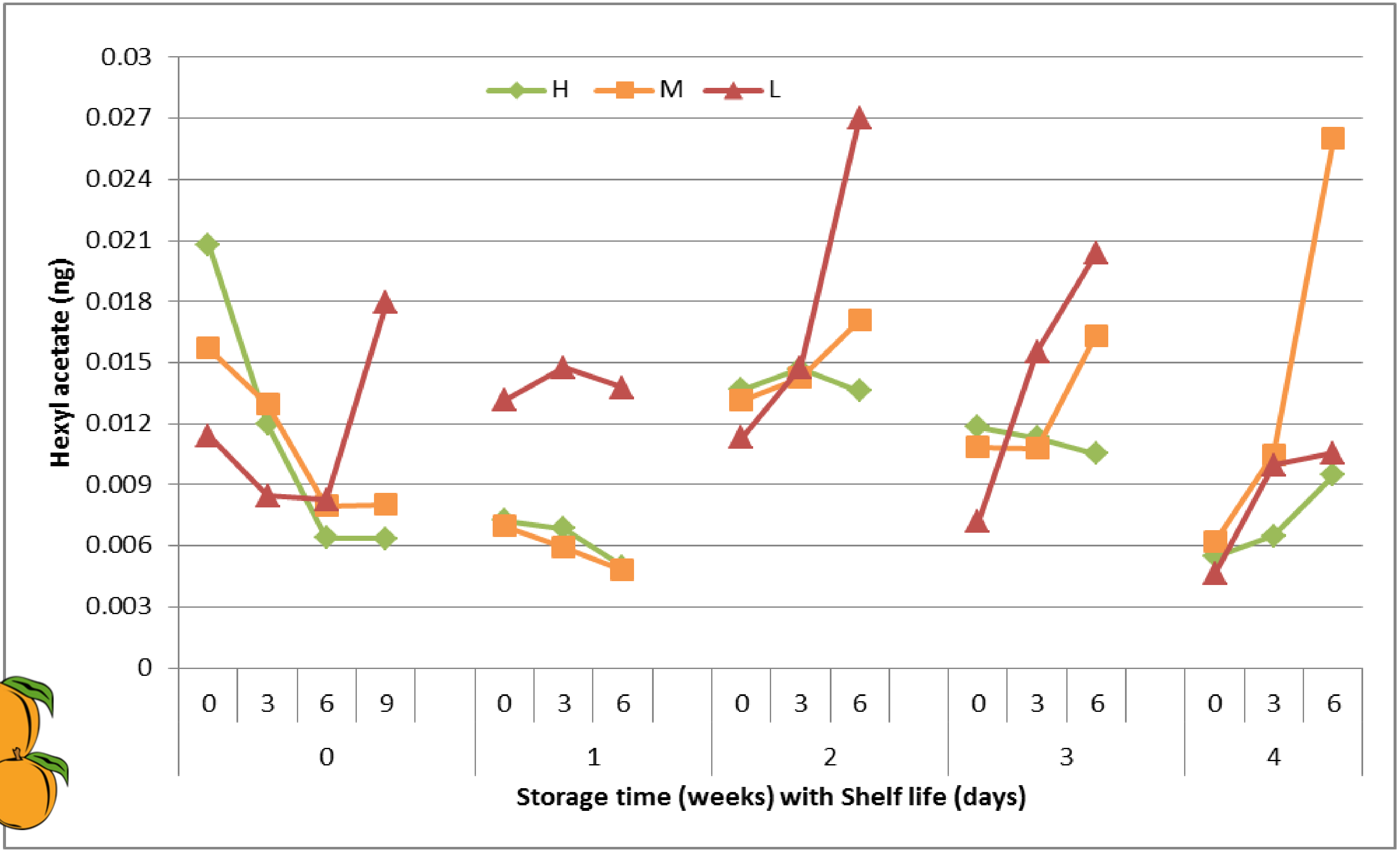


Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Cold storage

'Rose Bright' nectarine (2016-17)

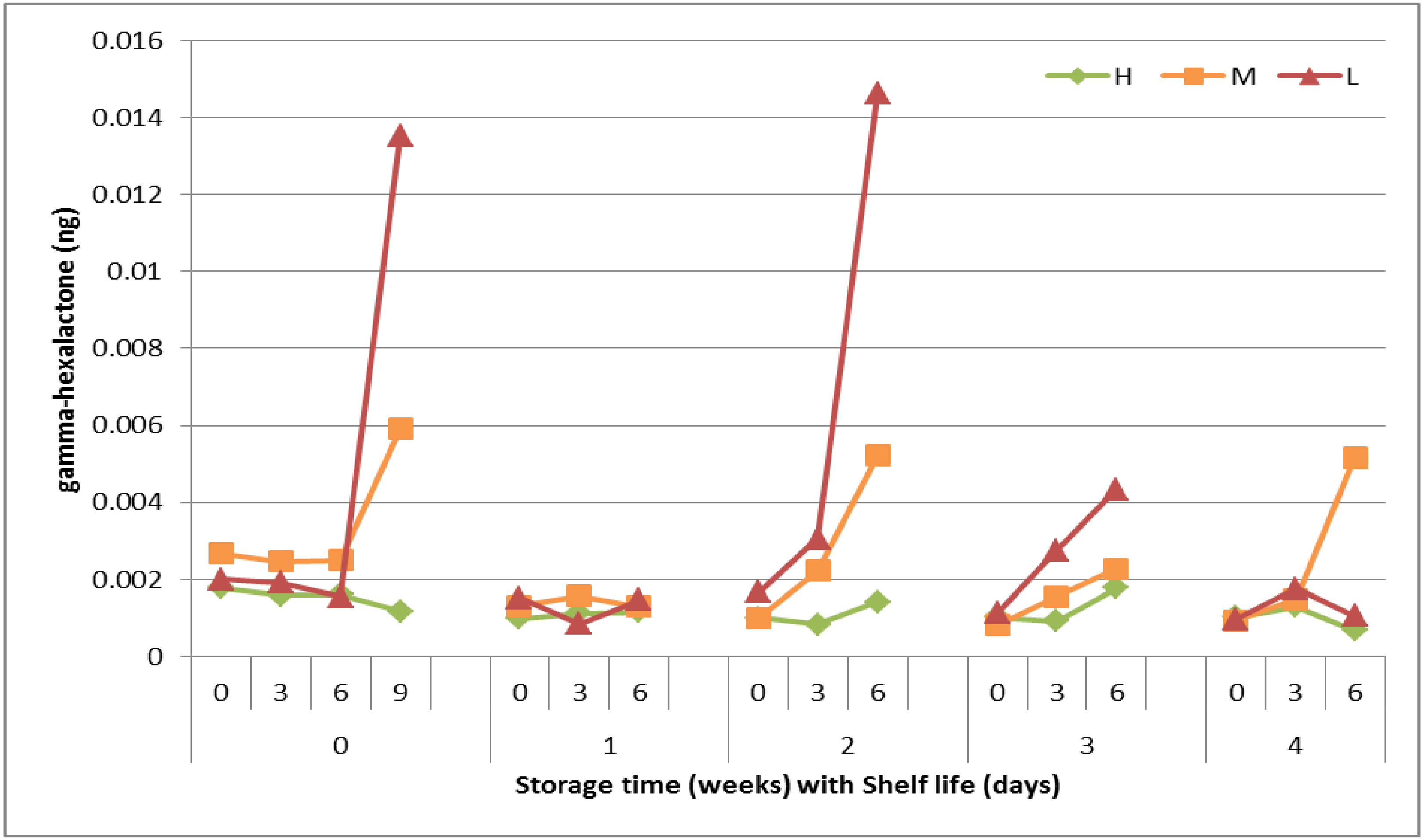


Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Cold storage

'Rose Bright' nectarine (2016-17)



What we think so far

Fruit physiological maturity can be determined by ethylene production and is important at harvest:

- For fruit to develop adequate peachy aroma perceived acceptable in the market place there must be ethylene production
- No ethylene means greater presence of unpleasant aroma compounds
- After cold storage and return to ambient temp some of the pleasant peachy aroma compounds show increased production

- Previous work shows difference between nectarines and peaches, and some cultivars within these, showing not all cultivars behave the same with regard to aroma development.

This coming season:

Continue sampling aromatic volatiles from individual cultivars during fruit development

Confirm: ‘Snow Flame 23’ peach;
‘August Flame’ peach;
‘September Bright’ nectarine

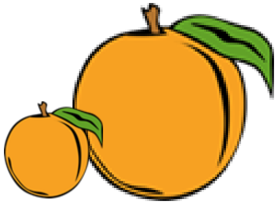
Collect: ‘Snow Flame 25’ peach;
‘August Bright’ nectarine;
‘Snow Fall’ peach;
‘Ice Princess’ peach

Conduct more Shelf Life trials:

Rose Bright nectarine
Snow Flame 23 peach
Snow Flame 25 peach
August Bright nectarine
August Flame peach

Conduct Storage Trial:

‘August Flame’ peach

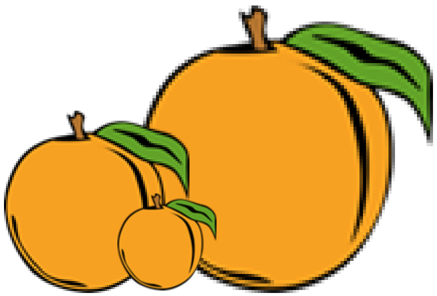


What's next?
Can we further improve market acceptance?

Not all cultivars behave the same.
Cultivar selection for your intended market may be important as does harvest time within the season - early, mid, late.

Other factors that can impact on fruit quality including aroma

- Chilling injury – is there an aroma compound that can be targeted to identify this
  - Electronic nose training
- Disinfestation



Questions ?

How many  did you see?!

Thank you

RMIT UNIVERSITY

Economic Development,
Jobs, Transport
and Resources

AGRICULTURE VICTORIA

Modelling peach and nectarine ripening during storage using the I_{AD} maturity index

Dario Stefanelli, John Lopresti, Glenn Hale, Janine Jaeger, Christine Frisina, Rod Jones, Bruce Tomkins

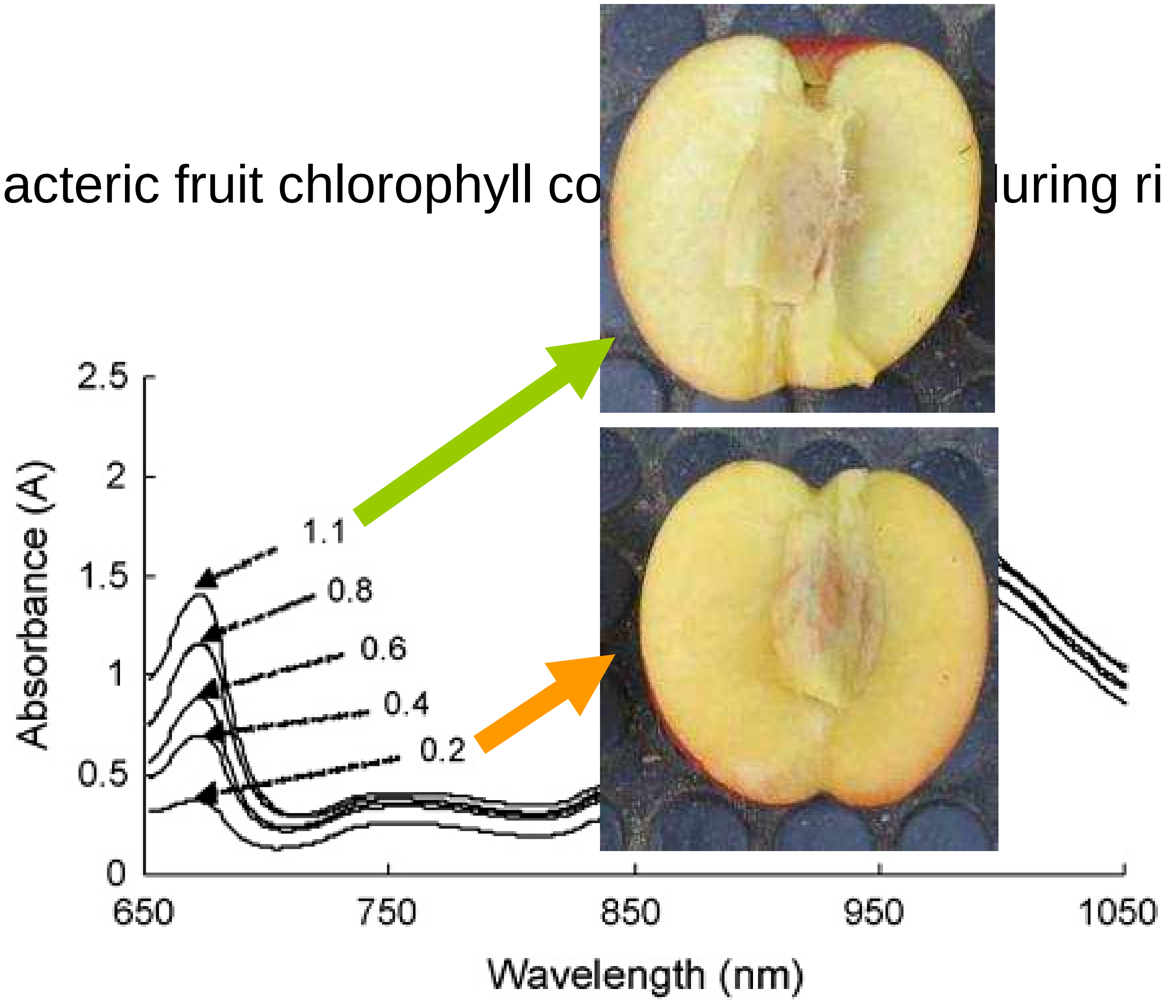
State Government Victoria

The DA (Difference of Absorbance) Technology

- Based on vis/NIR spectroscopy
- Measures Chlorophyll-a content in mesocarp (difference between 720 and 670 nm)
- Index of fruit physiological maturity stage
- It is proved for climacteric fruit (stone and pome)

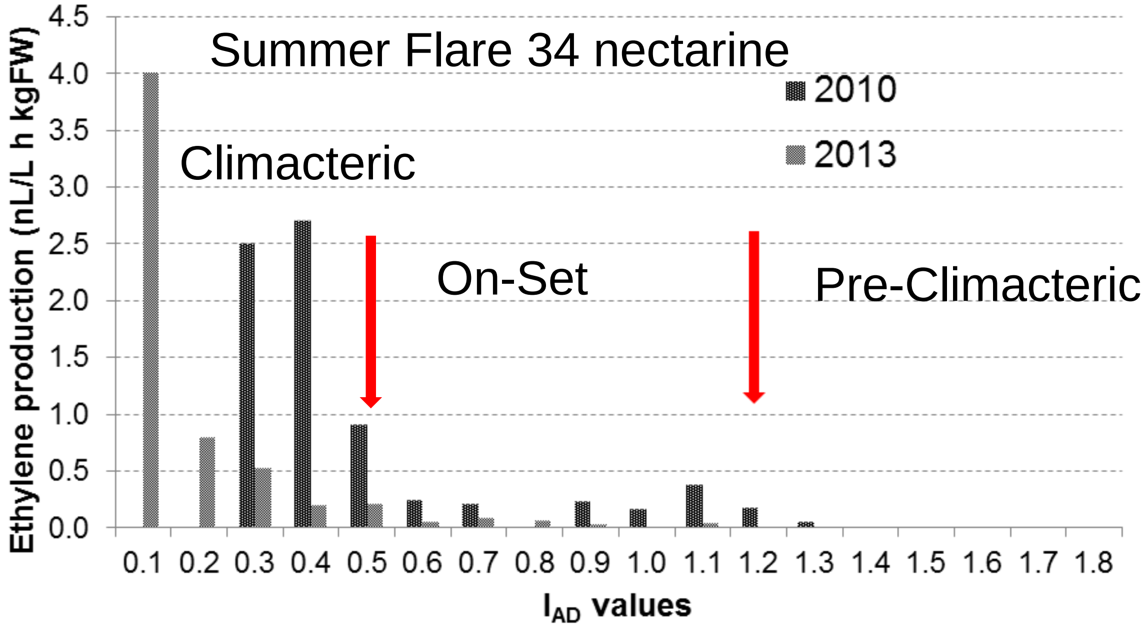
I_{AD} Index as measurement of maturity

In climacteric fruit chlorophyll co[…]luring ripening



The I_{AD} is related to the time course of ethylene production during fruit ripening

- I_{AD} classes and ethylene production per variety are **constant** every year; it is cultivar specific



Scope: to determine effect of T, Maturity class and variety on I_{AD} decay during storage

- Peach September Sun and Nectarine Summer Bright
  - Stored at 0°C and 7 °C
  - Monitored weekly for I_{AD} value for up to 42 days
- Nectarine Summer Flare 34
  - Stored at 18 °C
  - Monitored every 3 days for I_{AD} value for 10 days

I_{AD} Measured with DA-Meter

Fruit segregated by I_{AD} Value

Fruit numbered and prepared for storage

Fruit stored at either 0, 7 or 18 °C

Best fitting curve by SigmaPlot 12.5

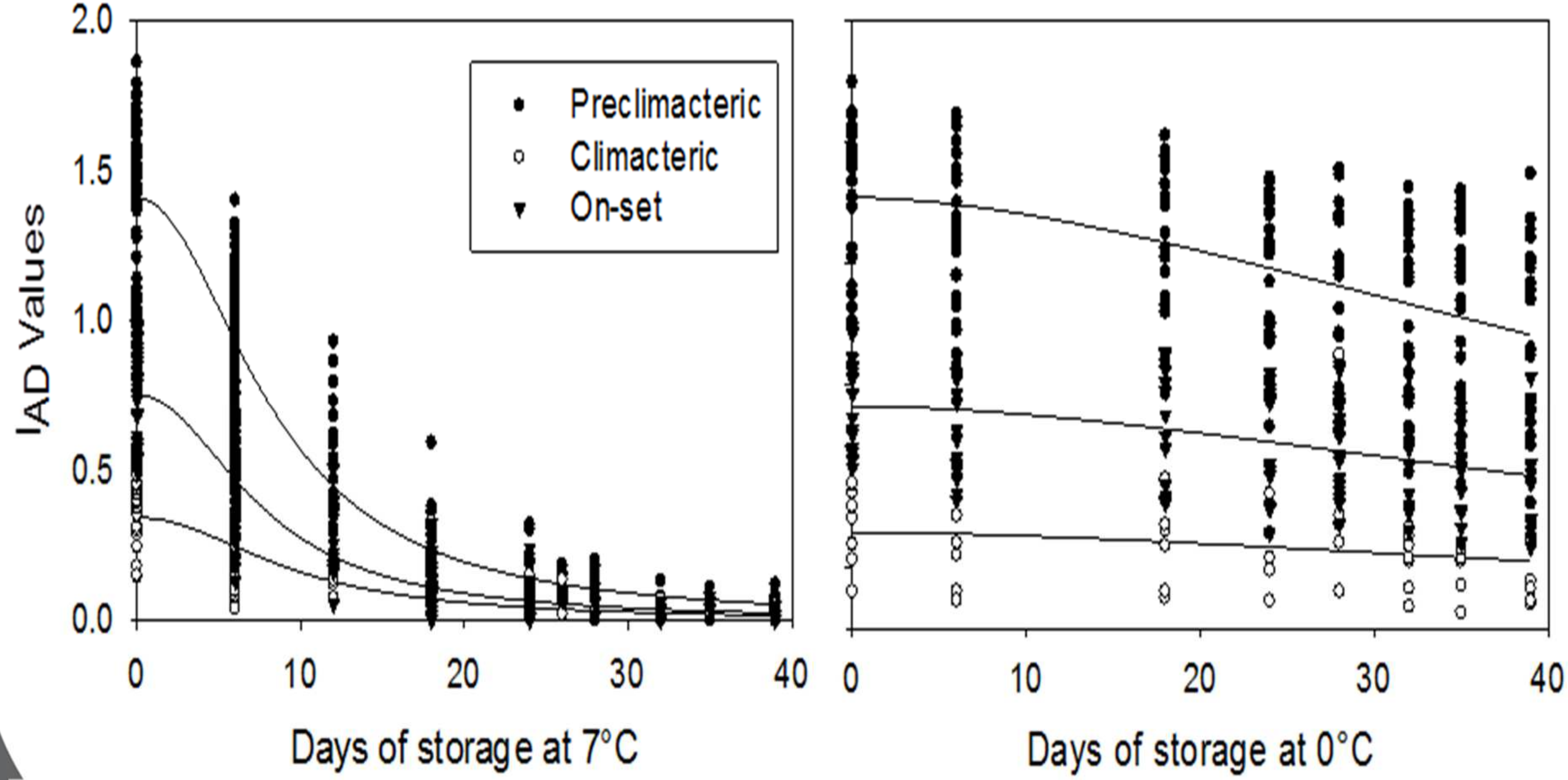
- Maturity class and storage T as sub-data sets within each variety
- Used Global Curve Fitting option
- Parameter “b” (curve slope) shared between sub-data sets

Best regression: sigmoidal following a logistic model with 3 parameters

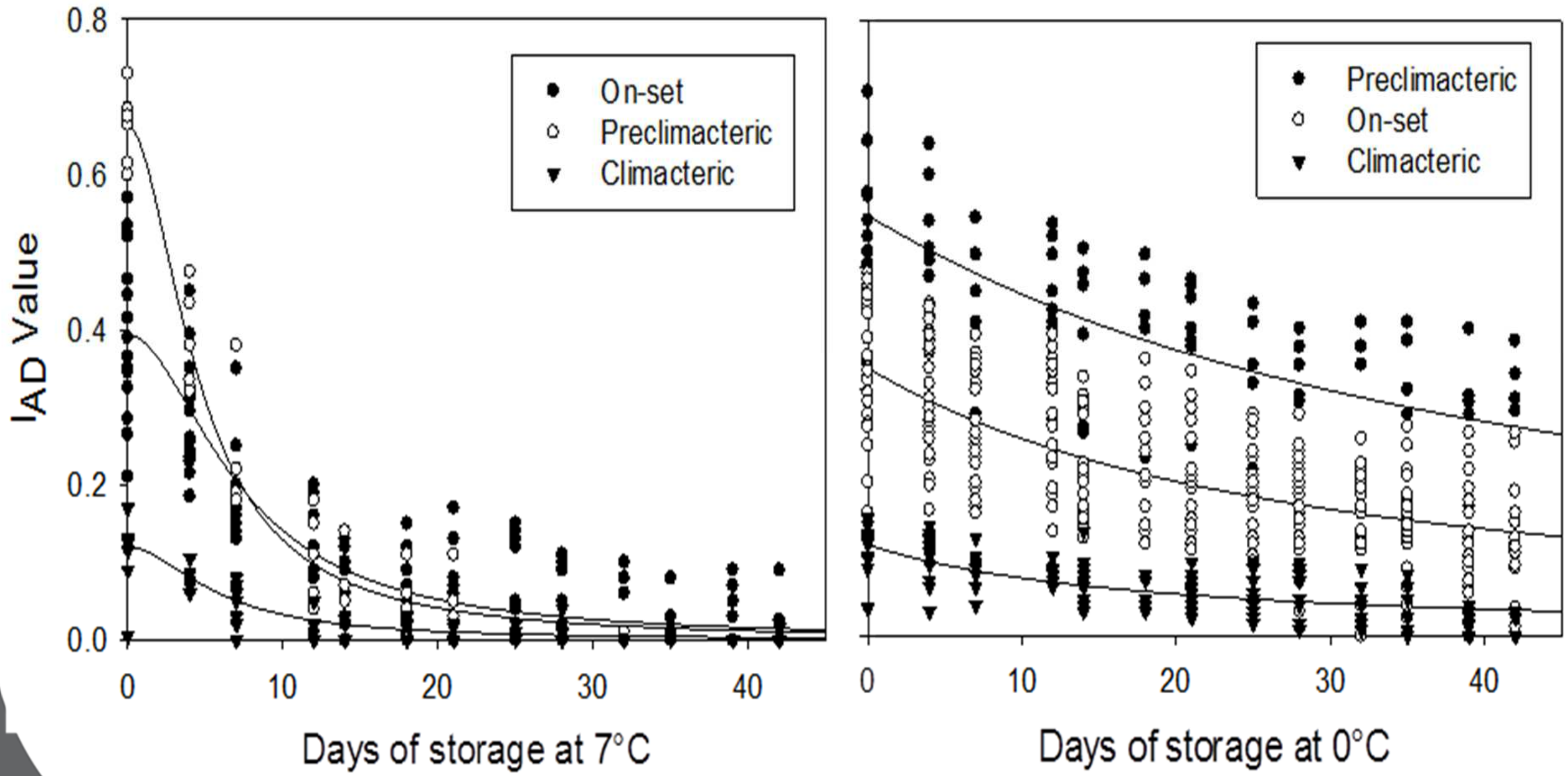
- $I_{AD} = \frac{a}{\left[1+\left(\frac{t}{x0}\right)^{b}\right]}$

- a = Intercept (average I_{AD} value within maturity class)
- t = Storage length in days
- X0 = Inflection point
- b = Curve slope (shared)

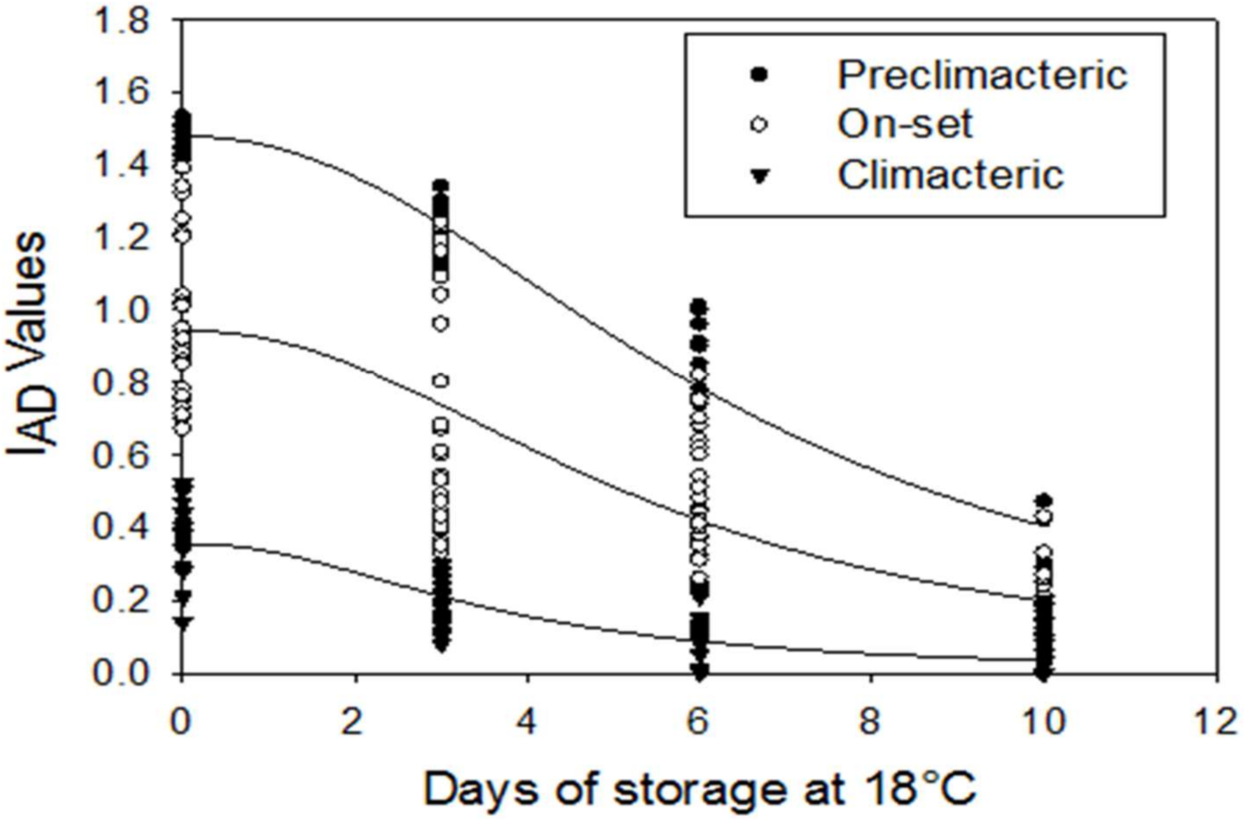
September Sun at 0 °C and 7 °C



Summer Bright at 0 °C and 7 °C



Summer Flare 34 at 18 °C



Regression parameters

‘September Sun’ peach - overall Adjusted R^2 = 0.88; P<0.0001								
Temperature	0°C				7 °C			
Parameter	b	a	X_0	Adj R^2	b	a	X_0	Adj R^2
Preclimacteric	2.055	1.404	56.167	0.26	2.055	1.408	8.263	0.90
On-set	2.055	0.734	52.458	0.27	2.055	0.753	7.645	0.83
Climacteric	2.055	0.322	51.780	0.14	2.055	0.342	9.424	0.60
‘Summer Bright’ nectarine - overall Adjusted R^2 = 0.87; P<0.0001								
Temperature	0°C				7 °C			
Parameter	b	a	X_0	Adj R^2	b	a	X_0	Adj R^2
Preclimacteric	1.567	0.639	41.163	0.48	1.567	0.671	4.207	0.95
On-set	1.567	0.399	24.818	0.52	1.567	0.405	6.689	0.81
Climacteric	1.567	0.137	20.640	0.56	1.567	0.122	5.495	0.77
‘Summer Flare 34’ nectarine - overall Adjusted R^2 = 0.84; P<0.0001								
Temperature					18°C			
Parameter					b	a	X_0	Adj R^2
Preclimacteric					2.158	1.478	6.359	0.94
On-set					2.158	0.943	5.412	0.62
Climacteric					2.158	0.356	3.577	0.60

Conclusions

- For stone fruit I_{AD} in postharvest decays following a sigmoidal logistic regression
- Cultivar, Temperature and Maturity class affect regression parameters
- More work needed to become a fully predicting model.
- I_{AD} could become the measurement of choice along the horticulture value chain.

Thanks to:

Growers

Colleagues

Funding:

VICTORIA State Government

Horticulture Innovation Australia

O4 Stone fruit ripening during storage measured by the IAD maturity index

Dario Stefanelli, *Department of Economic Development, Jobs, Transport & Resources, Bundoora, AU*
John Lopresti, *Department of Economic Development, Jobs, Transport & Resources, Bundoora, AU*
Glenn Hale, *Dedjtr, Bundoora, AU*
Janine Jaeger, *Dedjtr, Bundoora, AU*
Christine Frisina, *Dedjtr, Bindoora, AU*
Rod jones, *Dedjtr, Bundoora, AU*
Bruce tomkins, *Dedjtr, Bundoora, AU*

dario.stefanelli@ecodev.vic.gov.au

Consumer dissatisfaction due to poor and variable eating quality of peaches and nectarines is severely limiting sales growth on domestic and export markets of stonefruit producing countries. The effect of fruit maturity at harvest on ripening behaviour and eating quality during handling, storage and distribution is extremely important but not fully understood. A recent technology correlates the difference of absorbance in chlorophyll-a in the mesocarp tissue and fruit ethylene production. This provides an Index of absorbance difference (IAD), which non-destructively measures the physiological maturity stage of the fruit in relation to ethylene production. Through the IAD is possible to non-destructively monitor fruit ripening pre and postharvest. Several authors have reported the usefulness of the IAD in identifying and predicting optimal harvest time. In this experiment we have used the IAD value to monitor stone fruit ripening at different storage temperatures.
Fruit were segregated into classes based on ethylene production rate at harvest and then monitored during storage to understand the effect of harvest maturity on postharvest ripening behaviour. Two nectarine varieties (Summer Flare 34 and Summer Bright) and one peach variety (September Sun) were used in the experiment. Summer Bright and September Sun were monitored with a DA-Meter and the IAD measured twice weekly for up to 42 days during storage at both 0 and 7 °C to understand fruit ripening behaviour in different

temperature conditions. Summer Flare 34 ripening was monitored every three days for up to 10 days at 18 °C to understand fruit ripening at ambient temperatures.
The best regression curve describing changes in the IAD ripening index after harvest was a logistic sigmoidal curve. Storage temperature, variety and maturity class at harvest had an effect on the single curve parameters but not on the type of curve. A series of sigmoidal curves were identified of which variety and fruit maturity class at harvest were mostly responsible for the variability of the y-intercept while temperature was mostly responsible for the slope and inflection point. Curve R2 was variety dependent and decreased with temperature. Lowest values were found at 0 °C due to the slow changes in IAD which made fitting data to the curve difficult.
A common postharvest fruit ripening trend was identified from the observations reported here. After harvest the fruit IAD maturity index followed a sigmoidal curve independently of variety, maturity at harvest or storage temperature. Additional studies are necessary to add a predicting component to the model.

Economic Development,
Jobs, Transport
and Resources

The aromatic volatiles of summerfruit

Harvest maturity impacts on fruit aroma during storage and marketing

Christine Frisina &
Dario Stefanelli

AGRICULTURE VICTORIA

Stonefruit Maturity, Quality & Composition

Research to optimize fruit quality for domestic and export market

Problem

High variability fruit quality at harvest : ➡ Consumer dissatisfaction

- Maturity at harvest affects shelf life and storage period
- Cold storage affects fruit quality and composition

Approach

Use the Stonefruit Field Laboratory at Tatura to determine harvest maturity classes to optimise fruit quality and composition during field ripening and after cold storage

Ethylene for maturity classes and VOCs collection based on I_{AD} during fruit development

+

Fruit quality and VOCs as affected by cold storage and shelf life

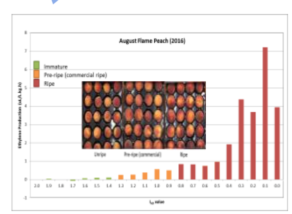
+

Biochemical analysis by GC and GC-MS

Stonefruit growers with knowledge to manage fruit quality:

- ✓ **Optimal harvest maturity for consumer preference**
- ✓ **Maturity class database for grower decision making**
- ✓ **Optimize cold storage and ripening protocols**

Ethylene protocol adopted



integrity FRUIT

Maturity classes adoption

⇒ ***Deliver high quality fruit to domestic and export markets***

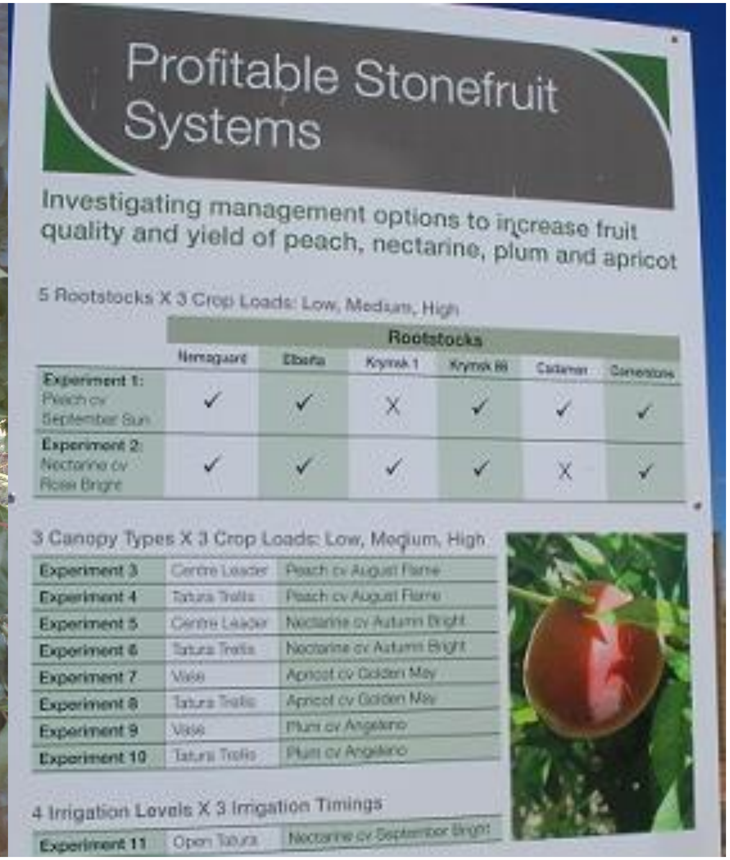
⇒ ***Improve marketability and increase profitability***

Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Stonefruit Maturity, Quality & Composition

Funding and support



15 varieties of summer fruit

- 9 Peach
- 4 Nectarine
- 1 Apricot
- 1 Plum (+ pollinator)

Focusing on Peach and nectarine

Hort Innovation
Strategic levy investment

SUMMERFRUIT FUND

RMIT UNIVERSITY

Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Background

Aromatic Volatile Organic Compounds

Consumer perception of poor quality fruit because doesn't smell peachy/fruity

Why?

What factors may affect fruit aroma?

- Maturity stage at harvest
- Shelf life
- Cold storage

Aroma is known to be combination of compounds

From previous research:

Identified 9 key aroma compounds for whole fruit aroma:
benzaldehyde, butyl acetate, hexyl acetate, linalool, γ-hexalactone, γ-octalactone, δ-octalactone, γ-decalactone, δ-decalactone

More than 90% of previous work has been done on bulked samples.

→ Individual fruit; I_{AD} as maturity index;

Maturity stage at harvest

Fruit were picked and checked for I_{AD} using a DA meter

- DA meter result is an indication of level of chlorophyll-a in the flesh of the fruit

Profiling of ethylene and volatiles:

Fruit were harvested across several days for each cultivar (up to 3 weeks).

All fruit used were also evaluated for flesh firmness and soluble solid concentration.

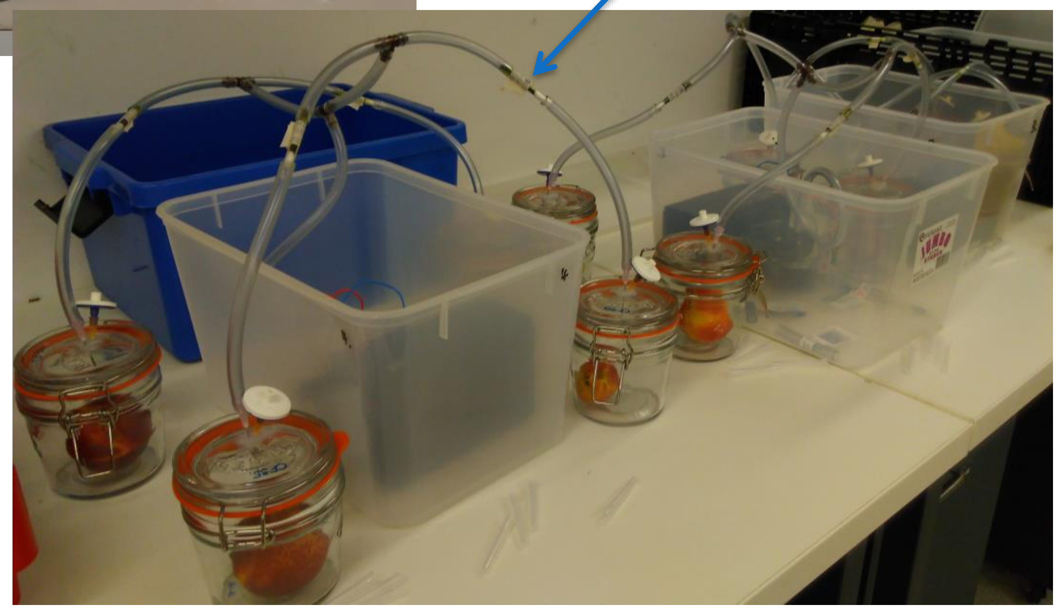
'August Flame' peach (2015-16)

DA classed: 0.3-0.6 Ripe (left), mid-ripe 0.8-1.1 (middle), 1.3-1.6 unripe (left)

Maturity stage at harvest

Evacuated vial

Charcoal trap



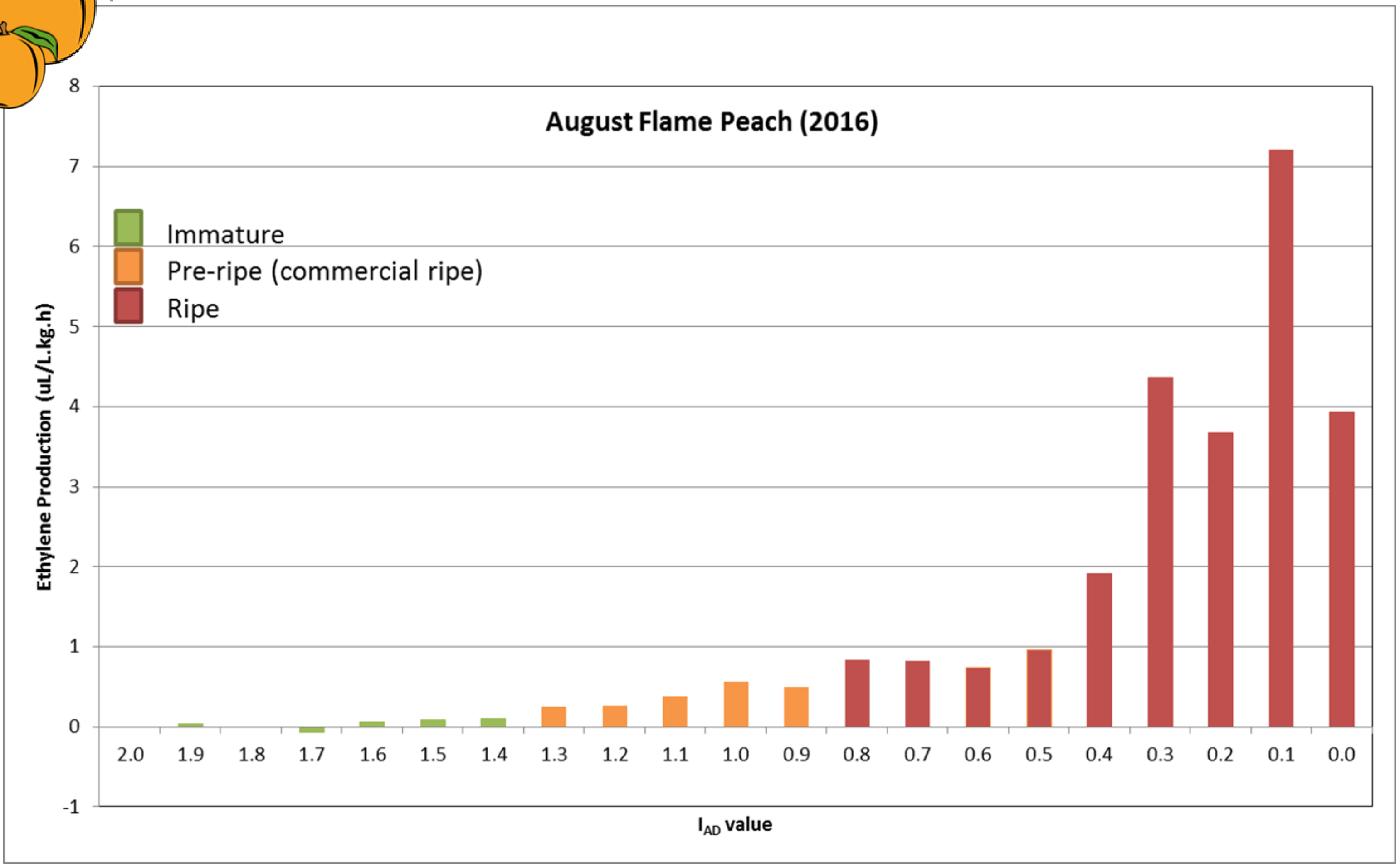
Ethylene and aromatic volatile sampling of individual fruit.

Evacuated vials were used to collect ethylene samples for later measurement by GC.

Charcoal traps were used to collect aromatic volatile samples for later measurement by GC-MS.

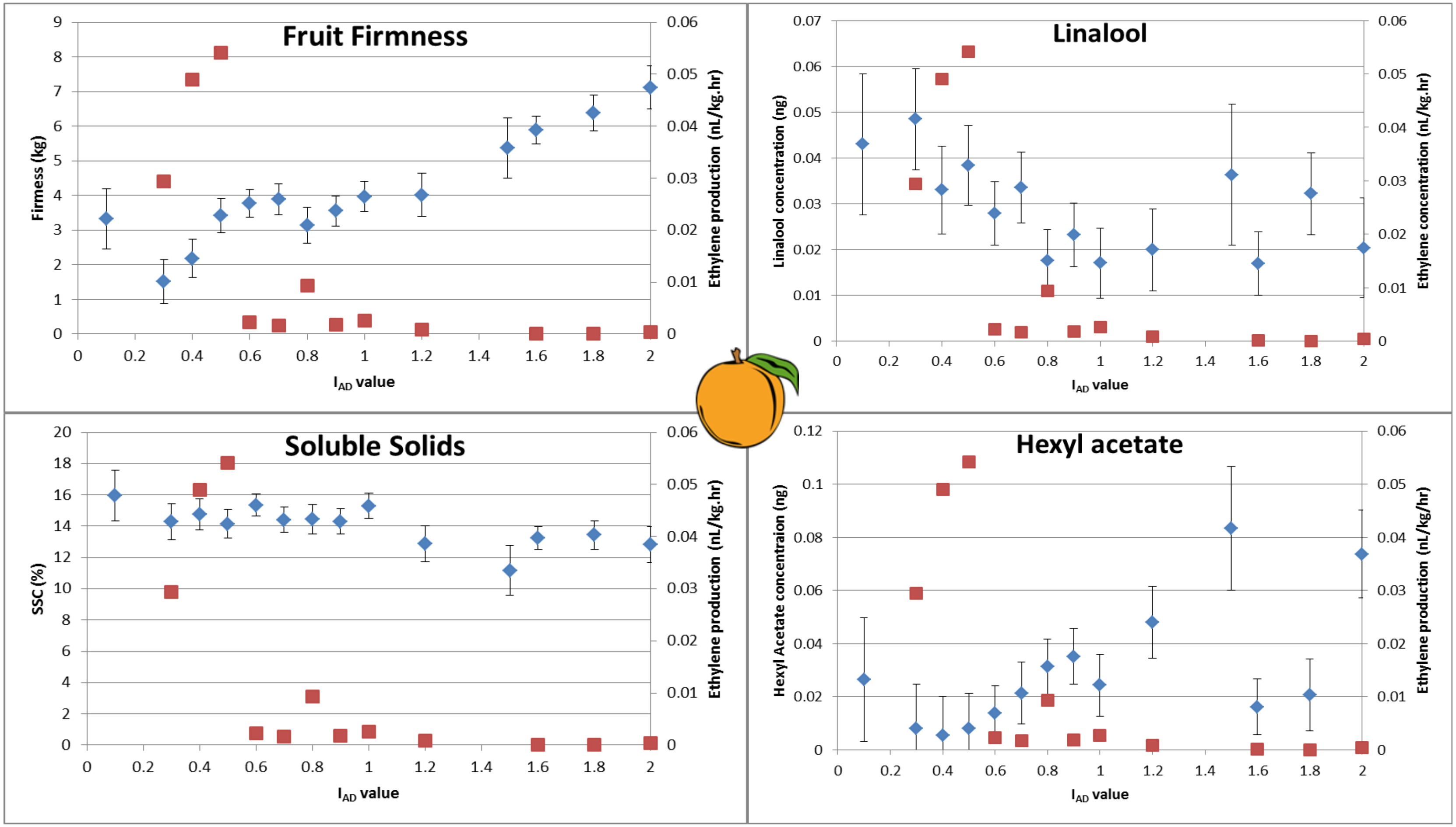
Maturity stage at harvest

'August Flame' peach (2015-16)



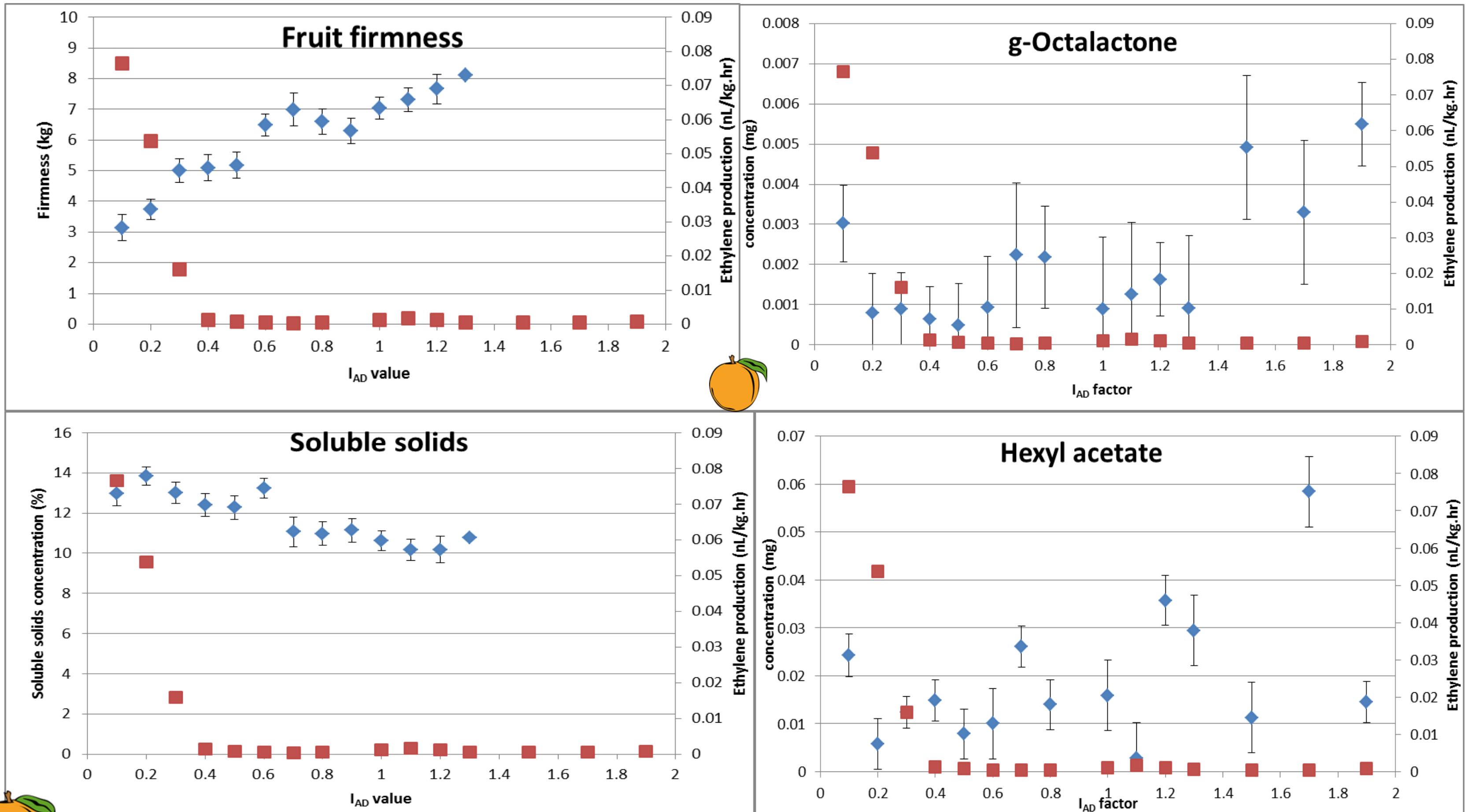
Maturity stage at harvest

‘Snow Flame 25’ peach (2016-17)



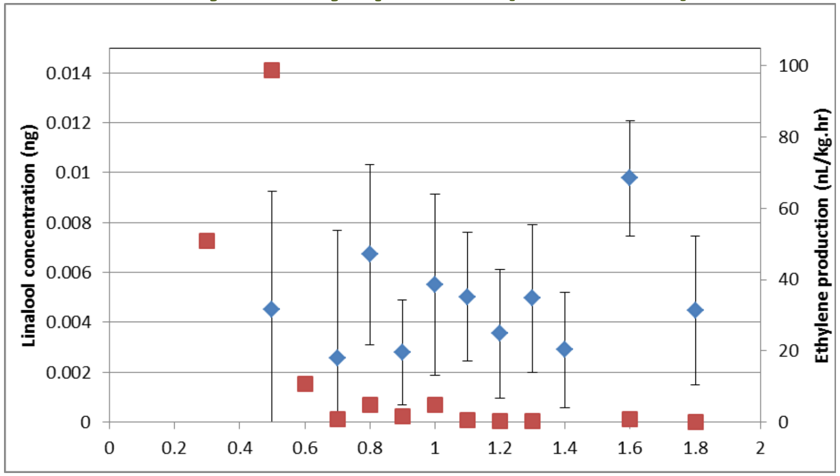
Maturity stage at harvest

'Rose Bright' nectarine (2016-17)

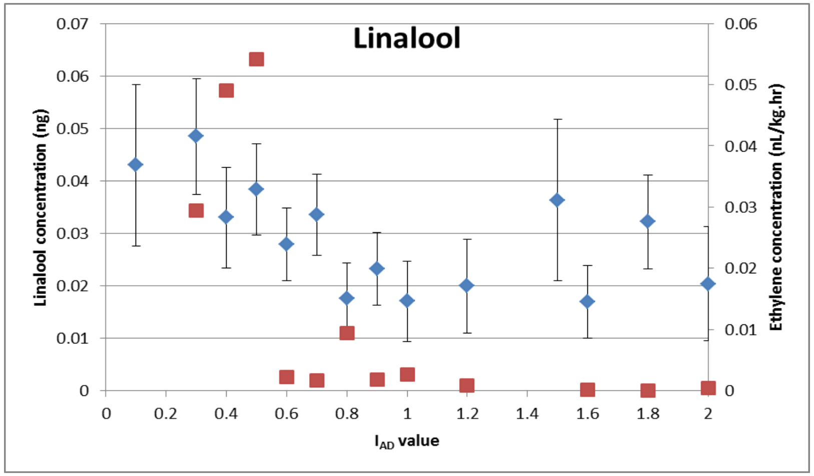


Linalool by cultivar and maturity stage

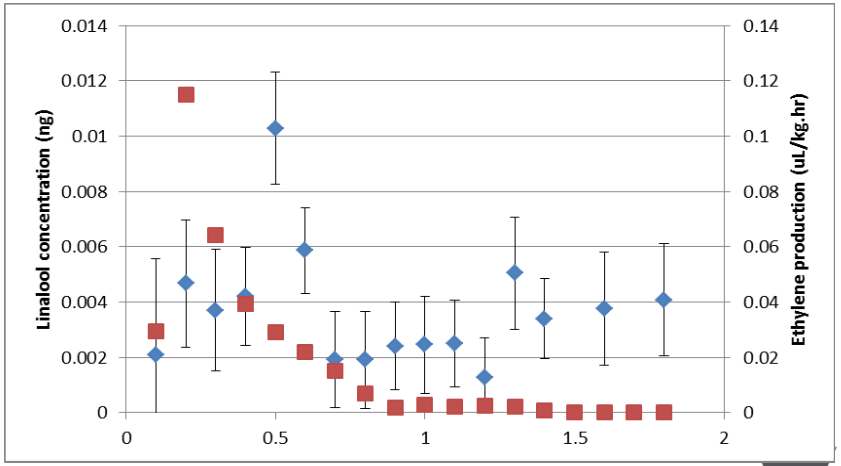
'O'Henry early' peach (2016-17)



'Snow Flame 25' peach (2016-17)



'August Flame' peach (2016-17)



Other compounds measured

Many of the key compounds had inconsistent levels.

They had detectable amounts as very unripe fruit, not mentioned in the literature. The exception being δ-octalactone.

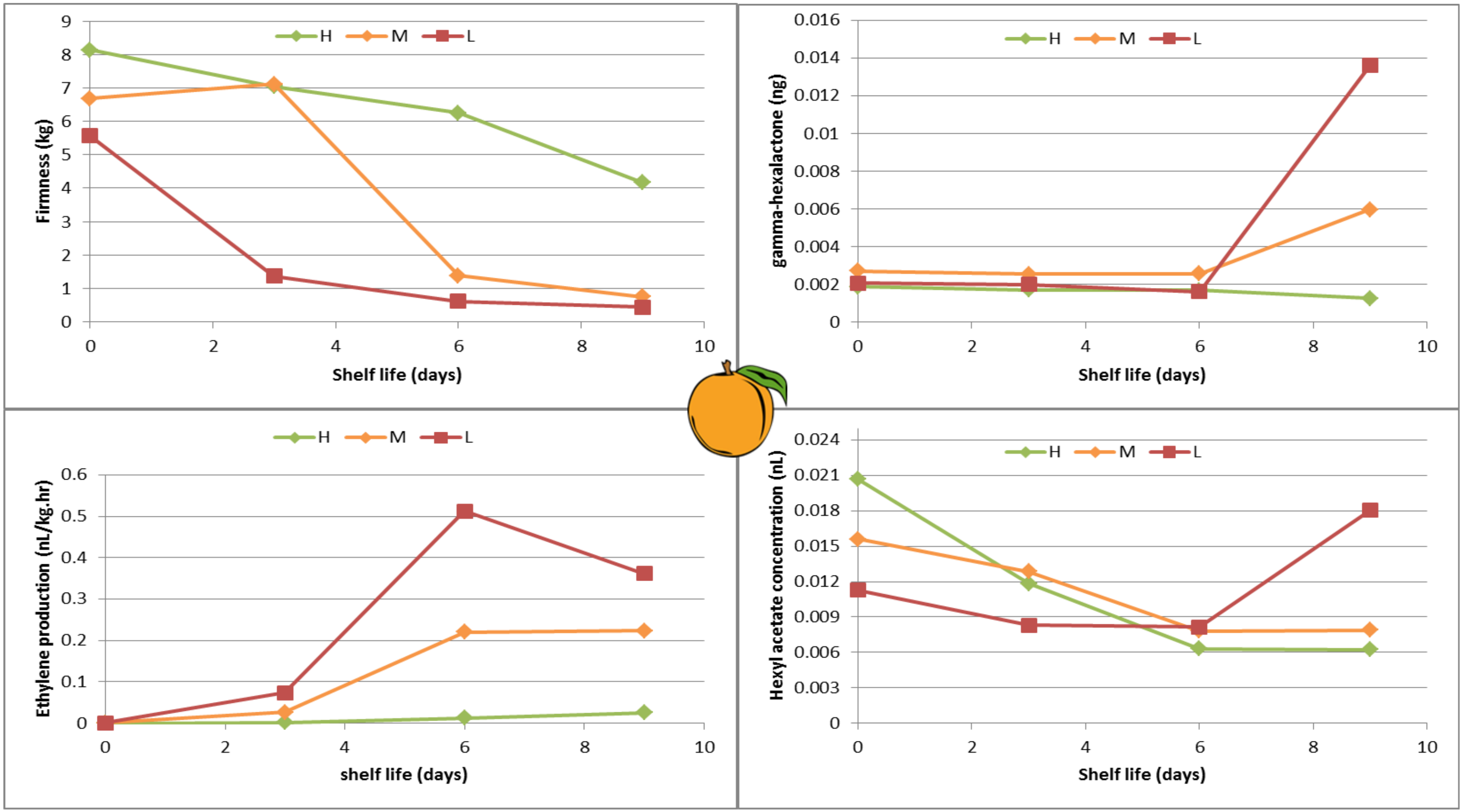
As the fruit developed on the tree:

Compound	Snow Flame 25	O'Henry	August Flame	Rose Bright
benzaldehyde	increase	Decrease*	Decrease*	Poor detection
butyl acetate	Decrease	Plateau*	increase	Poor detection
hexyl acetate	Decrease	Plateau	increase	Decrease
γ-hexalactone	Decrease	decrease	Increase	Poor detection
linalool	Increase	decrease*	increase/ decrease	Poor detection
γ-octalactone	Increase	decrease	Decrease	Decrease
δ-octalactone	decrease	Poor detection	Increase	Poor detection
γ-decalactone	Increase	decrease	Decrease	Poor detection
δ-decalactone	increase	Plateau*	Plateau*	Poor detection
		*High variation		

Shelf life

'Rose Bright' nectarine (2016-17)

H: unripe (DA 1.0 – 1.6); M: mid ripe (DA 0.5 – 0.9); L: full ripe (DA 0.1 -0.4)

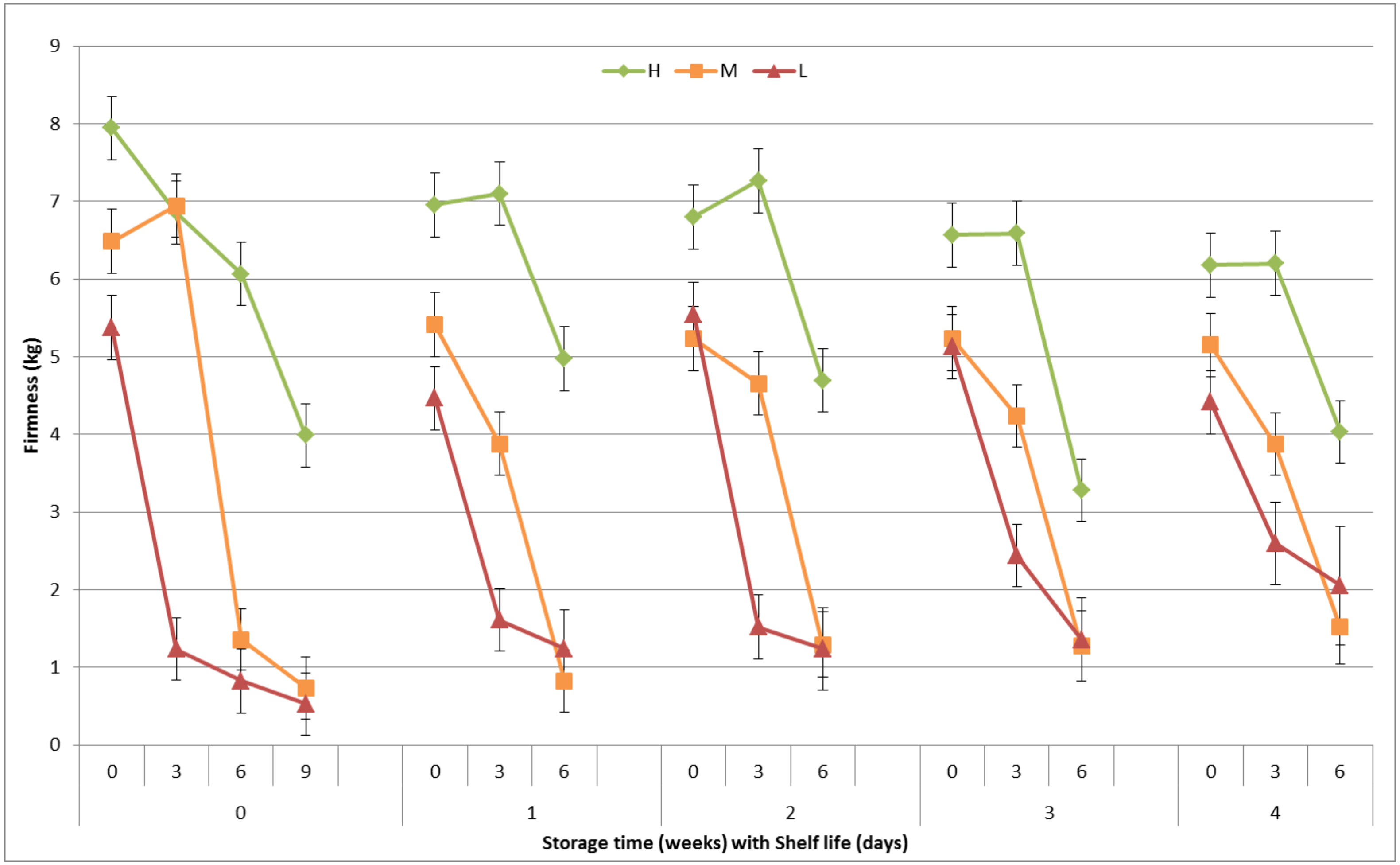


Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

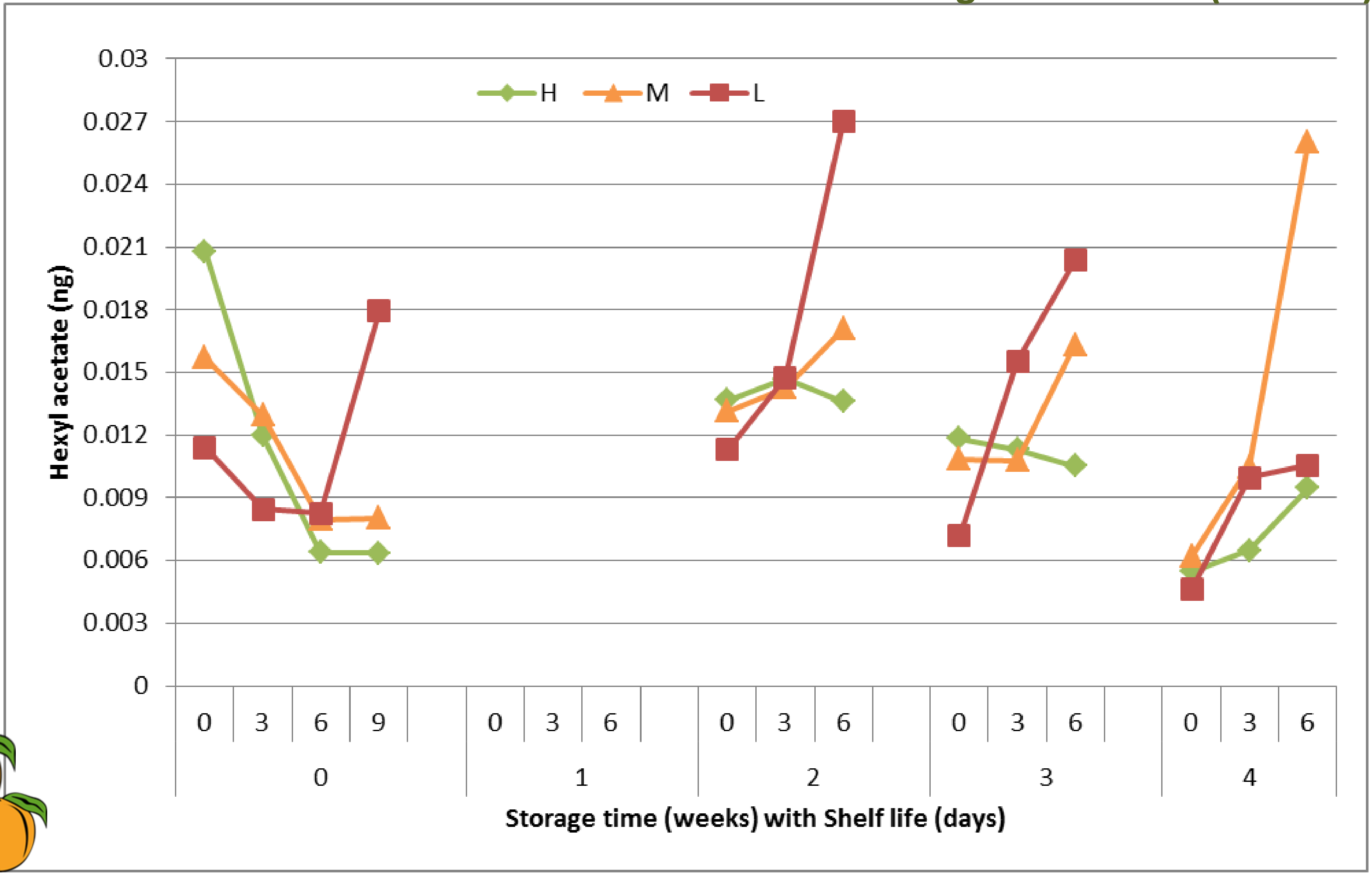
Cold storage

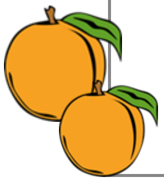
'Rose Bright' nectarine (2016-17)



Cold storage

'Rose Bright' nectarine (2016-17)



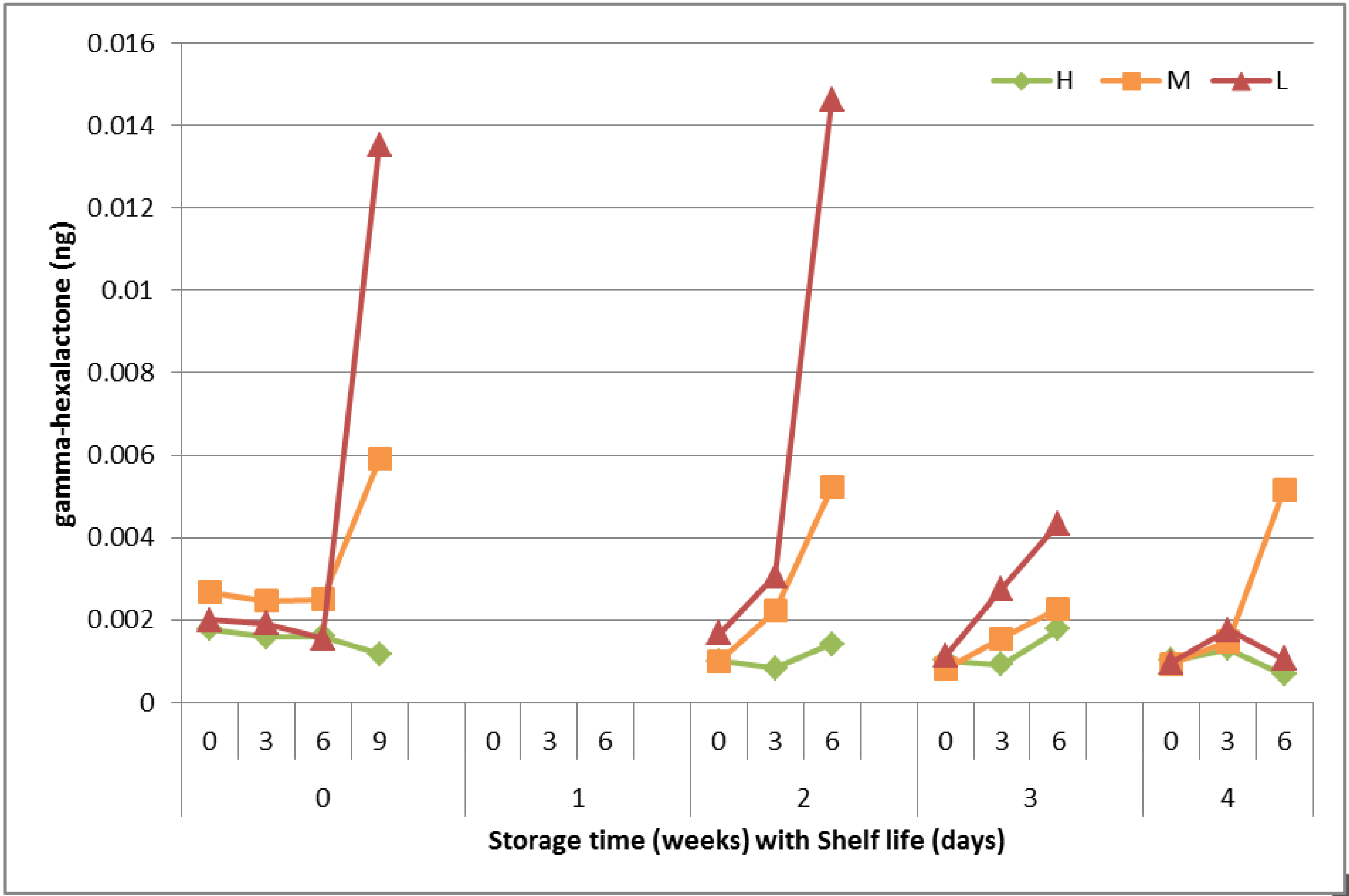


Economic Development, Jobs, Transport and Resources

AGRICULTURE VICTORIA

Cold storage

'Rose Bright' nectarine (2016-17)



Conclusions to date:

Fruit physiological maturity can be determined by ethylene production and is important at harvest:

This allows the utilisation of non-destructive instruments such as the DA meter to segregate fruit based on the physiological status

- For fruit to develop adequate peachy aroma perceived acceptable in the market place there must be ethylene production at harvest
- After cold storage and return to ambient temp some of the peachy aroma compounds show increased production
- There are difference between nectarines and peaches, and cultivars within these, showing not all cultivars behave the same with regard to aroma and fruit development.

Questions ?

How many  did you see?!

Thank you

Harvest Fact Sheet for 'August Bright' nectarine

(Draft June 2018)

'August Bright' is a clingstone, yellow flesh nectarine. It generally has crisp texture at harvest (6.0 to 7.0 kg/cm^2) that allows the fruit to be stored. 'August Bright' is harvested late January to mid February depending on the season and location. The DA-Meter should be used to monitor fruit ripening (minimum 60 fruit starting 4 weeks prior to harvest) to help identify optimal picking time.

Using the DA-Meter to estimate harvest

Optimal harvest has been identified by correlating fruit ethylene production and the I_{AD} maturity index obtained with the DA-Meter. 'August Bright' should be commercially harvested after the fruit has started to produce some ethylene with an I_{AD} maturity index between 1.19 and 0.50. Fruit above a maturity index of 1.20 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.49 are considered mature and should be sold immediately due to a short shelf life of 2 – 3 days.

Table 1. Ideal harvest quality indices for 'August Bight' nectarine.

Harvest period	Mid-late January to late February
Sweetness	13 – 17 °Brix
Firmness	6.0 – 7.0 kg/cm^2
DA-Meter maturity index	1.19 – 0.50

Harvest Fact Sheet for ‘September Bright’ nectarine

(Draft June 2018)

‘September Bright’ is a clingstone, yellow flesh nectarine. It generally has crisp texture at harvest (5.0 to 7.0 kg/cm^2) that allows the fruit to be stored. ‘September Bright’ is harvested early February to mid-March depending on the season and location. The DA-Meter should be used to monitor fruit ripening (minimum 60 fruit starting 4 weeks prior to harvest) to help identify optimal picking time.

<u>Using the DA-Meter to estimate harvest</u>

Optimal harvest has been identified by correlating fruit ethylene production and the I_{AD} maturity index obtained with the DA-Meter. ‘September Bright’ should be commercially harvested after the fruit has started to produce some ethylene with an I_{AD} maturity index between 1.29 and 0.50. Fruit above a maturity index of 1.30 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.49 are considered mature and should be sold immediately due to a short shelf life of 2 – 3 days.

Table 1. Ideal harvest quality indices for ‘September Bight’ nectarine.

Harvest period	Early February to mid-March
Sweetness	15 – 19 °Brix
Firmness	5.0 – 7.0 kg/cm^2
DA-Meter maturity index	1.29 – 0.50

Harvest Fact Sheet for 'Snow Flame 23' peach

(Draft June 2018)

'Snow Flame 23' is a clingstone, white flesh peach. It generally has crisp texture at harvest (4.0 to 5.0 kg/cm^2) that allows the fruit to be stored. 'Snow Flame 23' is harvested late November to mid-December depending on the season and location. The DA-Meter should be used to monitor fruit ripening (minimum 60 fruit starting 4 weeks prior to harvest) to help identify optimal picking time.

<u>Using the DA-Meter to estimate harvest</u>

Optimal harvest has been identified by correlating fruit ethylene production and the I_{AD} maturity index obtained with the DA-Meter. 'Snow Flame 23' should be commercially harvested after the fruit has started to produce some ethylene with an I_{AD} maturity index between 0.79 and 0.30. Fruit above a maturity index of 0.80 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.29 are considered mature and should be sold immediately due to a short shelf life of 2 – 3 days.

Table 1. Ideal harvest quality indices for 'Snow Flame 23' nectarine.

Harvest period	Late November to mid-December
Sweetness	12 – 13 °Brix
Firmness	4.0 – 5.0 kg/cm^2
DA-Meter maturity index	0.79 – 0.30
Shelf life	Up to 3 days

Harvest Fact Sheet for ‘Snow Flame 25’ peach

(Draft June 2018)

‘Snow Flame 25’ is a clingstone, white flesh peach. It generally has crisp texture at harvest (3.0 to 5.0 kg/cm^2) that allows the fruit to be stored. ‘Snow Flame 25’ is harvested late December to mid-January depending on the season and location. The DA-Meter should be used to monitor fruit ripening (minimum 60 fruit starting 4 weeks prior to harvest) to help identify optimal picking time.

<u>Using the DA-Meter to estimate harvest</u>

Optimal harvest has been identified by correlating fruit ethylene production and the I_{AD} maturity index obtained with the DA-Meter. ‘Snow Flame 25’ should be commercially harvested after the fruit has started to produce some ethylene with an I_{AD} maturity index between 0.99 and 0.50. Fruit above a maturity index of 1.0 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.49 are considered mature and should be sold immediately due to a short shelf life of 2 – 3 days.

Table 1. Ideal harvest quality indices for ‘Snow Flame 25’ peach.

Harvest period	Late December to mid-January
Sweetness	16 – 17 °Brix
Firmness	3.0 – 5.0 kg/cm^2
DA-Meter maturity index	1.0 – 0.50

Harvest and Storage Fact Sheet for 'Rose Bright' nectarine

(January 2018)

'Rose Bright' is a clingstone, yellow flesh nectarine. It generally has firm texture at harvest (6.5 to 8.0 kg/cm^2) that allows the fruit to be stored for up to 4 weeks. 'Rose Bright' is harvested mid-late November to mid-December depending on the season and location. The DA-Meter should be used to monitor fruit ripening (starting 4 weeks prior to harvest) to help identify optimal picking time. 'Rose Bright' does not show negative traits when stored for up to 4 weeks in air at 0.5°C. Fruit harvested at a DA-Meter maturity index between 0.89 and 0.50 has an acceptable shelf life length of 4 days (after 4 weeks storage) to 6 days (after harvest).

<u>Using the DA-Meter to estimate harvest</u>

Optimal harvest has been identified by correlating fruit ethylene production and the DA-Meter maturity index. 'Rose Bright' should be commercially harvested after the fruit has started to produce some ethylene with a DA-meter maturity index between 0.89 and 0.50. Fruit above a maturity index of 0.90 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.49 are considered mature and should be sold immediately due to a short shelf life of 2 – 3 days.

Table 1. Ideal harvest quality indices for 'Rose Bight' nectarine.

Harvest period	Late November to mid-December
Sweetness	11 – 15 °Brix
Firmness	6.5 – 8.0 kg/cm^2
DA-Meter maturity index	0.89 – 0.50

Table 2. optimal storage conditions for 'Rose Bright' nectarine

Temperature	0.5 - 2 °C
DA-Meter maturity index	0.89 – 0.50
Duration	up to 4 weeks in air
Shelf life at 20 °C	6 days after harvest; maximum of 4 days after 4 weeks storage

Harvest and Storage Fact Sheet for 'August Flame' peach

(Draft January 2018)

'August Flame' is a freestone, yellowish orange flesh peach. It generally has dense and crisp texture at harvest (5.5 to 8.0 kg/cm^2). 'August Flame' is harvested in February, depending on the season and location. The DA-Meter should be used to monitor fruit ripening, starting 4 weeks prior to harvest, to help identify optimal picking time. Fruit harvested at a DA-Meter maturity index between 1.39 and 0.90 has shown acceptable shelf life of up to 6 – 7 days after harvest. Preliminary storage trials at 0.5 °C showed presence of mealiness after 2 weeks of storage.

Using the DA Meter to Estimate Harvest:

Optimal harvest has been identified by correlating fruit ethylene production and the DA-Meter maturity index. 'August Flame' should be commercially harvested after the fruit has started to produce some ethylene with a DA-meter maturity index between 1.39 and 0.90. Fruit above a maturity index of 1.40 are too immature and should not be harvested due to difficulties in ripening correctly, and fruit with a maturity index below 0.90 are considered mature and should be sold immediately due to a short shelf life of 3 – 4 days.

Table 1. Ideal harvest quality indices for 'August Flame' peach

Harvest period	February
Sweetness	14 – 17
Firmness	5.5 – 8.0 kg/cm^2
DA-Meter maturity index	1.39 – 0.90

Table 2. optimal storage conditions for 'August Flame' peach

Temperature	0.5 -2 °C
DA-Meter maturity index	1.39 – 0.90
Duration	up to 2 weeks in air; longer storage duration may result in storage disorders.
Shelf life at 20 °C	6 days after harvest; maximum of 4 days after 4 weeks storage

Economic Development,
Jobs, Transport
and Resources

Premium Fruit to Asia Sub-project: Comparing stonefruit maturity, quality and volatile composition

Milestone report 11:
Annual Industry Technology Adoption Plan Implementation Report 2017-18

AGRICULTURE VICTORIA

Contents

Author: Sue McConnell and Mark Hincksman

Project CMI Number: 105224

Premium Fruit to Asia

INTRODUCTION

This report documents the implementation of the Premium Fruit to Asia 2017-18 Supplement to the Annual Industry Adoption Plan 2014-15 (McConnell and Hincksman 2017), in particular, the deliverables associated with the sub project 'Comparing stonefruit maturity, quality and volatile composition'. 2017-18 was the fourth and final year of the project and the activities documented in this report involved the development of a variety of communication tools, feedback loops and content creation tools developed by the project to accelerate industry adoption and gain feedback from growers.

Comparing stonefruit maturity, quality and volatile composition

Project outcome

Provide guidelines to identify maturity classes to optimise harvest timing depending on the market of choice, and to optimize cold storage and ripening protocols to consistently deliver high quality fruit to domestic and export markets.

Project background

Variable quality in stonefruit has been identified as a major impediment to producer profitability and sales in both domestic and export markets. The ability to optimize fruit maturity at harvest to meet expectations of the market of choice, to understand how fruit maturity affects storage and the impact on fruit composition and quality particularly sugar content, firmness and volatiles profile, will greatly improve fruit consistency and quality.

Variable maturity at harvest affects fruit responses to shelf life, cold storage and quality through the handling chain. Fruit composition and flavour volatiles in particular, are fundamental elements of fruit quality and therefore consumer acceptance. The fruit volatile profile is affected by fruit maturity on the tree, during postharvest storage and, subsequently, ripening. We aim to generate knowledge of the interaction between harvest maturity measured by the DA-Meter, storage and ripening behaviour and resultant effects on SSC, firmness and volatiles profile. This project is linked to the stonefruit trial orchard in Tatura (CMI 104603 & 104932) and to "optimal ripening protocols" (CMI 104937) which will deliver preliminary data on the varieties used and will add important information for the protocols.

This project will identify maturity classes to optimise harvest timing depending on the market of choice, with particular emphasis on export, for up to 10 nectarine and peach varieties. The project will generate cold storage and ripening protocols and the associated studies on volatile profiles will be done as part of a PhD degree. The results will provide knowledge to optimise fruit quality and consistency on domestic and export markets. Consistent, high quality fruit will increase consumer demand and provide a point of difference for Victorian stonefruit increasing both domestic and export markets by at least 5% equating to an increase in export value alone of $15 to $20 million per annum.

Project objectives

1. Identify I_{AD} index maturity classes for up to 8 commercial peach and nectarine cultivars used for export with correlations to other fruit maturity indices and fruit composition attribute such as ethylene production rate, firmness, sugar content and flavour (volatiles profile). (March 2018)
2. Develop cultivar-specific storage and ripening protocols to improve handling processes with particular emphasis on export while maximising fruit quality and aroma. (May 2018)
3. Contribute toward a PhD degree for a DEDJTR employee to further develop DEDJTR capabilities and expertise in fruit plant physiology. (May 2018)
4. Implement the annual information product and service delivery plan (May 2018)
5. Manage the project to deliver on time and on budget to an acceptable AR standard (June 2018)

MILESTONE ACHIEVMENT

This milestone report fulfils Milestone 11 of 'Comparing stonefruit maturity, quality and volatile composition' as it documents the implementation of the Premium Fruit to Asia, 2017-18 Supplement to the Annual Industry Adoption Plan 2014-15 (McConnell and Hincksman 2017).

Report against products and services plan

The proposed products and services for 2017-18 were documented in the Premium Fruit to Asia, 2017-18 Supplement to the Annual Industry Adoption Plan 2014-15 (McConnell and Hincksman 2017). Table A lists the deliverables relevant to the 'Comparing stonefruit maturity, quality and volatile composition' sub-project with a description of the activities undertaken. Samples of the deliverables are attached in Appendix A.

Table A

Deliverable(s)	Status	Description
Profitable stonefruit network E-newsletters	Completed	A new hin.com.au web platform was developed and went live in March 2018 hosting the Profitable stonefruit network incorporating >35 web pages of stonefruit research. http://www.hin.com.au/networks/profitable-stonefruit-research 4 e-newsletter for profitable stonefruit network completed and distributed to stonefruit network
Stonefruit video series including stonefruit field lab experiment reports	Completed	A series of 10 stonefruit videos were completed and loaded on the profitable stonefruit network on hin.com.au: • Continuous detection of plant water status in September Bright nectarines http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-irrigation-trials/continuous-detection-of-plant-water-status-in-high-density-september-bright-nectarines • Stonefruit sanitation through fumigation experiment for export opportunities • Harvest and cold storage impacts on the aroma volatile compounds of peach & nectarine https://www.youtube.com/watch?v=1aTocRFWsK8 • Control of Carpophilus Beetles with Dr Mofakhar Hossain http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-grower-events • Palmette and Cordon Tree Demonstrations https://www.youtube.com/watch?v=hbrkgJbJQgE • Stonefruit Crop load management for high quality fruit http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-canopy-crop-load-trials/2017-crop-load-management-results • Orchard establishment for high quality fruit https://www.youtube.com/watch?v=r_cmvCJdO0U • Stonefruit Irrigation experiment for high quality fruit https://www.youtube.com/watch?v=HAb9Tl8HPqE • Cutri Fruit http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-grower-events
Photo collection	Completed	23 time series videos were developed from photos of seasonal changes and growth stages of different cultivars/rootstocks/treatments and loaded on hin.com.au. http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-canopy-crop-load-trials/time-series-photos
DA meter data repository	Completed	DA meter IAD maturity classes database placed on hin.com.au http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-maturity-and-fruit-quality/da-meter-iad-maturity-classes-database
Roadshow to Renmark, Swan Hill & Cobram	Completed	42 growers and packers attended the Stonefruit Innovative Research roadshow in Renmark, Swan Hill & Cobram at the end of August 2017 (organised by Horticulture Services). Five AVR researchers presented at the roadshow. http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-grower-events

PFtA case study	Completed	Premium Fruit to Asia case study completed, in pdf & interactive online version, on the Horticulture Services component of the AVR sub-project 'Comparing stonefruit maturity, quality & volatile composition' 2014-2018. http://pub.lucidpress.com/d53cef3a-6b48-4843-b0f5-cc703ac9b51d/ Premium Fruit to Asia case study stonefruit

REFERENCES

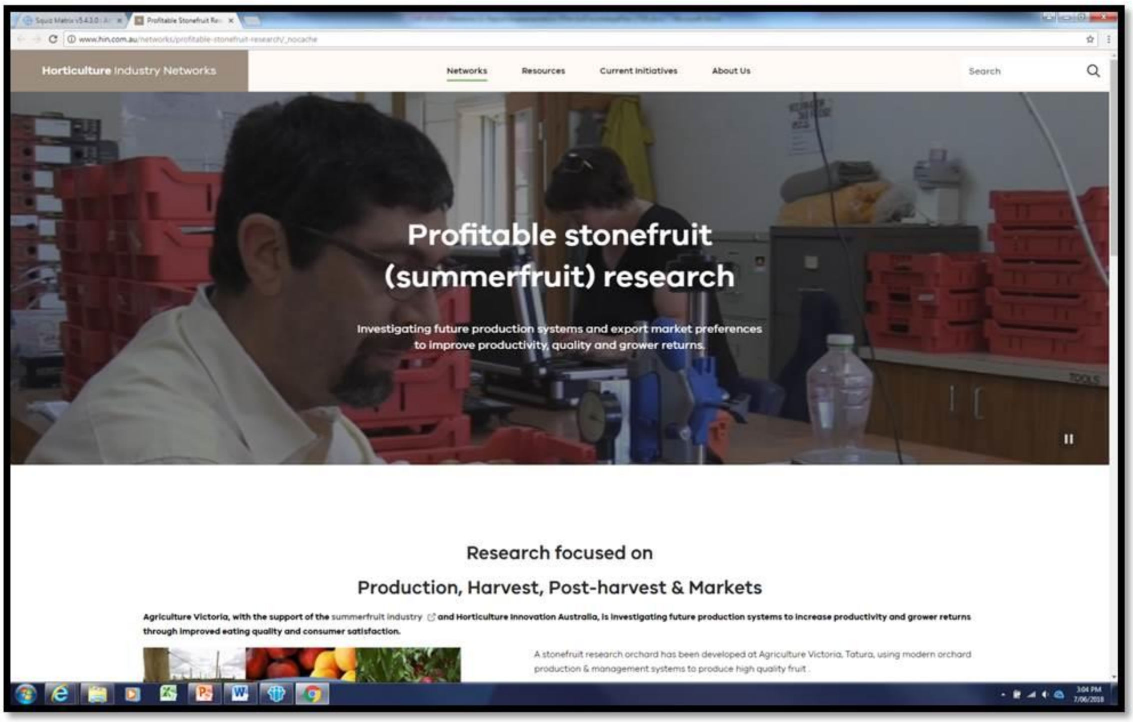
McConnell, S and Hincksman, M, 2017, 'Premium Fruit to Asia, 2017-18 Supplement to Annual Industry Technology Adoption plan 2014-15', Department of Environment and Primary Industries, Victoria.

APPENDICES

Appendix A: Examples of deliverables from Premium Fruit to Asia, 'Comparing stonefruit maturity, quality and volatile composition' sub-project.

1. New hin.com.au web platform – Profitable stonefruit network

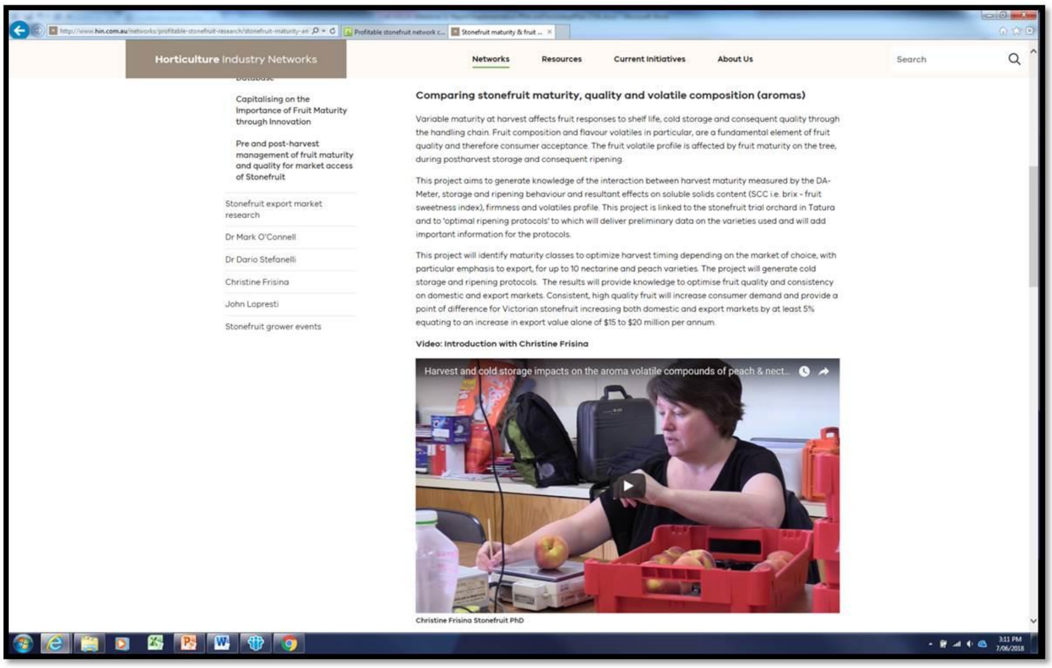
http://www.hin.com.au/networks/profitable-stonefruit-research



2. Profitable stonefruit network e-newsletter

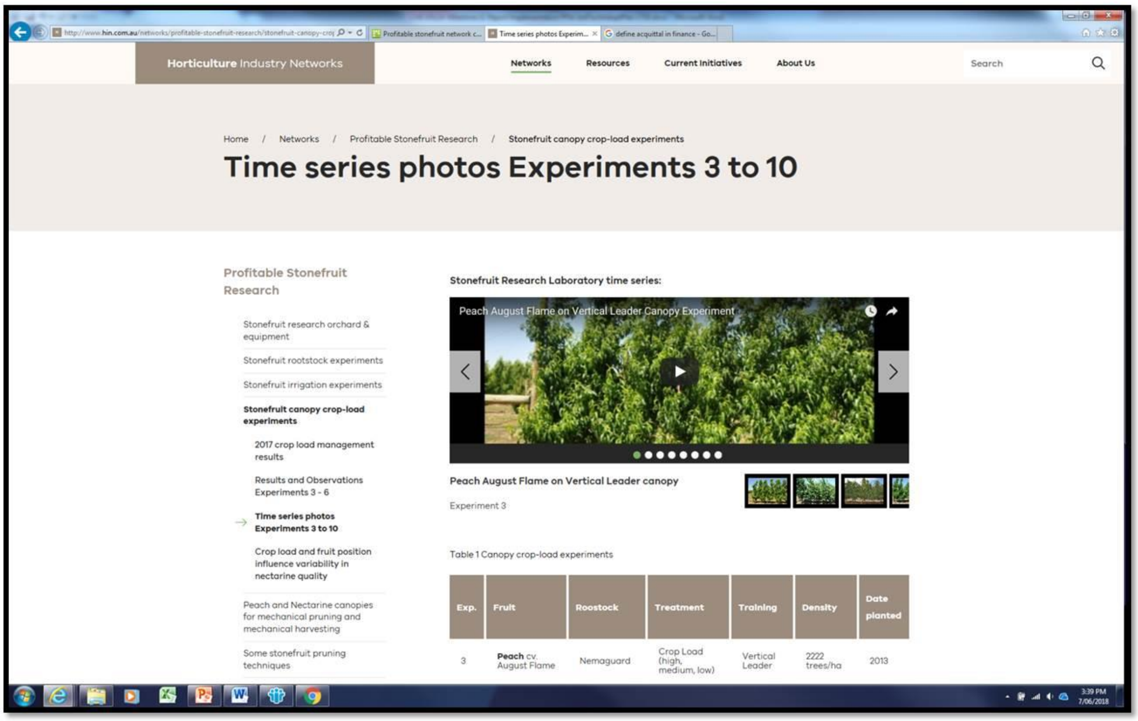
3. Stonefruit video series

http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-maturity-and-fruit-quality



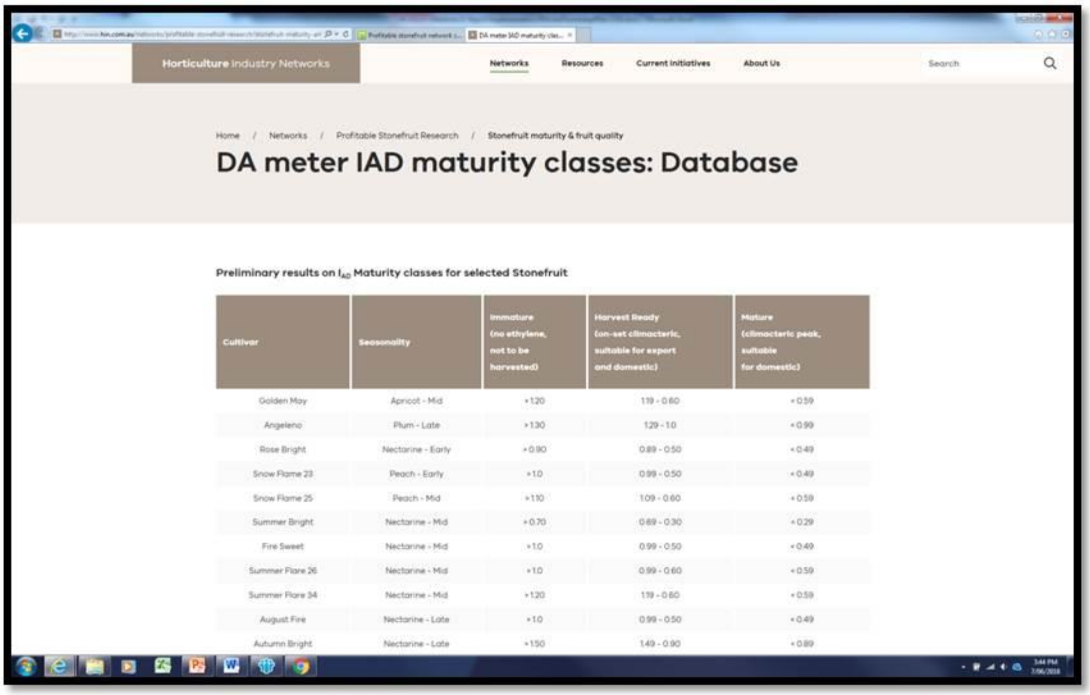
4.Time series videos

http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-canopy-crop-load-trials/time-series-photos



5. DA meter data repository

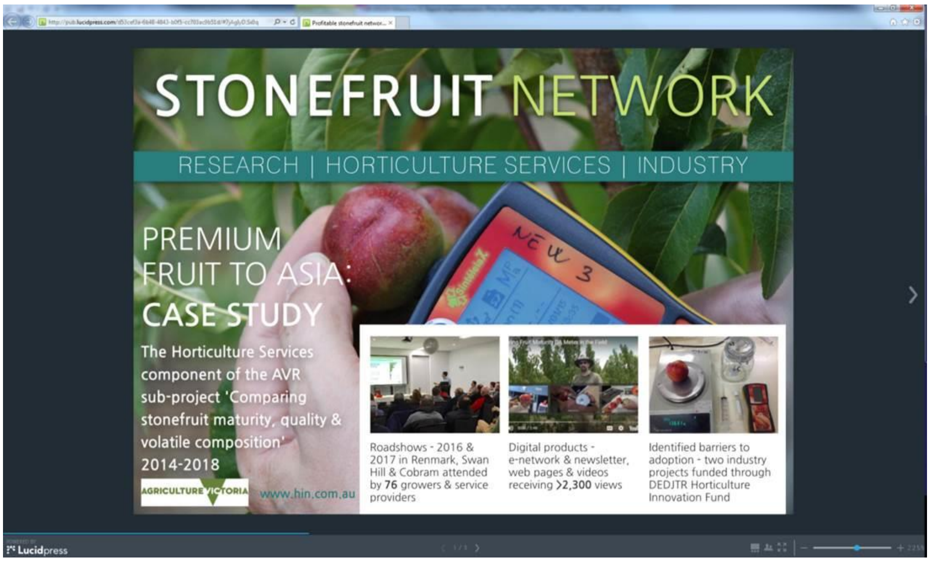
http://www.hin.com.au/networks/profitable-stonefruit-research/stonefruit-maturity-and-fruit-quality/da-meter-iad-maturity-classes-database



Cultivar	Seasonality	Immature (no ethylene, not to be harvested)	Harvest Ready (on-set climacteric, suitable for export and domestic)	Mature (climacteric peak, suitable for domestic)
Golden May	Apricot - Mid	>1.20	1.19 - 0.60	<0.59
Angeleno	Plum - Late	>1.30	1.29 - 1.0	<0.99
Rose Bright	Nectarine - Early	>0.90	0.89 - 0.50	<0.49
Snow Flame 23	Peach - Early	>1.0	0.99 - 0.50	<0.49
Snow Flame 25	Peach - Mid	>1.10	1.09 - 0.60	<0.59
Summer Bright	Nectarine - Mid	>0.70	0.69 - 0.30	<0.29
Fire Sweet	Nectarine - Mid	>1.0	0.99 - 0.50	<0.49
Summer Flare 26	Nectarine - Mid	>1.0	0.99 - 0.60	<0.59
Summer Flare 34	Nectarine - Mid	>1.20	1.19 - 0.60	<0.59
August Fire	Nectarine - Late	>1.0	0.99 - 0.50	<0.49
Autumn Bright	Nectarine - Late	>1.50	1.49 - 0.90	<0.89

6. Case study

http://pub.lucidpress.com/d53cef3a-6b48-4843-b0f5-cc703ac9b51d/



Stonefruit Roadshow Evaluation Summary

Location & date: Cobram 3/8/16

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Oscar Villalta

Present: 14 growers, 7 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- DA meter x4
    - We use it
- Management of brown rot (treatment with fungicides & how to spray) x3
- Consistency of fruit maturity and improving this
- Information regarding export nectarines to China
- Infection criteria table (brown rot)
- Examples of how to present powerpoint
- Virtual orchard walks
- What the best tree crop is
- Coming up to speed with the stonefruit projects and how to access the information

Is there anything you would like more information on?

- More information on the DA meter and the relevant unit measured for fruit on variety basis
- What is the best tree spacing for quality
- Tree spacing information
- What involvement of supermarkets in the project
- MRLs to China
- How is the best way to control brown rot
- Pruning techniques for different canopies both winter & summer
- Fertiliser & irrigation management
- PGR's for fruit growth
- MRL management throughout the season

Would you attend an event like this again?
7 YES ie 100%
0 No

Stonefruit Roadshow Evaluation Summary

Location & date: Renmark 26/7/16

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Jason Size

Present: 12 growers, 9 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- A way to measure how old fruit is and how it can help make decisions where to sell the fruit
- Use of DA to predict retain & harvest
- Ethylene protocol work
- DA meter x2
- The field laboratory – learning about what is happening at Tatura
- HIN website x2
- All
- Yield, quality, variety research

Is there anything you would like more information on?

- Rootstocks
- Assessment of fruit at fruit purchase point & influence of post-harvest treatments on ripening & managing delivery to the consumer
- Ethylene protocol
- Grower manual
- DA meter & varieties – will values be consistent across growing regions. Can we share cost of varieties as there are many
- DA meter
- Some experience on return on value from use of DA meter
- Another presentation next year if possible
- Brown rot research – as per video on Summerfruit SA website when made available

Would you attend an event like this again?

9 YES ie 100%

0 No

Stonefruit Roadshow Evaluation Summary

Location & date: Swan Hill 27/7/16

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Oscar Villalta

Present: 10 growers, 7 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- DA meter x6
  - Testing
  - How meter works
  - Use & info on the DA meter & how we can improve harvest
  - Being able to predict fruit maturity with the DA meter
- Ethylene correlation protocol
- Brown rot program x6
- HIN network

Is there anything you would like more information on?

- Management of brown rot and China MRL
- More basics regarding the stone fruit industry
- The research farm at Tatura
- Fruit ripening brown rot
- Trials

Would you attend an event like this again?

7 YES ie 100%
0 No

Stonefruit Roadshow Evaluation Summary

Location & date: Renmark 29/8/17 (7:20pm -10:30pm)

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Jason Size, Mofakhar Hossain, Christine Frisina

Present: 12 growers/packers/service suppliers, 8 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- Good talks from all speakers, well done
- All
- Information on China markets plus others
- Beetle control
- Picking for optimal flavour
- Rootstock
- Tree planting space seeing difference in crop/flower/size & cost per ha
- To grow fruit
- Confirmed DA work
- Confirmed that we need to pick at correct DA or maturity to get optimum aroma compound release & consumer (China) acceptance

Is there anything you would like more information on?

- Interested in China market demands as more info comes to hand
- More information on China markets
- Any topic to assist in growing better quality fruit
- Rootstock & crop
- Watering crop at different stages of the year
- DA meter
- Ethylene
- Specific aroma compound work

Would you attend an event like this again?

8 Yes = 100%
0 No

Do you receive the Profitable stonefruit newsletter?

2 Yes
6 No

If yes, has the newsletter provided any information that would be useful to implement in your business?

Do you use a DA meter?

3 Yes
5 No

If yes. Is there any information you require to make it more useable?

- Within the Qfm group we have one & we have a person who does all the DA meter work

If no, what are your reason(s) for not using it?

- I use my years of experience
- Don't have property yet
- The boss used it instead
- Cost
- No need

Stonefruit Roadshow Evaluation Summary

Location & date: Swan Hill 30/8/17 7pm-9:45pm

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Christine Frisina, John Lopresti

Present: 17 growers/packers/service suppliers, 10 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- The theories are good – not ready to use
- Consumers demand in China/consumer preference for fruit in China x2
- Variations of ethylene
- DA meter
- All of it/ in maturity's in china yellow nectarines are acceptable for consumer consumption
- All
- Don't pick too green
- DA meter x2

Is there anything you would like more information on?

- How we relate this science to the daily demands of supplying supermarket & export customers
- Results for irrigation to yield in T/ha & Meg/ha
- Consumer consumption
- China
- More DA numbers so we can actually use the technology
- DA meter

Would you attend an event like this again?

10 Yes = 100%
0 No
One Yes proviso, if it achieves the challenges of having the science ready to use

Do you receive the Profitable stonefruit newsletter?

5 Yes
5 No

If yes, has the newsletter provided any information that would be useful to implement in your business?

- Research currently happening – production figures on water & crop load

Do you use a DA meter?

1 Yes
9 No

If yes. Is there any information you require to make it more useable?

- The table on values that each variety reaches maturity – best per specific market. How to meet a (8 day best before) when supplying supermarket

If no, what are your reason(s) for not using it?

- Extra time needed in the day
- Cost due to us being small producers
- Don't have our varieties
- Cost to implement
- Time x3
- Not enough data
- Never used or seen before x2Don't need it

Stonefruit Roadshow Evaluation Summary

Location & date: Cobram 31/8/17 7pm-10pm

Presenters: Mark Hincksman, Dario Stefanelli, Mark O'Connell, Christine Frisina, John Lopresti

Present: 11 growers/packers/service suppliers, 7 evaluation responses

What did you hear tonight that would be useful to implement in your business?

- Information about fruit maturity and export market
- Post Harvest
- Maybe DA Meter

Is there anything you would like more information on?

- Fruit assessment for China
- Effects of cold storage on fruit quality
- Post harvest cooling
- Post harvest storage on stonefruit

Would you attend an event like this again?

7 Yes = 100%
0 No

Do you receive the Profitable stonefruit newsletter?

5 Yes
2 No

If yes, has the newsletter provided any information that would be useful to implement in your business?

Do you use a DA meter?

0 Yes
7 No

If yes. Is there any information you require to make it more useable?

- The table on values that each variety reaches maturity – best per specific market. How to meet a (8 day best before) when supplying supermarket

If no, what are your reason(s) for not using it?

- Prefer NIR meter
- Had a look into it. Not really required at the moment
- Haven't bought one yet
- Pricing
- Didn't know about it