Final Report

Building Resilience to Drupelet Disorder in Rubus

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Project:

Building Resilience to Drupelet Disorder in Rubus – RB14003

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Content

Summary

This project investigated the causes, mechanisms, and potential management strategies for red drupelet reversion (RDR) in blackberries (*Rubus* spp.). RDR is a physiological disorder of blackberries, whereby individual or groups of drupelets that are black at harvest revert to red, usually after the fruit has been harvested and placed into cool storage. RDR reduces the visual and physical quality of the fruit and is considered a major physiological disorder of commercial blackberries. This project examined the physiochemical changes that occur during RDR development and investigated pre and postharvest factors associated with the development of the disorder.

The project involved field and laboratory trials located in Tasmania over a three-year period. Laboratory trials were undertaken to establish and quantify the underlying physiochemical changes associated with RDR. Field trials were then designed to assess the effects of nitrogen application rate, harvest technique, environmental conditions at harvest, and postharvest storage conditions on the incidence and severity of RDR.

The colour change is associated with a decrease in anthocyanin concentration, reduced cellular integrity, reduced drupelet firmness and lower pH. The symptoms associated with the disorder are indicative of mechanical injury to affected drupelets. Susceptibility to RDR is genotypically influenced, with evidence that cultivar firmness, cell wall formation and weight loss can influence incidence and severity of expression. Abiotic stresses, particularly warm temperatures during harvest, were linked to high rates of RDR through increasing the mechanical injuries incurred during harvest and handling. Excessively high nitrogen application rates were associated with an increase in RDR incidence. Rapid temperature changes in postharvest storage were associated with more severe colour change in affected drupelets.

Outputs produced by the project included six published or submitted refereed journal articles, four fact sheets circulated to a growers at state or national industry conferences, and nine oral presentations at state, national, and international industry conferences or field days. The outputs of this project are collated in a thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy to the University of Tasmania, which is included as an appendix to this report.

The major recommendations from the project include:

- Harvesting techniques should be optimised to reduced double and rough handling of fruit. Where practical, fruit should be harvested directly into punnets, and care should be taken during transport to minimise vibrational damage
- Harvesting conditions should be managed to limit handling of blackberries at extreme temperatures. This includes harvesting during the early morning or evening and avoiding harvesting on extremely warm days. Fruit temperatures exceeding 23 °C during handling and transport will significantly increase the incidence and severity of RDR.
- Punnet design and postharvest technologies to reduce mechanical injury to fruit should be explored. Unnecessary fruit-on-fruit contact could be reduced through using punnets which contain only one layer of fruit. With the emergence of larger-fruiting cultivars, commonly used punnet designs may need to be adjusted to better suit these varieties.
- Once cooled, fruit should remain cool to reduce the incidence and severity of RDR.
- The development of cultivars with low susceptibility to RDR should be pursued. Skin firmness, texture, and water loss are correlated with RDR susceptibility.

Keywords

Blackberry; red drupelet reversion; drupelet disorder; postharvest; anthocyanin; shelf life

Introduction

Red drupelet reversion (RDR), sometimes referred to as red drupelet disorder, colour reversion, reddening, or red cell, is a physiological disorder of blackberries that causes individual or groups of drupelets which are black at harvest to turn red postharvest. Until recently, the disorder has not been well understood, with little research into the physiology or management techniques to reduce incidence of RDR. As Australian and worldwide blackberry production has increased over the last decade, reducing the incidence and severity of RDR has become an issue of more importance to producers and retailers.

The broad aim of this project was to advance the knowledge of the causes, mechanisms, and management practices for RDR in commercial blackberries. Following a review of the literature and a survey of Australian producers, four key goals were identified and research was designed to address these:

1. *To identify and quantify the physiochemical changes occurring in drupelets affected by RDR.*

The underlying physiological mechanisms associated with RDR had not previously been reported, and so establishing this was necessary to further investigate the disorder. This involved attempting to induce RDR in blackberries and investigating the physiochemical changes occurring at a fruit, drupelet, and cellular level. This work provided a basis for understanding susceptibility to RDR and further refined the direction of the research.

2. *To identify any physical or environmental factors involved in expression of RDR.*

Following the initial study identifying the physiochemical changes occurring during RDR, mechanical injury was identified as a key factor in the development of the disorder. To investigate this, multiple experiments examining the effects of handling, climatic conditions at harvest, and postharvest storage conditions on incidence and severity of RDR were undertaken.

3. *To identify plant nutrition that may be contributing to an increase in RDR.*

An anecdotal relationship between nutrition and RDR had been observed among blackberry producers within Australia and overseas. Specifically, the hypothesis that excess nitrogen fertiliser application during harvest can significantly increase the susceptibility of blackberries to red drupelet disorder was investigated.

4. *To identify and develop potential pre- or postharvest techniques to reduce the incidence of RDR.*

As well as investigating factors associated with high rates of the disorder, this project aimed to address practical techniques to reduce the incidence of RDR in commercial settings.

Methodology

Review of relevant literature and industry survey

A survey of Australian *Rubus* growers and an initial review of the relevant literature was conducted to establish the level of current knowledge of the disorder and further refine the research questions. The results of the grower survey and initial literature review were not published, but were used to refine the research direction of the project. A lack of a comprehensive review of previous literature was identified, and so a comprehensive review of the literature was updated for publication to include the results of this project (Appendix 1, Chapter 2).

Physiochemistry of blackberries affected by RDR

The first key goal of the project, as identified by the initial survey and literature review, was to identify and quantify the underlying physiochemical associated with RDR development. This work was necessary to provide a fundamental base of knowledge for the remainder of this research as well as future study in this field.

'Fully black', 'partially red', and 'fully red' drupelets were excised from fresh blackberry fruit and analysed fresh for structural properties, or frozen at -80 °C for later analysis of physiochemical properties. Analyses included: observation of cellular structural properties of drupelets by light microscopy, observation of skin structural properties by electron microscopy, anthocyanin pigment concentration and profile by HPLC, CIELAB colour profile of drupelets, titratable acidity, total soluble sugars, pH, electrolyte leakage, and drupelet skin firmness by penetrometer testing.

Effects of climatic conditions during harvest and handling on the postharvest expression of red drupelet reversion in blackberries

This trial was designed to investigate the effects of injury inferred by handling fruit during harvest, and how environmental conditions at harvest influenced this. Environmental conditions at harvest had also been suggested by previous research, and anecdotally by producers, as contributing to high rates of the disorder, but no studies had investigated this thoroughly.

Fruit were harvested on ten occasions over two days by one of two methods: either hand-harvested into shallow buckets and transferred to industry standard 125 g clamshell punnets (standard practice), or harvested carefully without handling by cutting the pedicel and placing each fruit into individual cotton wool-lined trays. The number of partially red and fully red drupelets per fruit was counted, firmness was measured by compression, and skin firmness was measured by a penetrometer. Air and fruit skin temperature, relative humidity, vapour pressure deficit and soil water tension were all influenced by the time of day.

Nitrogen application rate and harvest date affect red drupelet reversion and postharvest quality in 'Ouachita' blackberries

This two-year field trial was designed to address the third goal of the project; to identify plant nutrition that may be contributing to an increase in RDR. A potential relationship between excessive nitrogen application and increased susceptibility to the disorder was identified by multiple anecdotal grower survey responses.

The experimental layout for the trial consisted of a randomised complete block design, with each of the three polytunnels treated as a block. Each block contained three 106 m long rows of 'Ouachita' blackberry canes spaced at 2.5 m intervals. Each row was treated with a randomly allocated treatment of low (53 kg N ha^{-1}), medium (106 kg N ha^{-1}), or high (212 kg N ha^{-1}) rate of N fertiliser each season over a two-year period from 2016 to 2017. RDR incidence and severity, yield, fruit mass, firmness, and physiochemical quality were analysed at 11 harvests over the two seasons. Mineral analysis of fruit and post-season primocane leaves was undertaken.

Flesh temperature during impact injury and subsequent storage conditions affect the severity of colour change caused by red drupelet reversion in blackberries

The potential for rapid rates of cooling to exacerbate the disorder has been raised by producers repeatedly and investigated with mixed results by previous authors. In this trial, we investigated rapid versus slow cooling under laboratory conditions.

In order to induce RDR, individual fruit were subjected to mechanical injury from a steel ball dropped from a height of 25 cm at initial temperatures of 15, 25, and 35 °C. Following injury, fruit were either rapidly cooled to 2°C in a -24 °C cooler or slowly in a 2 °C cooler. The colour of the impact site and of the undamaged control fruit were measured 24 hours and 7 days after the initial impact injury using a colorimeter. 80 fruit were randomly assigned to each of the bruise temperature treatments. 40 fruit per treatment were bruised and 40 were unbruised controls. Of each group of 40 fruit, 20 were subjected to each cooling rate treatment after the impact injury.

The impact site on each fruit and a site on the side of each control fruit were assessed for colour change 24 h and 7 days after the initial impact injury. CIELAB colour space values were measured using a CR-400 colorimeter.

Communication and extension

The project involved continual communication of key results and recommendations to industry via fact sheet development and oral presentation at industry conferences and field days. Fact sheets are presented in Appendix 1-A, and oral presentations are listed in the Outputs section of this report.

Outputs

Refereed communications arising from this project

Published

Edgley, M., Close, D.C., and Measham, P.F. (2018). The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels. Acta Horticulturae. 1205, 885-890. DOI: 10.17660/ActaHortic.2018.1205.113.

Submitted

Edgley, M., Close, D.C., Measham, P.F., and Nichols, D.S. (Submitted). Physiochemistry of blackberries (Rubus L. subgenus Rubus Watson) affected by red drupelet reversion.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Effects of climatic conditions during harvest and handling on the postharvest expression of red drupelet reversion in blackberries.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Nitrogen application rate and harvest date affect red drupelet reversion and postharvest quality in 'Ouachita' blackberries.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Flesh temperature during impact injury and subsequent storage conditions affect the severity of colour change caused by red drupelet reversion in blackberries.

Prepared for submission

Edgley, M., Close, D.C., and Measham, P.F. Red drupelet reversion in blackberries: A complex of genetic and environmental factors.

Non-refereed articles, conference presentations, posters, and other outputs arising from this project

Edgley M., Close D.C., and Measham P.F. (2015), 'The use of modified atmosphere packaging to extend the shelf-life of a range of commercial raspberry varieties in cool storage', XI International Rubus and Ribes Symposium, Asheville, North Carolina, USA – *Poster presentation*

Edgley M., Close D.C., and Measham P.F. (2015), 'Red drupelet disorder in blackberries', Fruit Growers Tasmania Grower Field Day, Huonville, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2016), 'Red drupelet reversion: 2016 update', Fruit Growers Tasmania Annual Conference, Hobart, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2016), 'Managing red drupelet disorder', Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2016), 'The effects of nitrogen fertiliser on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries', I International Symposium on Protected Cultivation in Tropical and Temperate Climates & X International Symposium on Protected Cultivation in Mild Winter Climates, Cairns, Australia – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), 'Research into red drupelet disorder in Australia' Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia, USA – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), 'Red drupelet disorder in blackberries: 2017 update' Fruit Growers Tasmania Annual Conference, Launceston, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), 'Managing red drupelet disorder', Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2017), 'Causes and mechanisms of red drupelet reversion in commercial blackberries', 2017 School of Land and Food Annual Conference Program, Hobart, Tasmania – *Oral presentation*

Tasmanian Institute of Agriculture (2017), 'Blackberry reversion 2017', https://www.youtube.com/watch?v=IYxrA-PwYL8 – *Youtube video*

Edgley M., Close D.C., and Measham P.F. (2018), 'Causes and mechanisms of red drupelet reversion in commercial blackberries', BerryQuest International 2018, Launceston, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2018), 'Managing red drupelet disorder', Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2018), 'Causes and mechanisms of red drupelet reversion in commercial blackberries' III International Berry Fruit Symposium, Istanbul, Turkey – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2019), 'Causes and mechanisms of red drupelet reversion in commercial blackberries', Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia – *Oral presentation*

Outcomes

The findings presented in the various outputs contained within this report have relevance to commercial blackberry producers, retailers, and breeders, as well as implications for future research into both RDR and broader postharvest quality of blackberry fruit. The results from Appendix 1.4 show that the physiochemical symptoms associated with RDR are consistent with mechanical injury to fruit, resulting in cell decompartmentalisation and the subsequent degradation of anthocyanin pigments.

The results presented in Appendix 1.5 demonstrated that handling of fruit is strongly associated with RDR development, and that environmental conditions resulting in fruit skin temperatures exceeding 23 °C during handling can significantly exacerbate the incidence and severity of the disorder. These findings offer further support to the conclusions from Appendix 1.4, and strongly implicate cell disruption as a major mechanism involved in RDR development. Appendix 1.7 then demonstrated that storage conditions following mechanical injury can influence the severity of colour change associated with RDR. This suggests that postharvest storage may be able to be manipulated in order to reduce the severity of the disorder, which offers opportunity for further study.

The results from Appendix 1.6 show that incidence of RDR can be influenced by the N fertiliser application rate. This effect varied with harvest date but was significant in six harvests over the two-year trial. This finding offers some explanation for previous anecdotal observations and offers opportunity for further research into the effects of plant nutrition on RDR and broader blackberry fruit quality.

Due to the lack of a published comprehensive review of the literature detailing the extent of the current knowledge of RDR, the literature review contained in Appendix 1.2 of this report is of importance to further investigations in this field. The rapid expansion of the worldwide blackberry industry over the last two decades has not been fully matched with an increase in study into the fruit's physiology, highlighted by the lack of published data on RDR as well as other physiological disorders and plant-soil interactions. In recent years, this project and other concurrent studies have resulted in a significant growth in knowledge surrounding the genotypic variance, physiochemical mechanisms, and environmental influences on RDR expression. Appendix 1.2 consolidates the information generated from the previous sporadic studies and current work into a comprehensive article summarising the available data on RDR. It is intended that this work, when published, will be of interest to both academic and commercial parties, as well as promoting a deeper understanding of this complex and commercially important disorder.

The data presented in the thesis contained in Appendix 1 establish some key mechanisms and causes of RDR. This research has highlighted the importance of environmental factors, fruit handling practices, agronomic management and postharvest factors in RDR development. These findings will contribute to the development of management techniques and future studies incorporating a range of blackberry cultivars and growing environments.

Summary of key findings

- RDR in blackberries is associated with cellular disruption, loss of membrane integrity and decompartmentalisation in affected drupelets. These processes lead to the degradation of anthocyanin pigments and the resultant colour change associated with the disorder.
- Mechanical injury incurred during handling and transport of fruit is strongly associated with the development of RDR.
- Environmental conditions causing fruit temperatures to exceed 23 °C during harvest appear to significantly

exacerbate the degree of structural damage incurred by handling. Further study is needed to investigate the effects of other confounding environmental variables.

- Excessive N application during fruit development and harvest may be associated with increased incidence of RDR. However, rates typically applied in commercial production did not affect the incidence or severity of RDR.
- Inter and intra-seasonal variation in RDR incidence and severity is likely caused by variation in environmental conditions at individual harvest dates.
- Rapid cooling following mechanical injury may exacerbate the severity of colour change.

Monitoring and evaluation

The proposal for this project was submitted under the previous HAL project proposal system and as such did not contain a formal monitoring and evaluation plan. However, project success was addressed in the UTAS research plan, targeting academic outputs and industry engagement. Some key details for these are detailed below:

This project actively engaged industry stakeholders at state, national, and international conferences and events in order to disseminate the latest results, recommendations, and outputs. This process was essential to improve the knowledge surrounding RDR, as well as gain feedback from growers and researchers in this field. As well as formal events, regular discussion was had with industry partners during field trials, providing feedback which refined the research direction and trial design.

The project also generated significant international interest, with numerous industry figures contacting project researchers with input, feedback, and questions regarding the projects results and outputs. Presentations generated from the project have been circulated on two international caneberry blogs to a wide audience, and updates have been presented at five internationally attended conferences. A youtube video detailing results from the first two years of the project was developed and has generated 180 views in 18 months, and has been shared on Facebook by industry stakeholders.

The six publications from the project are expected to generate ongoing engagement with industry and researchers in this field. In particular, the comprehensive literature review and quantification of the physiochemical changes associated with RDR represent significant advancements in this field and deliver highly relevant findings for both industry and academic purposes.

Recommendations

The below recommendations are made to reduce the incidence and severity of RDR in Australian blackberry production:

- Harvesting techniques should be optimised to reduced double and rough handling of fruit. Where practical, fruit should be harvested directly into punnets, and care should be taken during transport to minimise vibrational damage
- Correctly training harvest workers should be a high priority for producers in order to reduce mechanical injuries incurred during harvest.
- Harvesting conditions should be managed to limit handling of blackberries at extreme temperatures. This includes harvesting during the early morning or evening and avoiding harvesting on extremely warm days. Fruit temperatures exceeding 23 °C during handling and transport will significantly increase the incidence and severity of RDR.
- Cane and field management should be designed around reducing the field heat that fruit are exposed to. Cane architecture to encourage fruit shading, the use of shade cloth, or shading structures should be considered.
- Punnet design and postharvest technologies to reduce mechanical injury to fruit should be explored. Unnecessary fruit-on-fruit contact could be reduced through using punnets which contain only one layer of fruit. With the emergence of larger-fruiting cultivars, commonly used punnet designs may need to be adjusted to better suit these varieties.
- Nitrogen application rates should be kept within recommended ranges to reduce susceptibility to RDR. Excessive nitrogen over multiple years may promote increased incidence and severity of the disorder.
- Agronomic management techniques should be investigated further to fully understand the nutrient-fruit quality relationships for specific cultivars and environments. Any links between agronomic management and fruit firmness should be explored.
- Postharvest storage including temperature during handling and rapid temperature changes can influence the severity of RDR, though reducing cooling rate should be thoroughly evaluated for further effects on shelf-life.
- Once cooled, fruit should remain cool to reduce the incidence and severity of RDR.
- The effect of temperature on the amount of vibration damage incurred during transport of fruit should be investigated.
- The development of cultivars with low susceptibility to RDR should be pursued. Skin firmness, texture, and water loss are correlated with RDR susceptibility.

Future research direction

RDR is an issue of growing importance to blackberry producers and researchers, as evidenced by the increasing number of research projects investigating various aspects of the disorder in recent years. This increase has seen substantial growth in the understanding of the underlying physiological mechanisms and causes of RDR. Despite this, considerable knowledge gaps still exist in this area of research.

Further study to clarify any underlying physiological reasons for the genotypic variance in incidence and severity will be of interest to breeders and growers in order to select cultivars with low susceptibility to RDR. As well as this, a better understanding of what physiological characteristics provide resistance to RDR may further confirm or clarify our conclusions as to the major factors causing RDR expression.

There are a growing number of studies investigating the effects of preharvest environmental factors on RDR expression, such as those shown in Appendix 1.5. While some consistency has been reported across disparate environments for the effects of temperature during harvest on RDR incidence, the effects appear to vary with genotype and are potentially confounded by other climatic variables. Additional data for a range of commercially important cultivars may clarify these conclusions, though care should be taken to assess a broad range of climatic variables to minimise any bias in results.

Nutritional links to RDR should be further investigated. This thesis established that increased N rates can influence RDR incidence; however, the underlying causes behind this remain unclear. This thesis offers substantial opportunities to continue and broaden this area of research to fully understand the influence of nutrient fertiliser application rates on RDR expression.

As discussed in Appendix 1.2 and 1.6, inconsistencies currently exist in the reported techniques used to assess the incidence and severity of RDR. In order to better enable future researchers and industry to be able to compare rates of the disorder across studies and environments, work should be done to develop a standard management technique for sampling the incidence and severity of RDR in practical settings. While counting the total number of affected drupelets per fruit and/or attempting to classify levels of severity in affected drupelets is time-consuming, a technique such as this offers the most in-depth data about severity of the disorder. Additionally, the incidence of affected drupelets per fruit at several different levels (e.g. 1+, 3+, or 5+ drupelets) can be reported in order to allow for comparison with most other studies. Alternatively, the use of imaging software to digitally assess RDR incidence may offer rapid, accurate, and unbiased evaluation, though such techniques may not be widely available or practical in all situations.

Refereed scientific publications

Journal article

Edgley, M., Close, D.C., and Measham, P.F. (2018). The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels. Acta Horticulturae. 1205, 885-890. DOI: 10.17660/ActaHortic.2018.1205.113.

Journal articles submitted for peer review

Edgley, M., Close, D.C., Measham, P.F., and Nichols, D.S. (Submitted). Physiochemistry of blackberries (Rubus L. subgenus Rubus Watson) affected by red drupelet reversion.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Effects of climatic conditions during harvest and handling on the postharvest expression of red drupelet reversion in blackberries.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Nitrogen application rate and harvest date affect red drupelet reversion and postharvest quality in ‘Ouachita’ blackberries.

Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Flesh temperature during impact injury and subsequent storage conditions affect the severity of colour change caused by red drupelet reversion in blackberries.

Edgley, M., Close, D.C., and Measham, P.F. (Prepared) Red drupelet reversion in blackberries: A complex of genetic and environmental factors.

Intellectual property, commercialisation and confidentiality

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

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Appendices

Appendix 1. Thesis titled, 'Causes and mechanisms of red drupelet reversion in blackberries', to be submitted in fulfilment of the requirements for the degree of Doctor of Philosophy to the University of Tasmania.

This thesis contains all relevant materials generated by the project, however material currently under the peer-review process has been omitted (Chapters 2, 4, 5, 6, 7), with only the abstracts presented for this report.

Causes and mechanisms of red drupelet reversion in blackberries

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B.Agr.Sci (Hons) University of Tasmania

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Tasmania

February 2019

Statements and Declarations

Declaration of originality

This thesis does not contain material which has been accepted for any degree or diploma by the University of Tasmania or any other or any other institution except by the way of background information, or where duly referenced. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where reference is made, nor does it contain any material that infringes copyright.

Max Edgley

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Statement regarding published work contained in this thesis

Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Max Edgley – Tasmanian Institute of Agriculture (84.5 %)

Dugald Close – Tasmanian Institute of Agriculture (10 %)

Penelope Measham – Tasmanian Institute of Agriculture (5 %)

David Nichols – Central Science Laboratory, University of Tasmania (0.5 %)

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Abstract

Red drupelet reversion (RDR) is a physiological disorder of blackberries, whereby individual or groups of drupelets that are black at harvest revert to red, usually after the fruit has been harvested and placed into cool storage. RDR reduces the visual and physical quality of the fruit and is considered a major physiological disorder of commercial blackberries. This thesis examined the physiochemical changes that occur during RDR development and investigated pre and postharvest factors associated with the development of the disorder.

The physiochemical properties of drupelets that were affected and unaffected by RDR were examined. The total anthocyanin concentration in black, partially red, and fully red drupelets was 1841 mg kg^{-1}, 1064 mg kg^{-1} and 769 mg kg^{-1} by fresh weight respectively. Anthocyanins containing acylated or disaccharide sugar moieties were more stable than anthocyanins with non-acylated and monosaccharide sugar moieties. The pH of partially red (3.05) and fully red drupelets (3.01) was lower than that of black drupelets (3.32). The firmness, measured by penetrometer, of partially red (1.90 N) and fully red drupelets (1.77 N) was lower than fully black drupelets (2.39 N). Electrolyte leakage over 24 hours was higher from partially red (84.8 %) and fully red drupelets (90.0 %) than fully black drupelets (64.9 %). Examination by light and electron microscopy showed consistent cell disruption, separation and loss of integrity in the upper mesocarp of affected drupelets. The physiochemical symptoms associated with the development of RDR were consistent with mechanical injury, causing cell decompartmentalisation and subsequent anthocyanin degradation.

The effects of handling fruit and climatic factors at harvest on RDR incidence and severity were investigated during ten harvests in 2017. Fruit that were handled during harvest had at least one drupelet develop RDR in 85 % of samples, while only 6 % of fruit that were not handled had any drupelets that developed the disorder.

The incidence and severity of RDR was significantly higher when fruit skin temperatures exceeded 23 °C during harvest, and these conditions were also associated with reduced skin firmness of drupelets that were affected and unaffected by RDR.

The degree of colour change following controlled, repeatable impact damage at a range of temperatures and subsequent storage conditions was measured by colourimeter. Impact injury caused a significant colour difference (ΔE) relative to the control fruit in 95 % of fruit. As temperature during impact and the subsequent rate of temperature change increased, the severity of colour change worsened.

The effects of nitrogen (N) application rate on RDR, fruit quality, and yield were investigated in a two-year trial. A high N application rate of 212 kg ha^{-1} produced fruit with significantly higher incidence and severity of RDR than medium (106 kg ha^{-1}) and low N (53 kg ha^{-1}) rates. The high N treatment increased yield through increasing the number of harvestable fruit in year one, and both the number of harvestable fruit and fruit mass in year two. Firmness and physiochemical fruit quality were not affected by N treatment.

The findings establish the major underlying physiochemical changes associated with RDR in blackberries and demonstrate the effects of abiotic factors associated with the development of the disorder in commercial settings. Future research directions and potential management techniques for reducing the incidence of RDR in commercial settings are also discussed.

Preface

Following a brief introductory chapter, this thesis is mainly composed of papers which have been published, submitted, or prepared for submission to refereed journals. Each chapter contains an explanatory preface detailing its publication status at the time of submission, its relevance to the project and thesis, and lists any relevant appendices. Each research chapter is presented with the preserved referencing style required by the targeted journal, with the numbering of headings, tables, and figures altered to reflect their position in the thesis. The first of the chapters intended for publication is a literature review that consolidates and discusses the knowledge to date on RDR in commercial blackberries. The following four chapters consist of research papers, each of which addresses one or more of the aims of the project, as outlined below. Following the research chapters, a general discussion, conclusions, and key recommendations of the project are presented.

Aims and structure

The broad aim of this project was to advance the knowledge of causes, mechanisms, and management practices for red drupelet reversion (RDR) in commercial blackberries. Following a review of the literature and a survey of Australian producers four key goals were identified and research was designed to address these:

1. *To identify and quantify the physiochemical changes occurring in drupelets affected by RDR.*

The underlying physiological mechanisms associated with RDR had not previously been reported, and so establishing this was necessary to further investigate the disorder. This involved attempting to induce RDR in blackberries and investigating the physiochemical changes occurring at a fruit, drupelet, and cellular level. This work provided a basis for understanding susceptibility to RDR and further refined the direction of the research. This aim is addressed in Chapter 4.

2. *To identify any physical or environmental factors involved in expression of RDR.*

Following the initial study identifying the physiochemical changes occurring during RDR, mechanical injury was identified as a key factor in the development of the disorder. To investigate this, multiple experiments examining the effects of handling, climatic conditions at harvest, and postharvest storage conditions on incidence and severity of RDR were undertaken. This is addressed in Chapters 5 and 7.

3. *To identify plant nutrition that may be contributing to an increase in RDR.*

An anecdotal relationship between nutrition and RDR had been observed among blackberry producers within Australia and overseas. Specifically, the hypothesis that excess nitrogen fertiliser application during harvest can significantly increase the susceptibility of blackberries to red drupelet disorder was investigated. This aim is addressed in Chapter 6.

4. *To identify and develop potential pre- or postharvest techniques to reduce the incidence of RDR.*

As well as investigating factors associated with high rates of the disorder, Chapters 5, 7, and 9 address practical techniques to reduce the incidence of RDR in commercial settings. This thesis concludes with the key findings, future research direction, and recommendations of the project.

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Refereed communications arising from this project

Published

Appendix D.1 Edgley, M., Close, D.C., and Measham, P.F. (2018). The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels. Acta Horticulturae. 1205, 885-890. DOI: 10.17660/ActaHortic.2018.1205.113.

Submitted

Chapter 4. Edgley, M., Close, D.C., Measham, P.F., and Nichols, D.S. (Submitted). Physiochemistry of blackberries (*Rubus* L. subgenus *Rubus* Watson) affected by red drupelet reversion.

Chapter 5. Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Effects of climatic conditions during harvest and handling on the postharvest expression of red drupelet reversion in blackberries.

Chapter 6. Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Nitrogen application rate and harvest date affect red drupelet reversion and postharvest quality in ‘Ouachita’ blackberries.

Chapter 7. Edgley, M., Close, D.C., and Measham, P.F. (Submitted). Flesh temperature during impact injury and subsequent storage conditions affect the severity of colour change caused by red drupelet reversion in blackberries.

Prepared for submission

Chapter 2. Edgley, M., Close, D.C., and Measham, P.F. Red drupelet reversion in blackberries: A complex of genetic and environmental factors.

Non-refereed articles, conference presentations, posters, and other outputs arising from this project:

Edgley M., Close D.C., and Measham P.F. (2015), 'The use of modified atmosphere packaging to extend the shelf-life of a range of commercial raspberry varieties in cool storage', XI International Rubus and Ribes Symposium, Asheville, North Carolina, USA – *Poster presentation*

Edgley M., Close D.C., and Measham P.F. (2015), 'Red drupelet disorder in blackberries', Fruit Growers Tasmania Grower Field Day, Huonville, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2016), 'Red drupelet reversion: 2016 update', Fruit Growers Tasmania Annual Conference, Hobart, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2016), 'Managing red drupelet disorder', Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2016), 'The effects of nitrogen fertiliser on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries', I International Symposium on Protected Cultivation in Tropical and Temperate Climates & X International Symposium on Protected Cultivation in Mild Winter Climates, Cairns, Australia – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), 'Research into red drupelet disorder in Australia' Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia, USA – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), ‘Red drupelet disorder in blackberries: 2017 update’ Fruit Growers Tasmania Annual Conference, Launceston, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2017), ‘Managing red drupelet disorder’, Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2017), ‘Causes and mechanisms of red drupelet reversion in commercial blackberries’, 2017 School of Land and Food Annual Conference Program, Hobart, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2018), ‘Causes and mechanisms of red drupelet reversion in commercial blackberries’, BerryQuest International 2018, Launceston, Tasmania – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2018), ‘Managing red drupelet disorder’, Tasmanian Institute of Agriculture – *Fact sheet*

Edgley M., Close D.C., and Measham P.F. (2018), ‘Causes and mechanisms of red drupelet reversion in commercial blackberries’ III International Berry Fruit Symposium, Istanbul, Turkey – *Oral presentation*

Edgley M., Close D.C., and Measham P.F. (2019), ‘Causes and mechanisms of red drupelet reversion in commercial blackberries’, Southeast Regional Fruit and Vegetable Conference, Savannah, Georgia – *Oral presentation*

Chapter 1. General introduction

This chapter includes a brief overview of blackberry fruit taxonomy, the blackberry industry in Australia and worldwide, and physiological topics relevant to the remainder of the thesis but are not covered in individual chapters. A review of any literature specific to RDR has been omitted for inclusion in the standalone literature review contained in Chapter 2.

1.1. The blackberry

Blackberries are an edible summer fruit from the complex *Rubus* L. genus subgenus *Rubus* Watson (Clark and Finn, 2011), which includes a wide variety of cultivated and wild fruit crops with species found on all continents except Antarctica (Hummer, 2017). The major cultivated *Rubus* fruit include red raspberries (*Rubus idaeus*), black raspberries (*Rubus occidentalis)*, and blackberries, which typically do not have a species epithet because cultivated species are nearly all derived from at least two or more species (Clark and Finn, 2011).

1.2. Worldwide industry

Blackberries have historically been consumed predominantly as a wild fruit, with commercial production being a recent but fast-growing industry. Major growing regions include Serbia, the USA, Mexico, Hungary, China and Costa Rica (Strik *et al.*, 2007). Worldwide production has grown steadily since the early ‘90s, driven by factors including the need for a stable year-round supply, breeding programs allowing shipping to distant markets, and increasing consumer awareness of the health benefits of antioxidant-containing foods (Clark and Finn, 2014; Keogh *et al.*, 2010; Strik *et al.*, 2007). Strik *et al.* (2007) reported that 140,292 tonnes were harvested worldwide from 20,035 ha of cultivated plantings in 2005 – an increase of 45 % from 1995 production levels, with recent production estimated to be in excess of 25,000 ha (Clark and Finn, 2014).

1.3. Australian industry

Commercial blackberries are a minor horticultural crop in Australia, often grown in conjunction with raspberries and other small fruit. There are approximately 140 *Rubus* growers across the country in all states except the Northern Territory, producing approximately 800 tonnes of *Rubus* fruit from 613 ha, of which blackberries account for less than 10 % (Keogh *et al.*, 2010). The major production areas are the Gippsland and Dandenong Ranges in Victoria, and throughout Northern Tasmania (ARGA, 2009). The season runs from November through to May, with peak production occurring throughout January and February.

1.4. Anatomy and fruit structure

Blackberry plants are perennial, with biennial canes called primocanes in their first year of vegetative growth and, after a dormant winter period, they are known as floricanes in their second year. Floricanes produce flowers and fruit, while new vegetative primocanes are grown for the following year's crop. Breeding programs throughout North America have now produced several primocane fruiting cultivars, which fruit during their first year and can also be double-cropped for a second year's production (Clark and Perkins-Veazie, 2011; Clark and Salgado, 2016). Primocane cultivars were first grown commercially in 2004 and have had a rapid uptake among growers, particularly throughout the USA (Strik *et al.*, 2007), offering the advantages the extension of the fruiting season, the ability to double-crop, and a significant reduction in cane maintenance costs (Strik *et al.*, 2007; Thompson and Strik, 2009).

Blackberry fruit are an aggregate fruit that consist of a central torus or receptacle surrounded by a number of fleshy drupelets (Takeda, 1993). Each drupelet consists of a thin, soft exocarp, a fleshy mesocarp, and a hard endocarp (pyrene) that contains a seed. The size of the blackberry is determined by a combination of drupelet number and size, with modern cultivars producing a barrel, round, blocky, irregular or conical shape fruit weighing 8–15g (Clark and Finn, 2011).

At fruit maturity, an abscission zone forms at the base of the blackberry from the pedicle and the entire aggregate including the receptacle remains tightly together after abscission (Perkins-Veazie *et al.*, 2000). When the fruit is mature it can be easily removed from the cane with a small amount of force and will fall to the ground when overripe.

1.5. Fruit ripeness

The maturity of the blackberry fruit is typically described in a number of stages of ripeness: green, partial redness, full redness, partial or mottled black, shiny black, and dull black or overripe (Perkins-Veazie *et al.*, 2000b). The development of the red and black colour throughout the process is directly caused by the accumulation of anthocyanins in the fruit and is accompanied by an increase in size, softening, and an accumulation of carbohydrates and other nutrients.

1.6. Production and harvest practices

Open field production is the predominant system used worldwide, but a growing number of producers are shifting to protected production under tunnels, shade cloth, or a combination of the two, especially for new plantings and high-value markets (Clark and Finn, 2014; Strik *et al.*, 2007). The benefits of tunnel production can vary with climate; however, tunnels generally provide a longer growing season, and canes produce more first-class fruit due to yield increases and reduced losses to pests, diseases and environmental stresses (Rodríguez *et al.*, 2012; Rom *et al.*, 2010; Thompson and Strik, 2009).

Fruit intended for fresh market consumption is recommended to be harvested directly into clamshell punnets, though it is not uncommon for producers to pick into shallow buckets and then transfer fruit to punnets in the field or pack house, particularly in areas with high labour costs such as Australia (personal communication, January 2016). Following harvest, fruit should be quickly force-air cooled to 0–5 °C at 85–95 % relative humidity for storage and transport (Strik *et al.*, 2007).

Most commercial cultivars are harvested at the ‘shiny black’ stage of development, where shelf-life and ability to transport the fruit is best, although some cultivars retain astringency into the dull black stage and are unsuitable for export markets (Perkins-Veazie *et al.*, 1996a; Walsh *et al.*, 1983). Canes are harvested for ripe fruit every 2–5 days depending on cultivar, production system, and time of the season. Fruit is not washed or treated prior to sale in order to reduce handling and rot incidence.

1.7. Ripening processes

Blackberries increase in soluble sugars and decrease in titratable acidity during the ripening process. The increase in soluble sugars occurs primarily during the partial and fully black stage, with no significant increase from the shiny black to the dull black stage. Fructose and glucose are the major sugars in the fruit, existing in roughly equal amounts with negligible amounts of sucrose throughout the entire fruit development process (Kafkas *et al.*, 2006; Perkins-Veazie *et al.*, 2000b; Wrolstad *et al.*, 1980). Titratable acidity decreases approximately 50 % between the partial and shiny black stages, and 10–30 % between the shiny and dull black stages (Perkins-Veazie *et al.*, 2000b). The major organic acids vary with cultivar, but are most commonly reported as citric, malic, isocitric and lactoisocitric; with shikimic, fumaric, and succinic acid present in trace quantities (Fan-Chiang and Wrolstad, 2010; Kafkas *et al.*, 2006; Kaume *et al.*, 2012; Perkins-Veazie *et al.*, 2000b; Perkins-Veazie *et al.*, 1996a; Wrolstad *et al.*, 1980).

1.8. Development of phytochemicals

Blackberries are a rich source of phytochemicals including anthocyanins, phenolic acids, flavonols and other antioxidants that contribute to their taste, colour, aroma and nutritional profile. Wang and Lin (2000) reported on the total anthocyanin content, total phenolic content, and the oxygen radical absorbance capacity (ORAC) of various blackberry cultivars throughout three stages of ripening (green, pink, and ripe). The authors concluded that total phenolic content and ORAC values were lowest in pink berries (227–262 mg/100 g and 13.7–17.6 µmol of TE/g respectively on a wet matter basis), highest in green fruit (226–308 mg/100 g and 23.4–25.1 µmol of TE/g), and with moderate to high levels in ripe fruit (204–248 mg/100 g and 20.3–24.66 µmol of TE/g). Anthocyanins increased from 0.5–1.3 mg/100 g in green fruit to 8.8–10.6 mg/100 g in pink fruit and 133.5–171.6 mg/100 g in ripe fruit. The study also found that a linear relationship exists between total phenolic content and ORAC activity in all growth stages, as well as total anthocyanin content and ORAC activity in ripe berries. This indicates that the compounds responsible for antioxidant capacity of the fruit shift from predominantly colourless phenols and acids at the green stage to coloured anthocyanin pigments as the fruit ripens.

1.9. Anthocyanins: biosynthesis and chemistry

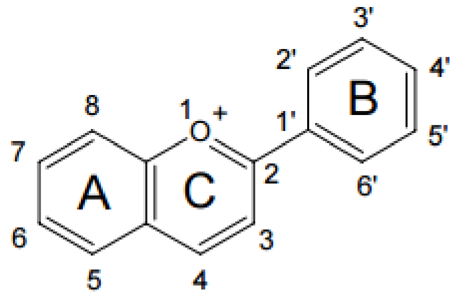
Anthocyanins, responsible for the attractive dark colour of blackberries, are water-soluble pigments belonging to a parent class of molecules called flavonoids, which are synthesised via the phenylpropanoid pathway (Cho *et al.*, 2004; Parker, 2010). These pigments can range from yellow and red to blue and dark purple depending on several factors including pH, co-pigmentation and functional groups (Welch *et al.*, 2008). They are produced by many organisms in the plant kingdom and have been observed to occur in all tissues of higher plants (Maharik *et al.*, 2009). Anthocyanins and related molecules are of significant interest to researchers and consumers due to their potential benefits for human health. Research indicates that anthocyanins and other flavonoid pigments have a wide range of biological effects including antioxidant, anti-inflammatory, antiallergenic, antiulcer, antibiotic and anti-carcinogenic properties (Cho *et al.*, 2004; Ding *et al.*, 2006; Maharik *et al.*, 2009). These properties arise from their high reactivity as hydrogen or electron donors and the ability of the polyphenol-derived radicals to stabilise and delocalise the unpaired electron, as well as their ability to chelate transition metal ions (Duan *et al.*, 2007).

Anthocyanins are biosynthesised from three molecules of malonyl CoA derived from fatty acid metabolism and one molecule of p-coumaroyl CoA synthesised from phenylalanine via the general phenylpropanoid pathway (Parker, 2010; Zhang *et al.*, 2014). Biosynthesis occurs in the cytoplasm with the major biosynthetic enzymes being located in the endoplasmic reticulum.

After synthesis, they are then transported across the tonoplast membrane into the vacuole by carrier enzymes, with glutathione-s-transferase thought to play a key role in this movement (Gomez *et al.*, 2011; Mueller and Walbot, 2001). They accumulate in the vacuole and play a number of roles in a wide range of plants including colouration to aid pollination, potential nutritional value, light absorbance and other physiological roles (Welch *et al.*, 2008).

Anthocyanidins or aglycons are the basic structures of anthocyanins, of which 23 are known to occur naturally (Castañeda-Ovando *et al.*, 2009; Welch *et al.*, 2008). These aglycons are inherently unstable and readily degrade to their corresponding aldehydes and phenolic acids or to the quinoid anhydrobase (Fleschhut *et al.*, 2006). Because of this, these molecules usually exist in nature in their glycosylated forms – anthocyanins – with sugars attached at the C3, C5, or C7 ring positions (Fig. 1-1.). Sugars found on the rings can include glucose, rhamnose, xylose, galactose, arabinose and fructose, with many anthocyanins also being acylated by aliphatic or aromatic acids (Castañeda-Ovando *et al.*, 2009; Fleschhut *et al.*, 2006; Welch *et al.*, 2008). Over 600 different anthocyanins have been identified as occurring naturally in a wide range of plants (Welch *et al.*, 2008).
Anthocyanin biosynthesis is one of the most studied and well understood pathways in plant secondary metabolism (Mueller and Walbot, 2001), although the effect of these compounds on human health as well as their chemical and biochemical interactions within the human body has not been as extensively researched.

Fig. 1-1. The basic flavylium ion structure – the backbone for anthocyanin pigments



1.10. Literature cited

Australian Rubus Growers Association, 2009, Strategic Plan for the Rubus Industry, 2009 - 2014, ARGA.

Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Pacheco-Hernández, M., Páez-Hernández, M.E., Rodríguez, J.A. & Galán-Vidal, CA 2009, 'Chemical studies of anthocyanins: A review', Food Chemistry, vol. 113, pp. 859-871.

Cho, M.J., Howard, L.R., Prior, R.L., & Clark, J.R., 2004, 'Flavanoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance chromatography/mass spectrometry', Journal of the Science of Food and Agriculture, vol. 84, pp. 1771-1782.

Cho, M.J., Howard, L.R., Prior, R.L., & Clark, J.R., 2004, 'Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry', Journal of the Science of Food and Agriculture, vol. 84, no. 13, pp. 1771-1782.

Clark, J.R. & Finn, C.E. 2014, 'Blackberry cultivation in the world', Revista Brasileira de Fruticultura, vol. 36, no. 1, pp. 46-57.

Clark, J.R. & Finn, C.E. 2011, 'Blackberry Breeding and Genetics', Fruit, Vegetable and Cereal Science and Biotechnology, vol. 5, no. 1, pp. 27-43.

Clark, J.R. & Perkins-Veazie, P. 2011, ''AFP-45' Primocane-fruiting Blackberry', HortScience, vol. 46, no. 4, pp. 670-673.

Clark, J.R. & Salgado, A. 2016, '‘Prime-Ark® Traveler’ Primocane-fruiting Thornless Blackberry for the Commercial Shipping Market', HortScience, vol. 51, no. 10, pp. 1287-1293.

Connor, A.M., Finn, C.E., McGhie, T.K., & Alspach, P.A. 2005, 'Genetic and environmental variation in anthocyanins and their relationship to antioxidant activity in blackberry and hybridberry cultivars', Journal of the American Society for Horticultural Science, vol. 130, no. 5, pp. 680-687.

Ding, M., Feng, R., Wang, S.Y., Bowman, L., Lu, Y., Qian, Y., Castranova, V., Jiang, B-H., & Shi, X. 2006, 'Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventative and chemotheraputic activity', The Journal of Biological Chemistry, vol. 281, no. 25, pp. 17359-17368.

Duan, X., Jiang, Y., Su, X., Zhang, Z., & Shi, J. 2007, 'Antioxidant properties of anthocyanins extracted from litchi (Litchi chinenesis Sonn.) fruit pericarp tissues in relation to their role in the pericarp browning', Food Chemistry, vol. 101, no. 4, pp. 1365-1371.

Fan-Chiang, H-J & Wrolstad, R.E. 2005, 'Anthocyanin Pigment Composition of Blackberries', Journal of Food Science, vol. 70, no. 3, pp. C198-C202.

Fan-Chiang, H-J & Wrolstad, R.E. 2010, 'Sugar and nonvolatile acid composition of blackberries', Journal of AOAC International, vol. 93, no. 3, pp. 956-965.

Fleschhut, J., Kratzer, F., Rechkemmer, G., & Kulling, S.E. 2006, 'Stability and biotransformation of various dietary anthocyanins in vitro', European Journal of Nutrition, vol. 45, no. 1, pp. 7-18.

Gomez, C., Conejero, G., Torregrosa, L., Cheynier, V., Terrier, N., & Ageorges, A. 2011, 'In vivo grapevine anthocyanin transport involves vesicle-mediated trafficking and the contribution of anthoMATE transporters and GST', The Plant Journal, vol. 67, no. 6, pp. 960-970.

Hummer, K.E. 2017, 'Blackberries: an introduction', in H.K. Hall & R.C. Funt (eds), Blackberries and their Hybrids. Crop Production Science in Horticulture, vol. 26, p. 1.

Kafkas, E., Koşar, M,. Türemiş, N. & Başer, K. 2006, 'Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey', Food Chemistry, vol. 97, no. 4, pp. 732-736.

Kaume, L., Howard, LR & Devareddy, L 2012, 'The Blackberry Fruit: A Review on Its Composition and Chemistry, Metabolism and Bioavailability, and Health Benefits', Journal of agricultural and food chemistry, vol. 60, no. 23, pp. 5716-5727.

Maharik, N., Elgengaihi, S. and Taha, H., 2009. Anthocyanin production in callus cultures of Crataegus sinaica boiss. Int J Acad Res, 1(1), pp.30-34.

Mueller, L.A & Walbot, V. 2001, 'Models for vacuolar sequestration of anthocyanins', Recent Adv. Phytochemistry, vol. 35, pp. 291-312.

Parker, J-L., 2010, 'What makes a cherry red? An investigation into flavonoid pathway regulation in sweet cherry (Prunus avium L.) fruit', Doctor of Philosophy thesis, University of Adelaide.

Perkins-Veazie, P., Clark, J.R., Huber, D.J., & Baldwin, E.A. 2000, 'Ripening physiology in 'Navaho' thornless blackberries: Color, respiration, ethylene production, softening, and compositional changes', Journal of the American Society for Horticultural Science, vol. 125, no. 3, pp. 357-363.

Perkins-Veazie, P., Collins, J.K. & Clark, J.R. 1996, 'Cultivar and maturity affect postharvest quality of fruit from erect blackberries', HortScience, vol. 31, no. 2, pp. 258-261.

RIRDC 2010, Pollination Aware Case Study: Rubus, by Keogh, R., Mullins, I., & Robinson, A.

Rodríguez, H.G., Popp, J., Thomsen, M., Friedrich, H. & Rom., CR 2012, 'Economic Analysis of Investing in Open-field or High Tunnel Primocane-fruiting Blackberry Production in Northwestern Arkansas', HortTechnology, vol. 22, no. 2, pp. 245-251.

Rom, C.R., Garcia, M.E., Johnson, D.T., Popp, J., Friedrich, H., & McAfee, J. 2010, 'High tunnel production of organic blackberries and raspberries in Arkansas', in pp. 269-276.

Sapers, G.M., Hicks, K.B., Burgher, A.M., Hargrave, D.L., Sondey, S.M. & Bilyk, A. 1986, 'Anthocyanin Patterns in Ripening Thornless Blackberries', Journal of the American Society for Horticultural Science, vol. 111, no. 6, pp. 945-950.

Siriwoharn, T., Wrolstad, R.E., Finn, C.E. & Pereira, C.B. 2004, 'Influence of cultivar, maturity, and sampling on blackberry (Rubus L. Hybrids) anthocyanins, polyphenolics, and antioxidant properties', Journal of Agricultural and Food Chemistry, vol. 52, no. 26, pp. 8021-8030.

Strik, B., Clark, J., Finn, C., & Banados, M.P. 2007, 'Worldwide blackberry production', HortTechnology, vol. 17, no. 2, pp. 205-213.

Takeda, F. 1993, 'Characterization of Blackberry Pyrenes', HortScience, vol. 28, no. 5, p. 488.

Thompson, E. & Strik, B.C. 2009, 'High Tunnel versus Open Field: Management of Primocane-fruiting Blackberry Using Pruning and Tipping to Increase Yield and Extend the Fruiting Season', HortScience, vol. 44, no. 6, pp. 1581-1587.

Walsh, C.S., Popenoe, J. & Solomos, T. 1983, 'Thornless blackberry is a climacteric fruit', HortScience, vol. 18, pp. 482-483.

Wang, S.Y. & Lin, H-S. 2000, 'Antioxidant Activity in Fruits and Leaves of Blackberry, Raspberry, and Strawberry Varies with Cultivar and Developmental Stage', Journal of agricultural and food chemistry, vol. 48, no. 2, pp. 140-146.

Welch, C.R., Wu, Q., & Simon, J.E. 2008, 'Recent Advances in Anthocyanin Analysis and Characterization', Current analytical chemistry, vol. 4, no. 2, pp. 75-101.

Wrolstad, R.E., Culbertson, J.D., Nagaki, D.A. & Madero, C.F. 1980, 'Sugars and nonvolatile acids of blackberries', Journal of agricultural and food chemistry, vol. 28, no. 3, pp. 553-558.

Wu, X. & Prior, R.L. 2005, 'Identification and Characterization of Anthocyanins by High-Performance Liquid Chromatography−Electrospray Ionization−Tandem Mass Spectrometry in Common Foods in the United States: Vegetables, Nuts, and Grains', Journal of agricultural and food chemistry, vol. 53, no. 8, pp. 3101-3113.

Zhang, Y., Butelli, E., & Martin, C. 2014, 'Engineering Anthocyanin Biosynthesis in Plants', Current Opinion in Plant Biology, vol. 19, pp. 81-90.

Chapter 2. Red drupelet reversion in blackberries: A complex of genetic and environmental factors

This chapter contains a review of the current literature with reference to RDR as of January 2019. Since no comprehensive review of the literature pertaining to RDR has been published previously, the initial literature review that was undertaken has been revised to include results generated from this project, with the intention of publication. Hence, this chapter contains references to the subsequent research chapters, as well as some repetition of methodologies, results and discussion points. Conversely, some discussion points from this review are repeated briefly in later chapters, though attempts have been made to limit repetition.

This chapter has been prepared for submission for peer review pending publication of the referenced research chapters.

Abstract

Red drupelet reversion (RDR) in blackberries is a physiological disorder that causes the postharvest reddening of individual or groups of drupelets, resulting in economic loss due to a reduction in marketability. This paper reviews recent advances in the understanding of RDR including the physiochemistry, causes of expression and genotypic variation in the incidence of RDR. The disorder is associated with a significant reduction in anthocyanin pigment concentration, which can vary in severity causing degrees of partial or full colour change. This is associated with observations of reduced cellular structural integrity and loss of membrane integrity. Susceptibility to the disorder is heavily genotypically influenced, with an identified link between cultivar texture, postharvest weight loss and RDR incidence. Current research indicates that RDR is primarily caused by mechanical injury to the fruit that has induced cell decompartmentalisation.

Further study is required to clarify the mechanism for pigment degradation, and to investigate confounding genotypic and environmental effects on RDR incidence.

Chapter 3. General materials and methods

3.1. Location of Field Trials

Field trials and fruit harvesting for all published work was undertaken at Costa Berries Dunorlan farm site, Dunorlan, Tasmania, Australia (41.5 °S, 146.6 °E). The region has a cool temperate climate and is a notable area for *Rubus* production, with rapid expansion of small fruit production in the area over the last decade. The region has a mean yearly rainfall of 995 mm, peaking in the winter months, which receives roughly double that of the summer months (Fig. 3-1).

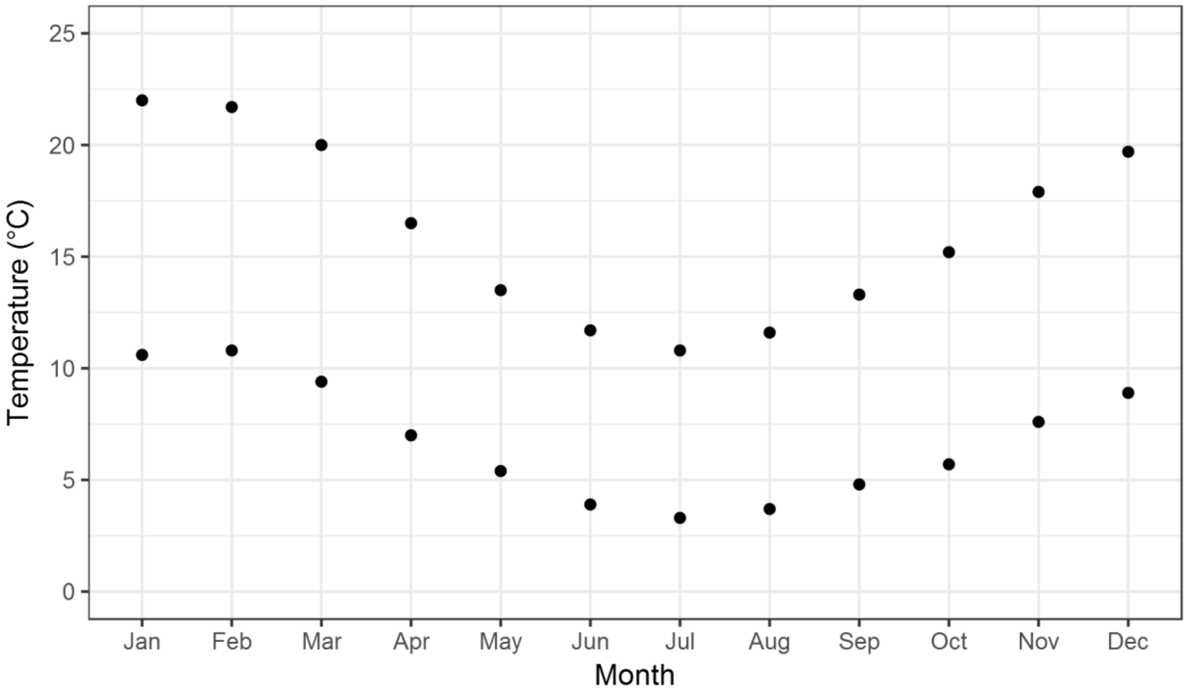


Fig. 3-1. Mean monthly minimum and maximum temperatures (°C) over the last 20 years at the Dunorlan field site. Data sourced from the Australian Bureau of Meteorology

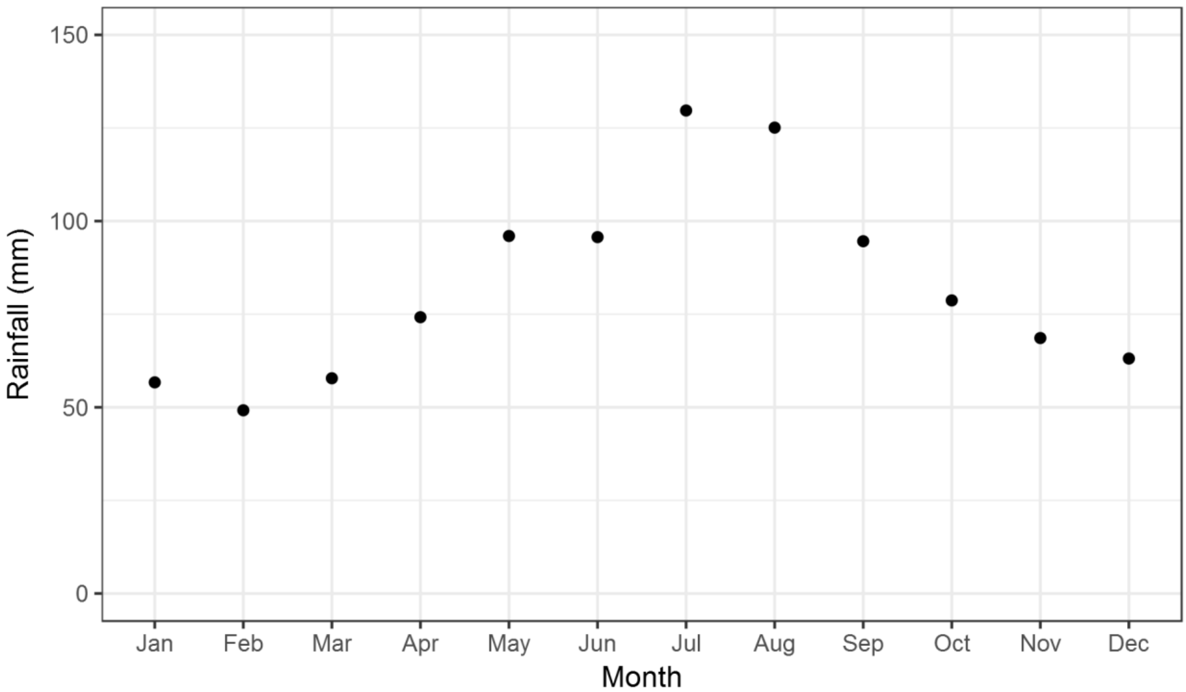


Fig. 3-2. Mean monthly rainfall (mm) over the last 20 years at the Dunorlan field site. Data sourced from the Australian Bureau of Meteorology

Some preliminary fieldwork was also carried out at Westerway Raspberry Farm, Westerway, Tasmania, Australia (42.7 °S, 146.8 °E). This site is in the Derwent Valley region, has a cool temperate climate and is a notable area for the production and processing of fresh market berry fruit. Data from these preliminary field harvests were not published; however they were used to guide the planning of larger trials.

Fig. 3-3. Map of Tasmania with the Dunorlan (A) and Westerway (B) field sites labelled.

3.2. Cultivar selection

The cultivar 'Ouachita' was selected for the majority of the experimental work for a number of reasons: the cultivar historically produces good quality fruit for a long season at the Dunorlan field site, it is the predominant cultivar grown in the state, the fruit produced has a medium-to-high susceptibility to red drupelet disorder and a relatively small incidence of other pests and diseases, and the block on which the cultivar is grown is flat with uniform soil and little wind exposure.

The cultivar is erect and thornless, producing blocky, conical fruit, which is non-uniform in shape. The cultivar was produced by the University of Arkansas blackberry breeding program, and when first released it produced fruit with an average weight of 5.8 g (Clark and Moore 2005). Fruit produced at the experimental site over the experimental period had a mean weight of 9.9 g over the three years of study.

Some preliminary work was undertaken on 'Navaho' and 'Loch Ness' fruit harvested from both sites.

3.3. Statistical analysis

R (R Core Team, 2017) versions 3.3.0 or later was used for all the statistical analysis undertaken. Specific statistical tests and R packages are described in the relevant experimental chapters. Unless otherwise stated, a significance level of $P<0.05$ was used for all statistical analysis. All graphs were generated using the package ‘ggplot2’ (Wickham, 2009).

Chapter 4. Physiochemistry of blackberries (Rubus L. subgenus Rubus Watson) affected by red drupelet reversion

This chapter addresses the first key goal of the project: to identify and quantify the underlying physiochemical associated with RDR development. This work was necessary to provide a fundamental base of knowledge for the remainder of this research as well as future study in this field.

This chapter has been submitted for peer review as an original research paper.

Abstract

Red drupelet reversion (RDR) is a physiological disorder causing individual or groups of drupelets on blackberries that are black at harvest to turn red during postharvest cool storage. The objectives of this study were to examine and quantify the physiochemical changes occurring in flesh affected by RDR. Drupelets were classified as 'fully black', 'partially red', or 'fully red'. The total anthocyanin concentration in black, partially and fully red drupelets was 1,841 mg kg^{-1}, 1,064 mg kg^{-1} and 769 mg kg^{-1} fresh weight respectively. Anthocyanins containing acylated or disaccharide sugar moieties were more stable than anthocyanins with non-acylated and monosaccharide sugar moieties. The pH of partially red (3.05) and fully red drupelets (3.01) was lower than black drupelets (3.32). Firmness of partially red (1.90 N) and fully red drupelets (1.77 N) was lower than that of fully black drupelets (2.39 N). Examination by light and electron microscopy showed cell disruption, separation and loss of integrity in the upper mesocarp of affected drupelets. Electrolyte leakage over 24 h was significantly higher from partially red (84.8 %) and fully red (90.0 %) than fully black drupelets (64.9 %). The data are consistent with RDR in blackberries arising from mechanical damage that causes cell decompartmentalisation and subsequent anthocyanin degradation.

Keywords: Anthocyanin; cell disruption; firmness; electrolyte leakage

Chapter 5. Effects of climatic conditions during harvest and handling on the postharvest expression of red drupelet reversion in blackberries

After mechanical injury causing cell disruption was identified as a potential mechanism involved in RDR development in Chapter 4, this trial was designed to investigate the effects of injury inferred by handling fruit during harvest. Environmental conditions at harvest had been suggested by previous research, and anecdotally by producers, as contributing to high rates of the disorder, but no studies had investigated this thoroughly.

This chapter addresses the second goal of the project: to investigate physical and environmental factors influencing RDR incidence and expression. The fourth goal of the project is also addressed: offering techniques to reduce incidence of the disorder in commercial settings.

This chapter has been submitted for peer review as an original research paper.

Abstract

Red drupelet reversion (RDR) causes individual drupelets on blackberries to revert from black at harvest to a red colour postharvest, reducing the quality and marketability of the fruit. The objective of this trial was to assess the effects of time of harvest and associated climatic variables, as well as the handling of fruit during harvest, on postharvest RDR expression and fruit quality.

Fruit were harvested on ten occasions over two days by one of two methods: either hand-harvested into shallow buckets and transferred to industry standard 125 g clamshell punnets (standard practice), or harvested carefully without handling by cutting the pedicel and placing each fruit into individual cotton wool-lined trays. The number of partially red (PR) and fully red (FR) drupelets per fruit was counted, firmness was measured by compression, and skin firmness was measured by a penetrometer. Air and fruit skin temperature, relative humidity, vapour pressure deficit and soil water tension were all influenced by the time of day. 85 % of fruit that were handled during harvest had at least one drupelet develop RDR, whilst only 6 % of fruit not handled during harvest had any RDR. In handled fruit, warmer skin temperature at harvest was associated with increased RDR incidence and severity ($P<0.001$). The skin firmness of fully black (FB) drupelets, measured by a penetrometer, also decreased significantly by an average of 0.56 N when harvested during warmer temperatures compared to fruit that was not handled. The data indicate that mechanical injury incurred during harvest is a major cause of RDR in fresh blackberries, and that harvest times associated with warmer temperatures result in significantly higher rates of RDR and reduced postharvest quality.

Keywords: Temperature, red drupelet, harvest time, bruising, firmness

Chapter 6. Nitrogen application rate and harvest date affect red drupelet reversion and postharvest quality in ‘Ouachita’ blackberries

This chapter addresses the second aim of the project: to investigate the effects of nitrogen application rates on RDR incidence. Supplementary data for this chapter is contained in Appendix D.

This chapter has been submitted for peer review as an original research paper.

Abstract

Background and Aims Red drupelet reversion (RDR) is a postharvest physiological disorder in blackberries that causes fruit that is black at harvest to subsequently turn red. This trial aimed to investigate the effects of nitrogen (N) fertiliser application rate on the expression of RDR and postharvest fruit quality.

Methods Nitrogen was applied weekly during the growing period via fertigation at a low, medium and high rate (53, 106, and 212 kg N ha^{-1} respectively) to ‘Ouachita’ blackberries in 2016 and 2017. Yield, RDR and postharvest quality were assessed.

Results Harvest date, N application rate and fruit mass were significant factors in the postharvest expression of RDR. In both years, fruit from the high N treatment exhibited significantly increased incidence and severity of RDR relative to the other two N application rates. Fruit temperatures during harvest of less than 23 °C were associated with lower incidence and severity of RDR in 2017, and smaller fruit were more likely to have no RDR in both years. The high N treatment produced more fruit than the low N treatment in 2016, and more and heavier fruit than both other treatments in 2017.

Keywords: Fertigation; temperature; fruit mass; mechanical injury

Chapter 7. Flesh temperature during impact injury and subsequent storage conditions affect the severity of colour change caused by red drupelet reversion in blackberries

This chapter investigates the effect of rapid cooling following impact injury to blackberries. The potential for rapid rates of cooling to exacerbate the disorder has been raised by producers repeatedly and investigated with mixed results by previous authors. In this trial, we investigated rapid versus slow cooling under laboratory conditions.

This chapter has been submitted for publication as a conference paper for the proceedings of the III International Berry Fruit Symposium, 2018, Istanbul, Turkey.

Abstract

Red drupelet reversion (RDR) is a physiological occurrence in blackberries where drupelets revert from black at harvest to red postharvest. The objectives of this trial were to assess the effects of temperature during mechanical injury and temperature changes following injury of blackberries on the subsequent development of RDR.

Individual fruit were subjected to mechanical injury from a steel ball dropped from a height of 25 cm at initial temperatures of 15, 25, and 35 °C. Following injury, fruit were either rapidly cooled to 2°C in a -24 °C cooler or slowly in a 2 °C cooler. The colour of the impact site and of the undamaged control fruit were measured 24 hours and 7 days after the initial impact injury using a colorimeter. Impact injury caused a significant colour difference (ΔE) compared to the control in 95 % of fruit. There were also significant interactions between initial temperatures and cooling rates on the colour of the impact site 24 hours and 7 days after treatment. Higher fruit temperatures at the time of mechanical injury and a faster cooling rate post-injury were associated with increased lightness and chroma. The

results confirm that mechanical injury to blackberry fruit leads to RDR, and that the temperature of fruit at the time of injury and subsequently can influence the severity of RDR.

Keywords: Bruising, impact injury, reversion, CIELAB, storage

Chapter 8. General discussion and conclusions

8.1. General discussion

This thesis delivers numerous findings that advance the current knowledge surrounding RDR, may impact on management strategies for commercial blackberry producers, and will be of relevance to future studies investigating this topic. The research chapters established the underlying physiochemical processes involved in the development of RDR and then identified several environmental and management factors contributing to RDR susceptibility and development. The implications of these findings offer potential management options to reduce the incidence and severity of RDR in commercial blackberry production, as well as stimulate further study in this area. This chapter addresses the four key research goals, summarises the findings of the project, and discusses the implications of this research for the blackberry industry and future research.

8.2. Research goals

8.2.1. To identify and quantify the physiochemical changes occurring in drupelets affected by RDR

The physiochemical changes associated with RDR are reported in Chapter 4. This chapter established that the colour change characterising RDR is induced by a degradation of anthocyanin pigments in affected tissue, which is accompanied by cellular disruption, loss of membrane integrity, decompartmentalisation, increased intracellular spaces, loss of firmness and reduced pH. The physiochemical changes were consistent with symptoms of mechanical injury to affected drupelets, which is in agreement with the recent similar findings and suggestions by Pérez-Pérez *et al.* (2018) and Salgado (2015). Further work is required to identify the exact mechanism of anthocyanin degradation; though the observed structural and chemical changes are consistent with decompartmentalisation of anthocyanins from cell vacuoles and their subsequent enzymatic degradation, which has been widely reported for many anthocyanin-containing foods (Castañeda-Ovando *et al.*, 2009; Lee and Wicker, 1991; Welch *et al.*, 2008).

Anthocyanin degradation from mechanical injury in fruit is generally associated with brown colour development due to the formation of brown polymers during the enzymatic degradation reaction. No authors have noted any visible browning associated with RDR, though this may be due to the anthocyanin concentration in reverted drupelets still being in the range of 700–1100 mg kg^{-1} FW, and the pH remaining less than 3.5. Given that the browning index in fruit juices from anthocyanin degradation has been demonstrated to be pH dependant (Dorris *et al.*, 2018; Jiang *et al.*, 2019), and that anthocyanin decolourisation at higher pH has been shown to contribute to tissue browning in fruit (Underhill and Critchley, 1994), these conditions may mask the formation of brown pigments. Thus, the reduction in anthocyanin concentration and the slight pH reduction favours the red colour development associated with RDR.

The data from Chapter 4 also indicate that in drupelets affected by RDR, anthocyanin species containing disaccharides or acylated sugar moieties are not degraded as readily as those containing monosaccharides and non-acylated sugars. This is consistent with previous reports for anthocyanin stability (Cevallos-Casals and Cisneros-Zevallos, 2004; Welch *et al.*, 2008), and may have further implications for researchers and breeders. It has been colloquially suggested that the colour change associated with RDR varies in severity and shade between cultivars; something that variation of species within the anthocyanin profile may influence. Whilst more data are needed to support this suggestion, an increase of acylated anthocyanin species within the profile of such cultivars may contribute to observable differences in the severity of colour change. This finding warrants further investigation in a range of cultivars grown in disparate environmental conditions to examine the effects of anthocyanin profile on RDR severity.

Chapter 4 established that RDR results in a significant softening of affected drupelets, most likely caused by the observed cellular structural damage and loss of turgor. This suggests that RDR may play a larger role in the postharvest quality and shelf-life of blackberries than has been established to date, given that fruit softening is a factor in mould development (Perkins-Veazie *et al.*, 1997).

Furthermore, fruit firmness can be a key indicator of freshness and quality to consumers (Redgwell and Fischer, 2002; Ross *et al.*, 2009), so decreasing RDR incidence will further impact quality perception. Fruit that were harvested without handling, such as those described in Chapter 5, displayed similar firmness values after 14 days storage to fruit that were hand-harvested after seven days in storage (data not shown). If a causative link exists between RDR and mould development, our findings and future work to reduce RDR incidence may further contribute to increased fruit quality, shelf-life and profitability of blackberry production.

The examination of the underlying physiochemical changes occurring in drupelets affected by RDR produced important findings to guide the direction of the following research chapters and will continue to contribute to the further study of RDR.

8.2.2. To identify any physical or environmental factors involved in expression of RDR

Chapters 5 and 7 demonstrated that damage incurred by handling or impacts to fruit can be a factor in RDR development, which was consistent with the conclusion from Chapter 4: that mechanical injury was associated with RDR development. This finding supports previous suggestions that mechanical injury may lead to RDR (Salgado 2015) with experimental evidence.

The data presented in Chapter 5 also indicate that conditions promoting fruit skin temperatures which exceed 23 °C were associated with increased RDR incidence and severity. These results support similar conclusions shown by McCoy *et al.* (2016) and Yin (2017): that harvest times with warm temperatures may exacerbate the disorder, though Lawrence and Melgar (2018) demonstrated that this effect can vary with genotype and other environmental factors. Despite this, it can be concluded that in order to reduce RDR incidence in commercial production, growers should aim to utilise harvest techniques and conditions that minimise fruit temperatures during harvest. In the Tasmanian climate, harvest times prior to midday offer these conditions, which provides growers with sufficient hours to complete daily harvests. However, this will vary with location.

The potential confounding effects of soil water status and VPD should be further investigated across a larger number of cultivars in order to make additional management recommendations relevant to a variety of production zones.

8.2.3. To identify any nutritional imbalances that may be contributing to an increase in RDR

The establishment of an interaction between nutrient fertiliser application rates and RDR incidence, as presented in Chapter 6, is a key finding of this project. This is the first reported link between nutrient fertiliser application rates and RDR incidence, so this work may encourage broader research into the physiological mechanisms behind this association as well as further investigations into nutrient-fruit quality interactions. The effects of the N fertiliser application rate and fruit N concentration on fruit yield and quality in blackberries is inconclusive in previous literature, where previous studies have reported conflicting or inconsistent results, possibly due to variations in cultivars, agronomic practices, soil, and the environment (Strik, 2008). Hence, whilst the findings from Chapter 6 are important in establishing the potential for nutrient fertiliser application rates to affect RDR incidence, no definitive management recommendations for individual production systems can be made from this study alone. This paper is relevant to the wider literature addressing nutrient-fruit quality relationships in *Rubus*, however, and may explain some grower observations of high N rates leading to increased RDR in commercial settings.

It was hypothesised that any interaction between N fertiliser rate and RDR incidence may emanate from changes in fruit firmness. No significant effect on firmness was observed in the data; however, as discussed in Chapter 6, compression firmness testing can produce inconsistent results, particularly for soft fruit. We recommend that penetration tests, or other alternative methodologies for assessing fruit firmness, should be explored in future studies that can examine the effects of nutrient fertiliser application rates on RDR and fruit quality in blackberries.

8.2.4. To identify and develop potential pre- or postharvest techniques to reduce incidence of RDR

Potential management techniques for reducing the incidence and severity of RDR were identified in Chapters 5, 6, and 7. Chapter 5 established that mechanical injury incurred during harvest and transport of fruit is an underlying factor in the development of RDR, and that fruit temperatures exceeding 23 °C during handling significantly increase incidence and severity. Aside from aiming to avoid harvest times associated with these conditions, the use of structures such as shade cloth to reduce heat exposure, or manipulation of cane architecture to shade the fruit are options that should be explored. Before these recommendations are put into practice, further study should be undertaken to fully understand any other effects of reducing light exposure on plant and fruit quality.

It is currently common practice in the Australian blackberry industry to pick blackberries into buckets and then transfer the fruit into punnets when the buckets are full. This reduces labour costs, which in Australia are a significant portion of production cost, though the practice is likely a major source of compression injury to fruit. In Chapter 5, 85 % of fruit harvested using this technique contained at least one reverted drupelet after 24 hours in cool storage, compared to just 6 % of fruit which was not handled during harvest. Whilst harvesting without handling is an impractical technique for commercial settings, as it would increase labour costs prohibitively, these data do demonstrate that even light handling can significantly reduce the postharvest quality of blackberries. This finding highlights the importance of reducing handling during harvesting, as well as proper picker training to reduce compression injury to fruit. No studies have investigated any finer points of harvest techniques such as ‘twisting’ versus ‘pulling’, and while it is common for growers to recommend that pickers use a ‘twist’ technique, it is often hard to enforce this due to the low levels of training and seasonal nature of the workforce (personal communication, January 2018).

Chapter 2 highlights the significant variation in RDR expression between cultivars, and it can be concluded that cultivar selection for the specific growing environment is vital for reducing losses to

RDR. Given the difficulty of importing new cultivars into Australia due to rigid biosecurity restrictions, depending on RDR-resistant cultivars is not an easy, short-term solution. However, breeding or importing cultivars with low susceptibility in Australian conditions should be considered for the longer-term management of RDR.

8.3. Other implications and findings arising from this project

Due to the lack of a published comprehensive review of the literature detailing the extent of the current knowledge of RDR, the literature review contained in Chapter 2 of this thesis is of importance to further investigations in this field. The rapid expansion of the worldwide blackberry industry over the last two decades has not been fully matched with an increase in study into the fruit's physiology, highlighted by the lack of published data on RDR as well as other physiological disorders and plant-soil interactions. In recent years, this project and other concurrent studies have resulted in a significant growth in knowledge surrounding the genotypic variance, physiochemical mechanisms, and environmental influences on RDR expression. Chapter 2 consolidates the information generated from the previous sporadic studies and current work into a comprehensive article summarising the available data on RDR. It is intended that this chapter will be of interest to both academic and commercial parties, as well as promoting a deeper understanding of this complex and commercially important disorder.

The published data contained in this thesis are solely from experiments carried out with the cultivar 'Ouachita'. This was necessary due to the relatively small-scale blackberry production industry in Tasmania, limiting the available experimental sites, as well as to allow for a broader range of experiments without replicating for multiple cultivars. Whilst this presents some limitations given the genotypic variance in RDR susceptibility and development demonstrated throughout the wider literature and discussed in Chapter 2, there are obvious trends in the physiochemical and structural observations between our data and other published studies, as demonstrated in Chapters 2 and 4. This suggests that the underlying physiological mechanisms involved in the development of and susceptibility to the disorder remain consistent across cultivars. Specifically, reports of anthocyanin reduction by 40–60 %, loss of cellular structural integrity, and an association with mechanical injury are consistent across cultivars and climatically disparate environments.

8.4. Future research direction

RDR is an issue of growing importance to blackberry producers and researchers, as evidenced by the increasing number of research projects investigating various aspects of the disorder in recent years (Edgley *et al.*, in press; McCoy *et al.*, 2016; Pérez-Pérez *et al.*, 2018; Worthington *et al.*, 2017; Yin, 2017). This increase has seen substantial growth in the understanding of the underlying physiological mechanisms and causes of RDR. Despite this, considerable knowledge gaps still exist in this area of research.

Further study to clarify any underlying physiological reasons for the genotypic variance in incidence and severity will be of interest to breeders and growers in order to select cultivars with low susceptibility to RDR. As well as this, a better understanding of what physiological characteristics provide resistance to RDR may further confirm or clarify our conclusions as to the major factors causing RDR expression.

There are a growing number of studies investigating the effects of preharvest environmental factors on RDR expression, such as those shown in Chapter 5. While some consistency has been reported across disparate environments for the effects of temperature during harvest on RDR incidence, the effects appear to vary with genotype and are potentially confounded by other climatic variables.

Additional data for a range of commercially important cultivars may clarify these conclusions, though care should be taken to assess a broad range of climatic variables to minimise any bias in results.

Nutritional links to RDR should be further investigated. This thesis established that increased N rates can influence RDR incidence; however, the underlying causes behind this remain unclear. This thesis offers substantial opportunities to continue and broaden this area of research to fully understand the influence of nutrient fertiliser application rates on RDR expression.

As discussed in Chapters 2 and 6, inconsistencies currently exist in the reported techniques used to assess the incidence and severity of RDR. In order to better enable future researchers and industry to be able to compare rates of the disorder across studies and environments, work should be done to develop a standard management technique for sampling the incidence and severity of RDR in practical settings. While counting the total number of affected drupelets per fruit and/or attempting to classify levels of severity in affected drupelets is time-consuming, a technique such as this offers the most in-depth data about severity of the disorder. Additionally, the incidence of affected drupelets per fruit at several different levels (e.g. 1+, 3+, or 5+ drupelets) can be reported in order to allow for comparison with most other studies. Alternatively, the use of imaging software to digitally assess RDR incidence may offer rapid, accurate, and unbiased evaluation, though such techniques may not be widely available or practical in all situations.

8.5. Conclusions

The findings presented in this thesis have relevance to commercial blackberry producers, retailers, and breeders, as well as implications for future research into both RDR and broader postharvest quality of blackberry fruit. The results from Chapter 4 show that the physiochemical symptoms associated with RDR are consistent with mechanical injury to fruit, resulting in cell decompartmentalisation and the subsequent degradation of anthocyanin pigments.

Chapter 5 demonstrated that handling of fruit is strongly associated with RDR development, and that environmental conditions resulting in fruit skin temperatures exceeding 23 °C during handling can significantly exacerbate the incidence and severity of the disorder. These findings offer further support to the conclusions from Chapter 4, and strongly implicate cell disruption as a major mechanism involved in RDR development. Chapter 7 then demonstrated that storage conditions following mechanical injury can influence the severity of colour change associated with RDR. This suggests that postharvest storage may be able to be manipulated in order to reduce the severity of the disorder, which offers opportunity for further study.

The results from Chapter 6 show that incidence of RDR can be influenced by the N fertiliser application rate. This effect varied with harvest date but was significant in six harvests over the two-year trial. This finding offers some explanation for previous anecdotal observations and offers opportunity for further research into the effects of plant nutrition on RDR and broader blackberry fruit quality.

The data presented in this thesis establish some key mechanisms and causes of RDR. This research has highlighted the importance of environmental factors, fruit handling practices, agronomic management and postharvest factors in RDR development. These findings will contribute to the development of management techniques and future studies incorporating a range of blackberry cultivars and growing environments.

8.6. Summary of key findings

- RDR in blackberries is associated with cellular disruption, loss of membrane integrity and decompartmentalisation in affected drupelets. These processes lead to the degradation of anthocyanin pigments and the resultant colour change associated with the disorder.
- Mechanical injury incurred during handling and transport of fruit is strongly associated with the development of RDR.
- Environmental conditions causing fruit temperatures to exceed 23 °C during harvest appear to significantly exacerbate the degree of structural damage incurred by handling. Further study is needed to investigate the effects of other confounding environmental variables.
- Excessive N application during fruit development and harvest may be associated with increased incidence of RDR. However, rates typically applied in commercial production did not affect the incidence or severity of RDR.

- Inter and intra-seasonal variation in RDR incidence and severity is likely caused by variation in environmental conditions at individual harvest dates.
- Rapid cooling following mechanical injury may exacerbate the severity of colour change.

8.7. Summary of recommendations for managing RDR in commercial blackberry production

- The development of cultivars with low susceptibility to RDR should be pursued.
- Harvesting techniques should be optimised to reduced double and rough handling of fruit.
- Correctly training harvest workers should be a high priority for producers in order to reduce mechanical injuries incurred during harvest.
- Harvesting conditions should be managed to limit handling of blackberries at extreme temperatures. This includes harvesting during the early morning or evening and avoiding harvesting on extremely warm days.
- Cane and field management should be designed around reducing the field heat that fruit are exposed to. Cane architecture to encourage fruit shading, the use of shade cloth, or shading structures should be considered.
- Punnet design and postharvest technologies to reduce mechanical injury to fruit should be explored. Unnecessary fruit-on-fruit contact could be reduced through using punnets which contain only one layer of fruit. With the emergence of larger-fruiting cultivars, the common punnet design may need to be adjusted to better suit these varieties.
- Agronomic management techniques should be investigated further to fully understand the nutrient-fruit quality relationships for specific cultivars and environments. Any links between agronomic management and fruit firmness should be explored.
- Postharvest storage including temperature during handling and rapid temperature changes can influence the severity of RDR, though reducing cooling rate should be thoroughly evaluated for further effects on shelf-life.

- The effect of temperature on the amount of vibration damage incurred during transport of fruit should be investigated.

8.8. Literature cited

Castañeda-Ovando A, Pacheco-Hernández MdL, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA (2009) Chemical studies of anthocyanins: A review. Food Chemistry 113: 859-871.

Cevallos-Casals BvA, Cisneros-Zevallos L (2004) Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. Food Chemistry 86: 69-77. doi: https://doi.org/10.1016/j.foodchem.2003.08.011.

Dorris MR, Voss DM, Bollom MA, Krawiec-Thayer MP, Bolling BW (2018) Browning Index of Anthocyanin-Rich Fruit Juice Depends on pH and Anthocyanin Loss More Than the Gain of Soluble Polymeric Pigments. Journal of Food Science 83: 911-921. doi: doi:10.1111/1750-3841.14106.

Edgley M, Close DC, Measham P (in press) The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels. Acta Horticulurae.

Jiang T, Mao Y, Sui L, Yang N, Li S, Zhu Z, Wang C, Yin S, He J, He Y (2019) Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. Food Chemistry 274: 460-470. doi: https://doi.org/10.1016/j.foodchem.2018.07.141.

Lawrence B, Melgar JC (2018) Harvest, Handling, and Storage Recommendations for Improving Postharvest Quality of Blackberry Cultivars. HortTechnology 28: 578-583. doi: 10.21273/horttech04062-18.

Lee HS, Wicker L (1991) Anthocyanin Pigments in the Skin of Lychee Fruit. Journal of Food Science 56: 466-&. doi: DOI 10.1111/j.1365-2621.1991.tb05305.x.

McCoy JE, Clark JR, Salgado A, Jecmen A (2016) Evaluation of harvest time/temperature on postharvest incidence of red drupelet reversion development and firmness of blackberry (*Rubus* L subgenus *Rubus* Watson). The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences 17: 59-65.

Pérez-Pérez GA, Fabela-Gallegos MJ, Vázquez-Barrios ME, Rivera-Pastrana DM, Palma-Tirado L, Mercado-Silva E, Escalona V (2018) Effect of the transport vibration on the generation of the color reversion in blackberry fruit. 1194 edn. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Perkins-Veazie P, Collins JK, Clark JR (1997) Quantifying Loss of Firmness in Blackberries. HortScience 32: 598.

Redgwell RJ, Fischer M (2002) Fruit texture, cell wall metabolism and consumer perceptions. Fruit quality and its biological basis Sheffield: Sheffield Academic Press: 46-88.

Ross CF, Chauvin MA, Whiting M (2009) Firmness evaluation of sweet cherries by a trained and consumer sensory panel. Journal of texture studies 40: 554-570.

Salgado A (2015) Applying molecular and phenotypic tools to characterize flesh texture and acidity traits in the Arkansas peach breeding program and understanding the crispy texture in the Arkansas blackberry breeding program. University of Arkansas.

Strik B (2008) A review of nitrogen nutrition of Rubus. IX International Rubus and Ribes Symposium 777.

Underhill S, Critchley C (1994) Anthocyanin decolourisation and its role in lychee pericarp browning. Australian Journal of Experimental Agriculture 34: 115-122.

Welch CR, Wu Q, Simon JE (2008) Recent Advances in Anthocyanin Analysis and Characterization. Current analytical chemistry 4: 75-101.

Worthington M, Clark J, Threlfall R, Sebesta B (2017) Evaluation of Red Drupelet Reversion in Blackberries using High-Throughput Digital Image Analysis. Smallfruits.org.
Yin MH (2017) Studies in Blackberry: Development and Implementation of a Phenotyping Protocol for Blackberry Seedling Populations and Impact of Time of Day of Harvest on Red Drupelet Reversion for University of Arkansas Blackberry Genotypes. University of Arkansas.

Chapter 9. Appendices

Appendix A: Fact sheets arising from this project

1. Edgley M, Close DC, and Measham PF (2016), 'Managing Red Drupelet Disorder, Tasmanian Institute of Agriculture – Fact sheet

2. Edgley M, Close DC, and Measham PF (2017), 'Managing Red Drupelet Disorder', Tasmanian Institute of Agriculture – Fact sheet

3. Edgley M, Close DC, and Measham PF (2017), 'Managing Red Drupelet Disorder', Tasmanian Institute of Agriculture – Fact sheet

4. Edgley M, Close DC, and Measham PF (2018), 'Managing Red Drupelet Disorder', Tasmanian Institute of Agriculture – Fact sheet

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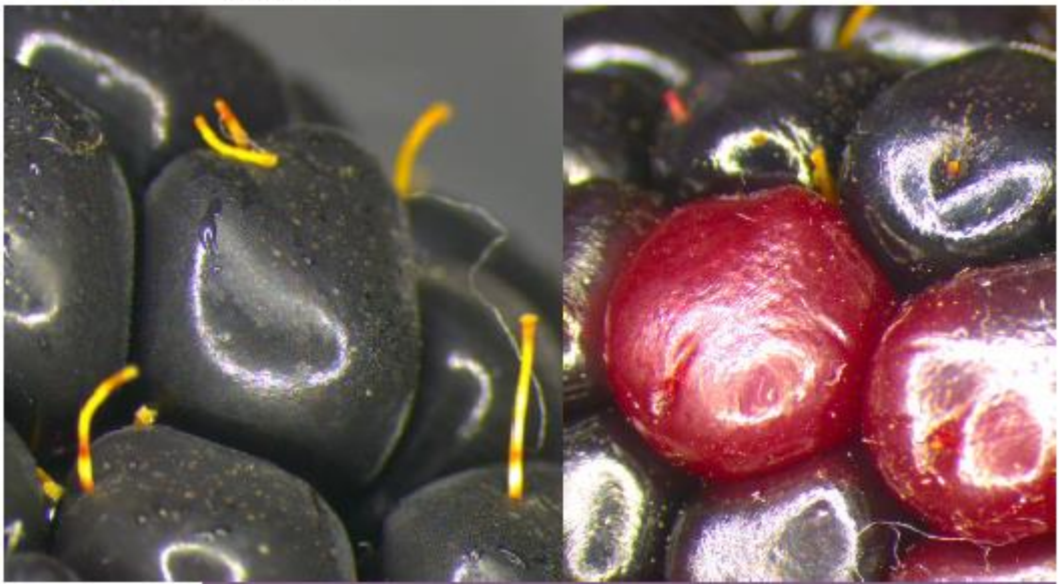

Perennial Horticulture Fact Sheet

Key Points

- A three year project, commenced in August 2015
- The project aims to understand the physiology behind drupelet reversion and develop management techniques to reduce the incidence of the disorder
- Research will assess both pre and post-harvest contributing factors
- Preliminary results indicate that fruit cooled at a slower rate is less prone tothe disorder, and the disorder is much more prevalent when fruit is damaged at very cold or very warm temperatures.
- Excess nitrogen during harvest has been shown to produce higher rates of the disorder

Managing Red Drupelet Disorder

Introduction

Red drupelet disorder (RDD), sometimes referred to as drupelet reversion or reddening, is a physiological disorder of blackberry fruit. Individual drupelets that appear uniform in colour with the rest of the fruit at harvest revert to a red colour following cool storage. Although there are a number of other causes for blackberries to change colour including UV damage, freeze damage, leakage, and insect damage, RDD is thought to be independent of these. Drupelet disorder can affect up to 50% of a crop and is one of the least understood postharvest problems in blackberry fruit production.

More Information

Red drupelet disorder can affect up to 50% of a crop , with anywhere from single drupelets to almost whole fruit being prone. Because the disorder generally appears following harvest and storage, the financial loss from severely affected fruit can be significant.

Affected fruit does not taste any different, however the disorder is off-putting to consumers. Reverted drupelets also appear to be more prone to other damage from leakage and attack by pathogens.

The disorder is heavily influenced by cultivar, with moderate environmental and seasonal variability.

Image 1: Blackberry fruit affected by red drupelet disorder

Underlying Physiology

The underlying physiology of the disorder is not fully understood, but research is underway in this area. The end result of the disorder is a loss of around 50% of the anthocyanin pigment that gives blackberries their dark colour (cyanidin-3-glucoside). It is not clear what reaction is causing this loss. Enzyme activity, pH changes, and physical rupture of the cells are known to cause pigment destruction in other fruit and it is likely that one or more of these play a role in red drupelet disorder.

Year one trials

The first year of field and lab trials were based around inducing the disorder and understanding what contributing factors may be causing high rates of the disorder. This involved:

- Evaluating the impact of storage conditions and physical damage on expression of the disorder
- Investigating the role of nutrition management in red drupelet disorder
- Understanding the underlying physiology of what is happening inside the cells during loss of colour

Preliminary Results

Preliminary results indicate significant factors in drupelet reversion are fruit temperature at harvest and during storage when fruit is most likely to be physically damaged. Fruit that is cooled at a slower rate is less prone to the disorder, and the disorder is much more prevalent when fruit is damaged at very cold or very warm temperatures. As well as this, excess nitrogen application during summer was shown to increase incidence of the disorder.

Image 2: Control fruit (A), fruit physically damaged at 2°C (B), and fruit damaged at 10°C (C)

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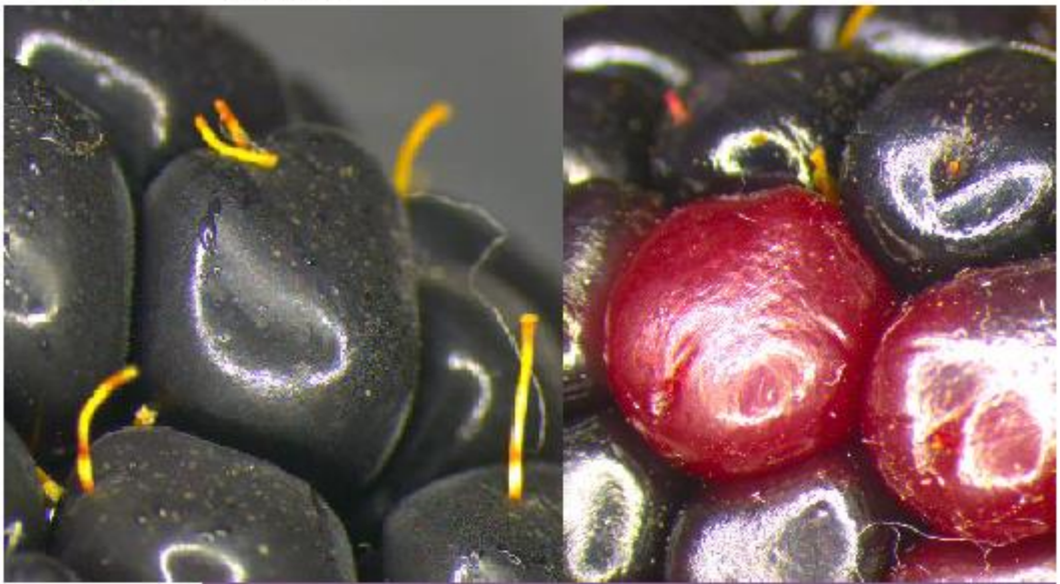

Perennial Horticulture Fact Sheet

Key Points

- Physical damage during harvest and shipping is the main cause of red drupelet disorder; but fruit is more prone under certain conditions
- High nitrogen fertigation during harvest can significantly increase the amount of fruit with red drupelet disorder post-harvest
- Fruit core temperatures exceeding 23C at harvest significantly increase the amount of red drupelet post-harvest
- Harvest times, techniques, and shipping conditions can be manipulated to reduce incidence of red drupelet disorder
- A step-cooling process reducing the rate of cooling post-harvest has been effective in reducing incidence of the disorder

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Managing Red Drupelet Disorder

Introduction

Red drupelet disorder (RDD), sometimes referred to as drupelet reversion or reddening, is a physiological disorder of blackberry fruit. Individual drupelets that appear uniform in colour with the rest of the fruit at harvest revert to a red colour following cool storage. Although there are a number of other causes for blackberries to change colour including UV damage, freeze damage, leakage, and insect damage, RDD is thought to be independent of these. Drupelet disorder can affect up to 50% of a crop and is one of the least understood postharvest problems in blackberry fruit production.

Underlying Physiology

The underlying mechanism responsible for the disorder is a degradation of the anthocyanin pigments which give blackberry fruit their colour. This happens when the cells of the fruit are damaged at harvest or during transport, and is exacerbated by certain environmental conditions such as rapid changes of temperature after damage.

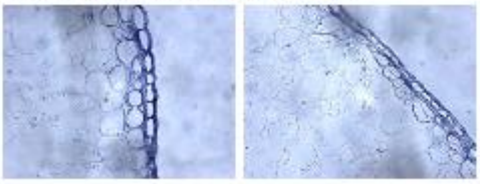

Image 1: Healthy blackberry cells (left) compared to damaged cells in drupelets affected by red drupelet disorder (right)

Harvest and Post-harvest Conditions

Environmental conditions such as temperature, humidity, and plant water status at harvest. Fruit which has a higher core temperature at harvest is significantly more prone to developing red drupelet post-harvest.

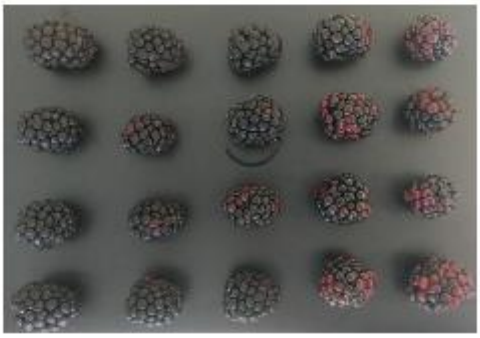

Image 2: Fruit harvested at increasing core temperatures (left to right)

Rate of Cooling

The rate at which fruit is cooled post-harvest has also been shown to play a role in the expression of the disorder. Fruit which is cooled at a slower rate in a 'step-cooling' process had significantly lower incidence of red drupelet in one trial. It is thought that rapid temperature change following physical damage to the fruit can worsen the structural damage.

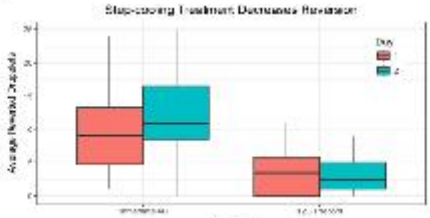

Figure 1: Results from a two day trial comparing storage techniques

Nitrogen Fertigation

The project has included a two-year field trial looking into the effects of nitrogen fertigation rates on post-harvest expression of the disorder. The results of this study include:

- High nitrogen application rates during harvest produced higher rates of red drupelet disorder
- Higher nitrogen rates also produced larger fruit for parts of the season, and higher overall yields

Ongoing Work

Work is ongoing to shed further light on the physiology of the disorder, as well as assessing potential management techniques to reduce incidence.

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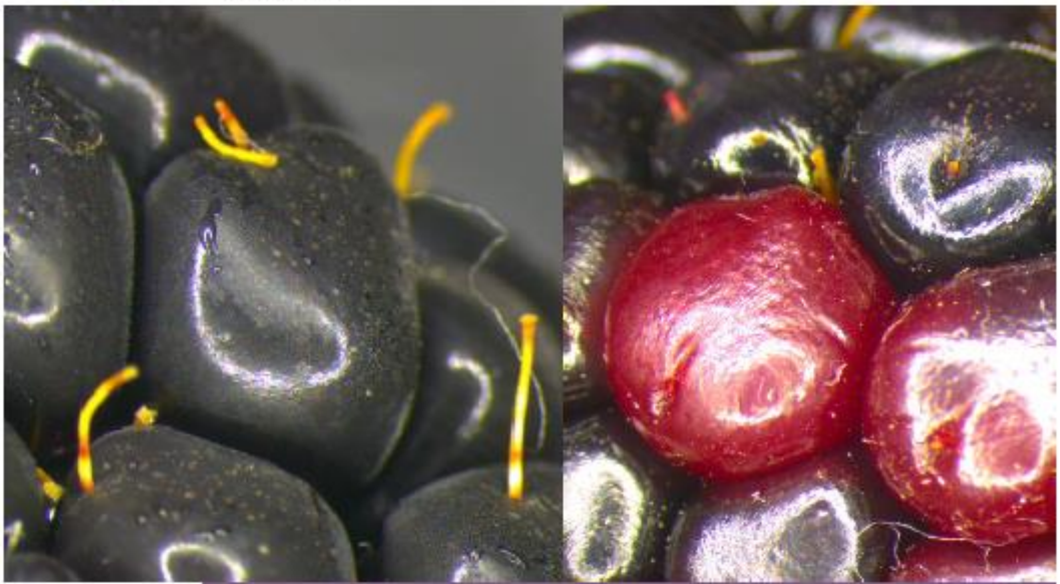

Perennial Horticulture Fact Sheet

Key Points

- Mechanical injury during harvest and shipping is the main cause of red drupelet reversion; but fruit is more prone under certain conditions
- High nitrogen fertigation during harvest can significantly increase the amount of fruit with red drupelet reversion post-harvest
- Fruit flesh temperatures exceeding 23°C at harvest significantly increase the amount of red drupelet post-harvest
- Harvest times, techniques, and shipping conditions can be manipulated to reduce incidence of reversion
- A step-cooling process reducing the rate of cooling post-harvest has been effective in reducing incidence of the disorder

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Managing Red Drupelet Reversion

Introduction

Red drupelet reversion (RDR), sometimes referred to as drupelet reversion or reddening, is a physiological disorder of blackberry fruit. Individual drupelets that appear uniform in colour with the rest of the fruit at harvest revert to a red colour following cool storage. Although there are a number of other causes for blackberries to change colour including UV damage, freeze damage, leakage, and insect damage, RDR is thought to be independent of these. The disorder can affect up to 50% of a crop and is one of the least understood postharvest problems in blackberry fruit production.

Underlying Physiology

The underlying mechanism responsible for the disorder is a degradation of the anthocyanin pigments which give blackberry fruit their colour. This happens when the cells of the fruit are damaged at harvest or during transport, and is exacerbated by certain environmental conditions such as rapid changes of temperature after damage.

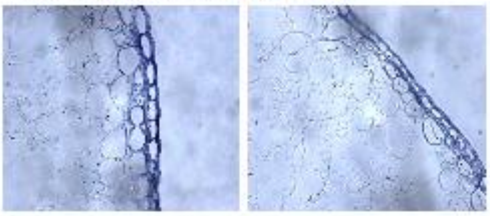

Image 1: Healthy blackberry cells (left) compared to damaged cells in drupelets affected by red

Harvest and Post-harvest Conditions

Environmental conditions such as temperature, humidity, and plant water status at harvest may influence expression of RDR. Fruit which has a higher skin temperature at harvest is significantly more prone to developing red drupelet post-harvest.

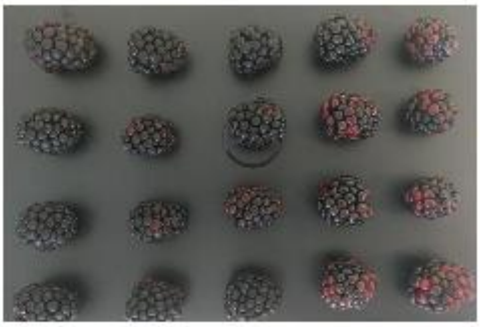

Image 2: Fruit harvested at increasing skin temperatures (left to right)

Rate of Cooling

The rate at which fruit is cooled post-harvest has also been shown to play a role in the expression of the disorder. In one trial, fruit which was cooled extremely quickly after being damaged had more severe colour change than fruit which was cooled at a slower rate.

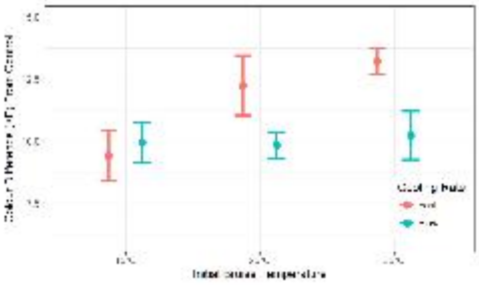

Figure 1: Results comparing bruise temperature

Nitrogen Fertigation

The project has included a two-year field trial looking into the effects of nitrogen fertigation rates on post-harvest expression of the disorder. The results of this study include:

- High nitrogen application rates during harvest produced higher rates of red drupelet reversion
- Higher nitrogen rates also produced larger fruit for parts of the season, and higher overall yields

Ongoing Work

Further analysis and results from the project are ongoing and will be available later this year.

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Hort Innovation Strategic levy investment

RASPBERRY AND BLACKBERRY FUND

This project has been funded by Hort Innovation, using the raspberry and blackberry 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

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Perennial Horticulture Fact Sheet

Key Points

- Physical damage during harvest and shipping is the main cause of red drupelet disorder; but fruit is more prone under certain conditions
- High nitrogen fertigation during harvest can significantly increase the amount of fruit with red drupelet disorder post-harvest
- Fruit core temperatures exceeding 23C at harvest significantly increase the amount of red drupelet post-harvest
- Harvest times, techniques, and shipping conditions can be manipulated to reduce incidence of red drupelet disorder
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Managing Red Drupelet Disorder

Red drupelet disorder (RDD), sometimes referred to as drupelet reversion or reddening, is a physiological disorder of blackberry fruit. Individual drupelets that appear uniform in colour with the rest of the fruit at harvest revert to a red colour following cool storage. Although there are a number of other causes for blackberries to change colour including UV damage, freeze damage, leakage, and insect damage, RDD is thought to be independent of these. Drupelet disorder can affect up to 50% of a crop and is one of the least understood postharvest problems in blackberry fruit production.

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Underlying Physiology

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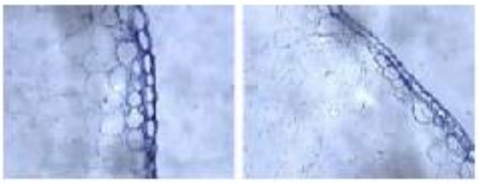

Image 1: Healthy blackberry cells (left) compared to damaged cells in drupelets affected by red drupelet disorder (right)

Rate of Cooling

The rate at which fruit is cooled post-harvest has also been shown to play a role in the expression of the disorder. Fruit which is cooled at a slower rate in a 'step-cooling' process had significantly lower incidence of red drupelet in one trial. It is thought that rapid temperature change following physical damage to the fruit can worsen the structural damage.

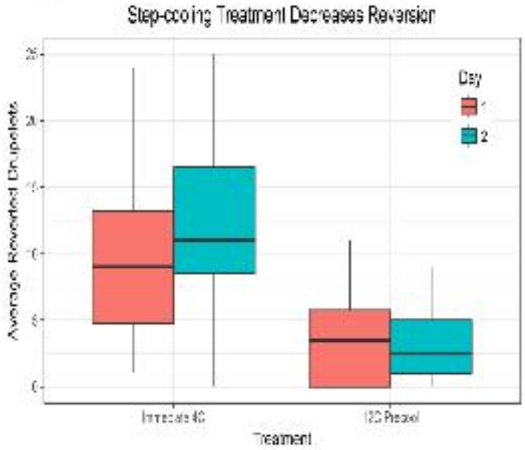

Figure 1: Results from a two day trial comparing storage techniques

Harvest and Post-harvest Conditions

Environmental conditions such as temperature, humidity, and plant water status at harvest can influence red drupelet development. Fruit which has a higher core temperature at harvest is significantly more prone to developing red drupelet post-harvest.

Image 2: Fruit harvested at increasing core temperatures (left to right)

Nitrogen Fertigation

The project has included a two-year field trial looking into the effects of nitrogen fertigation rates on post-harvest expression of the disorder. The results of this study include:

- **High nitrogen application rates during harvest produced higher rates of red drupelet disorder**
- **Higher nitrogen rates also produced larger fruit for parts of the season, and higher overall yields.**

Ongoing Work

Work is ongoing to shed further light on the physiology of the disorder, as well as assessing potential management techniques to reduce incidence.

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Appendix B: Additional material pertaining to Chapter 4

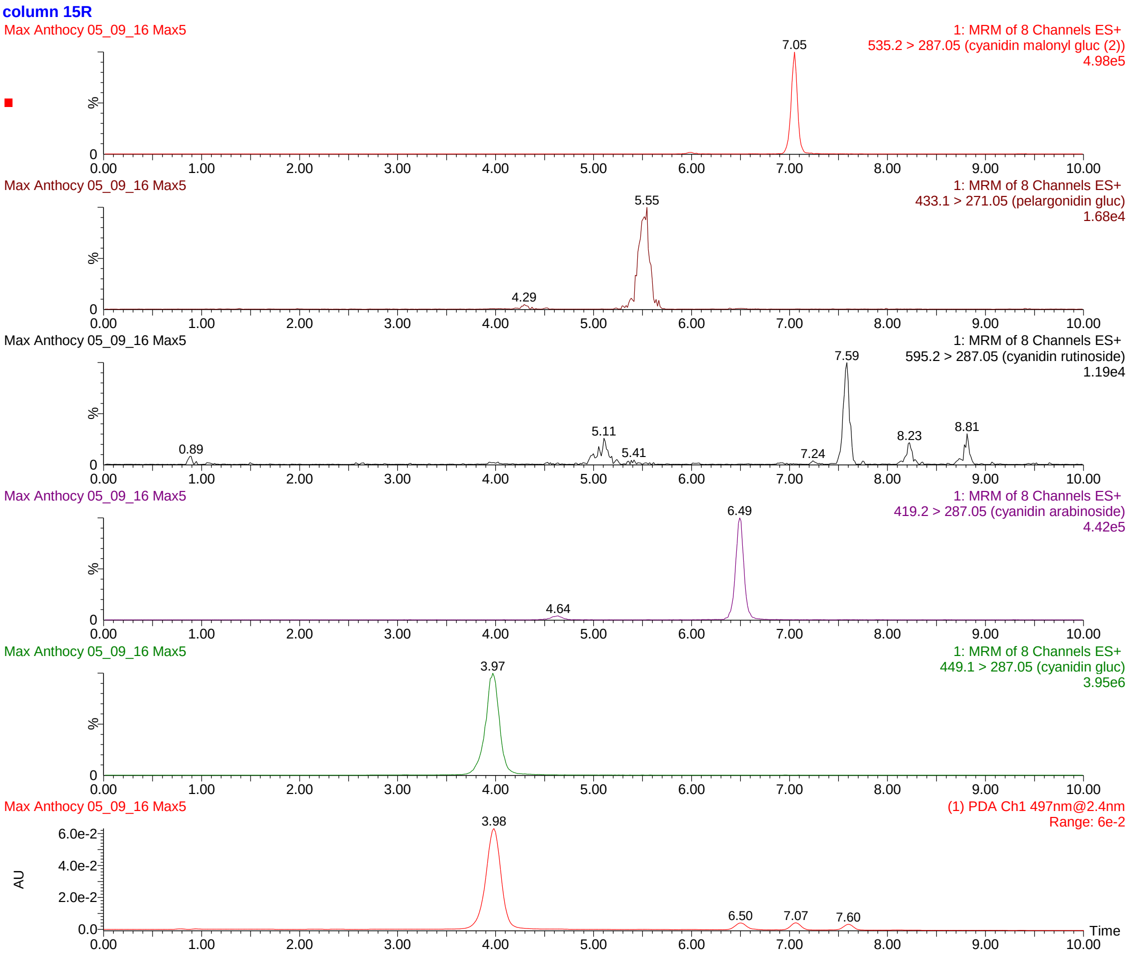


Fig. B-1. Chromatogram of anthocyanins identified in 'Ouachita' samples

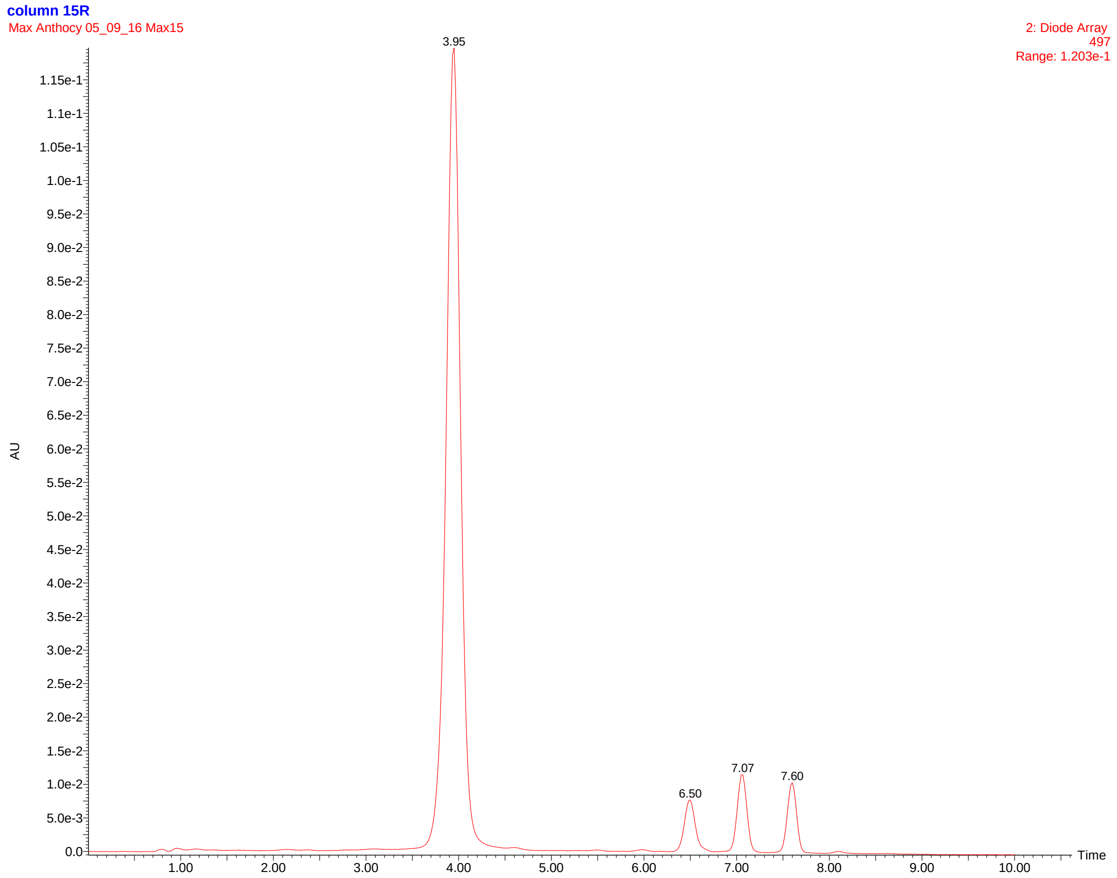


Fig. B-2. Chromatogram of minor anthocyanins identified in 'Ouachita' samples

Table B-1. Retention times of each anthocyanin extracted via UPLC

Anthocyanin:	**Retention time (min)**
Cyanidin-3-glucoside	3.9
Cyanidin-3-arabinoside	4.4
Cyanidin-3-rutinoside	5.0
Pelargonidin-3-glucoside	5.3
Cyanidin-3-(3''-malonylglucoside)	5.8
Cyanidin-3-xyloside	6.5
Cyanidin-3-(6''-malonylglucoside)	7.0
Pelargonidin-3-glucoside	7.5

Appendix C: Additional material pertaining to Chapter 5

Fig. C-1. Fruit from harvest treatment 1 (bottom) and harvest treatment 2 (top).

Fig. C-2. Fruit from harvest treatment 2 (undamaged) 24 days after harvest. Little to no mould was observed and fruit retained high firmness.

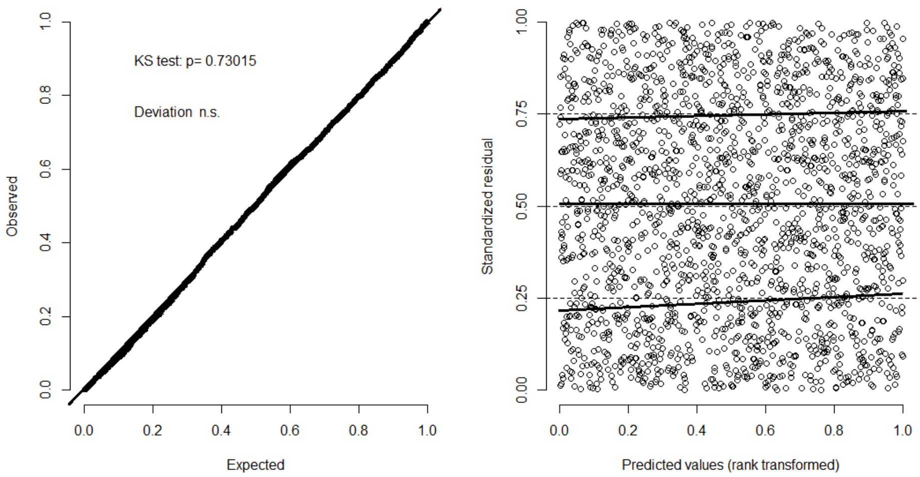
Appendix D: Additional material pertaining to Chapter 6

D.1 Supplementary figures and tables

Supplementary table D-1. Model coefficient estimates and significance.

Effect	Estimate	Std. Error	Z Value	Pr(>|z|)
Nitrogen Treatment	3.21	1.44	2.23	0.03
Harvest Date	-0.10	0.08	-1.44	<0.01
Mass	-0.67	0.23	-2.98	<0.01

[1]Model: $RDI = N\ Treatment + Harvest\ Date + Mass$
[2]Log-likelihood: -3597 on 29 df



Supplementary fig. D-1. Residual diagnosis for the best-fitting zero-inflated negative binomial model. QQ plot with KS test statistic is shown on the left, and residuals versus predicted values on the right.

Supplementary table D-2. Means of TA, TSS, TSS:TA, and monomeric anthocyanin (ACN) concentrations in 2016.

Harvest	N Treatment	TA (% citric acid)	TSS (°brix)	TSS:TA	ACN (mg 100g^{-1} fresh weight)
1	High	0.90 ^{abcde}	11.3 ^{a}	12.7 ^{abcdef}	58.2 ^{c}
	Medium	0.84 ^{abcde}	11.5 ^{a}	13.8 ^{abcd}	63.3 ^{bc}
	Low	0.77 ^{bcde}	11.3 ^{a}	15.0 ^{abc}	66.2 ^{bc}
2	High	1.05 ^{ab}	10 ^{abc}	9.6 ^{ef}	70.0 ^{abc}
	Medium	0.98 a^{bcde}	9.5 ^{bc}	9.9 ^{ef}	72.7 ^{abc}
	Low	1.02 ^{ab}	10.3 ^{abc}	10.1 ^{def}	77.6 ^{abc}
3	High	1.01 ^{abc}	10.3 ^{abc}	10.4 ^{cdef}	67.8 ^{bc}
	Medium	1.09 a	10.8 ^{abc}	9.9 ^{ef}	65.0 ^{bc}
	Low	0.99 ^{abc}	11.0 ^{ab}	11.2 ^{bcdef}	70.0 ^{abc}
	High	1.06 ^{ab}	9.2 c	8.7 ^{f}	83.6 ^{ab}

4	Medium	0.99 [abc]	9.5 [bc]	9.6 [ef]	67.9 [bc]
	Low	1.00 [abc]	9.1 c	9.2 [ef]	73.9 [abc]
	High	0.69 [de]	10.5 [abc]	15.4 [ab]	81.3 [abc]
5	Medium	0.72 [cde]	10.7 [abc]	14.9 [abcd]	80.8 [abc]
	Low	0.67 [e]	10.1 [abc]	16.1 [a]	92.5 [a]

[1]Means followed by different letters in each column were significantly different at P<0.05.

Supplementary table D-3. Means of pH, TA, TSS, TSS:TA, and ACN concentrations in 2017.

Harvest	N Treatment	TA (% citric acid)	TSS (°brix)	TSS:TA	ACN (mg 100g^{-1} fresh weight)
	High	0.94 [a]	12.3 [abc]	13.3 [ab]	79.2 [abc]
1	Medium	1.00 [a]	12.0 [abc]	11.9 [ab]	82.7 [a]
	Low	0.93 [a]	12.5 [abc]	14.0 [ab]	81.2 [ab]
	High	0.85 [a]	13.0 [a]	15.5 [a]	63.2 [abcd]
2	Medium	0.85 [a]	12.6 [ab]	14.9 [a]	63.9 [abcd]
	Low	0.77 [a]	12.9 [a]	16.7 [a]	65.4 [abcd]
	High	0.95 [a]	11.9 [abc]	12.6 [ab]	38.9 [cd]
3	Medium	1.02 [a]	12.0 [abc]	11.8 [ab]	41.0 [bcd]
	Low	0.91 [a]	12.1 [abc]	13.5 [ab]	30.9 [d]
	High	0.92 [a]	10.1 [bcd]	11.4 [ab]	45.8 [bcd]
4	Medium	0.99 [a]	10.8 [abcd]	11.0 [b]	63.8 [abc]
	Low	1.06 [a]	11.8 [abc]	11.1 [b]	41.6 [bcd]
	High	0.88 [a]	11.0 [abcd]	12.7 [ab]	47.5 [abcd]
5	Medium	0.89 [a]	10.5 [abcd]	11.8 [ab]	78.5 [abc]
	Low	0.92 [a]	10.7 [abcd]	11.7 [ab]	55.9 [abcd]
	High	0.99 [a]	9.1 [d]	9.2 [b]	63.5 [abcd]
6	Medium	0.88 [a]	9.8 [cd]	11.2 [b]	45.9 [abcd]
	Low	0.93 [a]	10.1 [bcd]	11.0 [b]	54.6 [abcd]

[1]Means followed by different letters in each column were significantly different at P<0.05.

Supplementary table D-4. Mean macronutrient concentration in fruit over the course of each season.

2016				
Harvest	Treatment	P (%)	K (%)	Ca (%)
	212 kg N ha^{-1}	0.17	1.10	0.22
1	106 kg N ha^{-1}	0.16	1.10	0.20
	53 kg N ha^{-1}	0.15	0.97	0.16
	212 kg N ha^{-1}	0.15	1.08	0.18
3	106 kg N ha^{-1}	0.16	1.22	0.20
	53 kg N ha^{-1}	0.15	1.06	0.20
	212 kg N ha^{-1}	0.15	0.99	0.21
5	106 kg N ha^{-1}	0.15	1.08	0.19
	53 kg N ha^{-1}	0.16	1.02	0.18
2017				
	212 kg N ha^{-1}	0.15	1.18	0.16
1	106 kg N ha^{-1}	0.14	1.04	0.19

	53 kg N ha^{-1}	0.15	1.14	0.17
3	212 kg N ha^{-1}	0.15	1.15	0.22
	106 kg N ha^{-1}	0.13	0.90	0.17
	53 kg N ha^{-1}	0.14	0.97	0.19
5	212 kg N ha^{-1}	0.14	0.97	0.20
	106 kg N ha^{-1}	0.13	0.93	0.18
	53 kg N ha^{-1}	0.14	1.04	0.23

[1]Means followed by different letters in each column and year were significantly different at P<0.05.

Supplementary table D-5. Phosphorus (P), potassium (K), and calcium (Ca) concentrations of primocane leaf samples taken two weeks postharvest.

Treatment	P (%)	K (%)	Ca (%)
2016			
212 kg N ha^{-1}	0.17 [a]	1.77 [a]	NA *
106 kg N ha^{-1}	0.19 [a]	1.82 [a]	NA *
53 kg N ha^{-1}	0.19 [a]	1.78 [a]	NA *
2017			
212 kg N ha^{-1}	0.14 [b]	1.05 [b]	1.28
106 kg N ha^{-1}	0.15 [b]	1.02 [b]	0.93
53 kg N ha^{-1}	0.13 [b]	0.82 [b]	1.23

1Means followed by different letters in each column and year were significantly different at P<0.05.
2 Analysis for Ca concentration was not available for the 2016 season.

D.2. Acta Horticulturae article

The following research article was published in Acta Horticulturae as a refereed conference paper. The article was written after the first year of the two-year nitrogen experiment. Chapter 6 supersedes this article; thus it is included as an appendix and not a stand-alone chapter.

Edgley, M., Close, D.C. and Measham, P.F. (2018). The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels. Acta Hortic. 1205, 885-890. DOI: 10.17660/ActaHortic.2018.1205.113

The effects of N fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels

M. Edgley, D.C. Close and P.F. Measham

Perennial Horticulture Centre, Tasmanian Institute of Agriculture, University of Tasmania, Sandy Bay, Tasmania, Australia.

Abstract

Red drupelet disorder (RDD), sometimes called reversion, red cell, or reddening, is a physiological occurrence in blackberries (*Rubus fruticosus*) which causes individual drupelets to revert to a red colour from black, reducing marketable yield. The disorder usually develops during postharvest cool storage. The objective of this trial was to assess the impact of nitrogen (N) application on fruit and foliar N concentration, with the aim to identify any link between N availability and RDD. Three levels of N (52.85, 105.7, 211.4 kg ha^{-1} total) were applied via weekly fertigation during the harvest period to 'Ouachita' blackberries grown under 150 µm high-UV transmittance polythene tunnels in northern Tasmania, Australia. The design consisted of three blocks of three 105.7 m long rows arranged in a complete randomised block design, with each row receiving a N application treatment. Fruit was sampled at five dates every 15 days between January 11 and March 11, 2016 by harvesting every fruit from four randomly selected 3 m sections of cane, from which subsamples of 20 fruit per row were taken for further analysis. Fruit were assessed for drupelet disorder by counting individual drupelets expressing RDD then weighed and homogenised to measure pH, titratable acidity, and total soluble solids (TSS) (°Brix). 'High' N treatment fruit had significantly increased average number of drupelets expressing RDD relative to the fruit of 'low' and 'medium' N treatments in the first four harvest dates. Over the season the average number of drupelets per fruit showing any level of RDD was 2.2, 1.63, and 1.41 for high, medium, and low N treatments, respectively. Early season fruit across all treatments had the highest levels of reversion averaging 3.19 drupelets per fruit in pick 1 with levels declining significantly at each subsequent harvest date (2.61, 1.41, 1.17, and 0.69 drupelets per fruit in picks 2-4 respectively). This study has shown that there is an association between high N fertigation during harvest and RDD expression, and time of the season and RDD expression.

Keywords: *Rosaceae*, green house, protected cropping, fruit quality

INTRODUCTION

Red drupelet disorder (RDD), sometimes referred to as reversion, red cell, or reddening, is a physiological disorder in blackberries which causes individual drupelets to revert from black to red, causing fruit to appear mottled and defective. The underlying mechanism and causes of the disorder are not currently known; however, it is thought within the industry that physical damage from handling during harvest and packing is linked to expression of reversion. The disorder is commonly developed postharvest after fruit goes through rapid temperature changes, though incidence is also reported for fruit on canes in some cultivars. There is currently little research into the disorder, with the underlying physiological mechanisms unknown, although it is known to be influenced by cultivar and environment (Clark and Finn, 2011). It has been suggested that the mechanism responsible for the loss of colour is a vacuolar rupture inside the cell, causing anthocyanin pigments to be degraded (Salgado and Clark, 2016). Salgado and Clark (2016) also demonstrated that 'crispy' blackberry cultivars, of high firmness, were particularly resistant to the disorder, which may suggest a link between the disorder and susceptibility of a cultivar to physical damage. Anecdotal evidence throughout growing regions in Australia and North America suggests that excess N during the harvest period can contribute to expression of the disorder, particularly to fruit grown in protected conditions, though a cause for this effect has not been established.

Plant nutrition has been shown to have a significant influence on fruit quality throughout a range of horticultural crops, with N, phosphorus (P), potassium (K) and calcium (Ca) all having been reported to affect various aspects of postharvest quality of berry fruit (Ali et al., 2012; Goldman et al., 1999). Nitrogen is the most abundant plant nutrient and is required in the highest concentrations for plant health with an adequate supply needed for bud and fruit development and nutritional quality (Ali, 2012). Over-supply of N has been reported to significantly decrease fruit antioxidant levels including anthocyanins due to the allocation of plant resources to increased vegetative growth resulting in a reduction in secondary metabolite production (Ali, 2012). Increased levels of N application have also been linked to decreased fruit background colour in cherries (Swarts et al., 2016), and decreased firmness in apples and strawberries (Miner et al., 1997; Nava et al., 2007).

Blackberries are increasingly being produced under tunnels, particularly in temperate growing regions, due to the ability to protect the fruit from adverse weather conditions or pests, manipulate the microclimate, and advance or extend the fruiting season to target high value markets (Strik et al., 2007). As producers increasingly shift towards protected cultivation for blackberries, new challenges have emerged in regards to pests, diseases, and nutrition management such as the loss of fruit to RDD. The objective of this study was to investigate the effects of N fertiliser rates on fruit quality in blackberry production. Specifically, we aimed to test how increasing N fertigation before and throughout the key growth and harvest period of blackberries would affect RDD expression and other fruit quality attributes.

MATERIALS AND METHODS

Site and cultivar selection

The fertigation trial was conducted at Dunorlan Farm in Northern Tasmania on a 5-year-old planting of 'Ouachita' blackberries trellised with a two wire trellising system. Rows were 105.7 m long and spaced at 2.5 m intervals, with three rows per tunnel. Tunnels were 4 m high at the apex with 3 m spacing of rows between tunnels, and covered with 150 µm high-UV transmittance polythene. 'Ouachita' was selected based on grower observations; uniformity of growth and fruit quality historically throughout the experimental block; a medium to high susceptibility to RDD; and a relatively small incidence of other pests and diseases in the cultivar at the experimental site historically. The cultivar is erect and thornless, producing blocky and conical fruit which is often non-uniform in shape, with drupelets commonly being uneven in size or protruding from the receptacle (Clark and Moore, 2005).

Experimental design

The experimental layout for the trial consisted of a randomised complete block design, with each of three tunnels treated as a block and each row in each block given a fertigation treatment of low (52.84 kg ha^{-1}), medium (105.74 kg ha^{-1}), or high (211.4 kg ha^{-1}) N as fertiliser grade $Ca(NO_3)_2$ which was applied once weekly via drip fertigation. This provided three replicates of each treatment. Treatments were applied as per industry standard, which commenced on November 9, 2015 through to March 2016.

Fruit harvest and quality analysis

Fruit was sampled five times throughout the season, every 15 days between January 11 and March 11 by harvesting every ripe fruit from each side of three randomly selected 4 m sections of cane, not including a 5 m buffer zone at each end of the rows. Fruit was harvested mid-morning directly into 125 g clamshell punnets, transported in a cooler with ice to the Tasmanian Institute of Agriculture in Hobart and stored at 4°C. Temperature (°C) and relative humidity (%) was logged hourly throughout the season in each tunnel by iButton DS1923 Hygrochron data loggers which were hung from the middle cable of the trellis system in places where minimal canes were located. Subsamples of 20 fruit at the 'shiny black' level of maturity were taken to evaluate for quality characteristics after 24 h in 4°C storage. Fruit were weighed on Mettler Toledo scientific balance scales, with average mass and total fruit count from harvested sections used to approximate yield (g m^{-1} of cane). Fruit firmness was measured using a Firmtech II firmness tester (Bioworks Inc, Wamego, KS, USA) and expressed as g mm^{-2} depressed. The number of drupelets expressing any symptoms of RDD was counted on each fruit and subsamples were then frozen at -24°C for further analysis post season. For analysis, samples were brought to 4°C then homogenized using a Retsch Grindomix GM200 for 30 s and centrifuged for 10 min at 4000 rpm at 4°C to obtain a clarified juice sample. Total soluble solids concentration (°brix) was measured using a Shibuya Optical hand held refractometer, titratable acidity (TA) and pH were measured using a Metrohm 702 SM Titrino automated titrater with TA being expressed as % citric acid equivalent. A second subsample of fruit was sent to AgVita Analytical for macronutrient concentration analysis (N, P, K, Ca) at pick 1 (January 11), 3 (February 14), 5 (March 11), and primocane leaf samples were also analysed for macronutrient concentration postharvest (March 30).

Data analysis

RDD expression data were analysed using a generalised linear mixed models (GLMM) approach in R version 3.3.1 (R Core Team, 2016) with a log-link function and a quasi-poisson distribution to account for over-dispersed count data. Effects of N treatment and pick date on fruit quality measurements from subsamples were tested by two-way ANOVAs with Tukey HSD post hoc tests at significance level $P<0.05$.

RESULTS AND DISCUSSION

The overall trend for the number of drupelets per fruit that expressed any symptoms of RDD (both 'partial' and 'full') was highest at the start of the season within all treatments. The total incidence of RDD across all N treatments declined significantly at each pick, with almost no incidence in pick 5 (Figure 1A). This trend was not explained by any covariates measured and may be due to other fruit biochemical changes, environmental conditions, or the conditions of the canes. High fruit flesh temperature combined with high humidity and subsequent rapid cooling are often linked to RDD expression, though this effect was not observed in this study. The temperature and humidity of the dates of harvest and surrounding days in this study did not reach high levels (data not shown), which is likely why this relationship was not observed.

There was a significant difference ($P<0.01$) in RDD expression between the high N treatment and both other treatments on every pick date but the final pick (Figure 1A). This difference came mostly from the increased number of fruit from the high N treatment having low levels of RDD (1-2 drupelets), whilst low and medium treatments had higher amounts of fruit with no incidence of the disorder (Figure 1B). As incidence of reversion increased per fruit, the difference in occurrence between N treatments lessened. These data appear to confirm the industry suggestion that increasing N application to blackberries can increase expression of RDD. It should be noted that the high levels used in the trial were significantly above that commonly seen in commercial production and that there were no significant differences between the low and medium levels used. The 'medium' level of N application used in this study is in line with commercial rates commonly applied. As these data are from a single year it may be that continued use of high N rates could compound the issue to produce this effect after a number of years at commercial N rates.

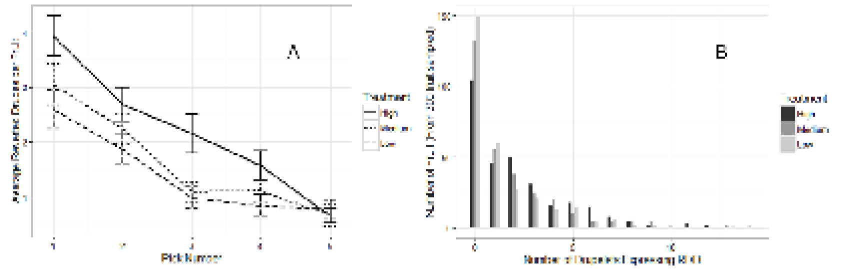


Figure 1. Mean number of drupelets showing any level of red drupelet disorder per pick with treatment (A) and total count numbers of red drupelet incidence per fruit for each nitrogen treatment (B).

Across the season, average yield per pick rose from pick 1 to pick 2 where it peaked before sharply decreasing at pick 3 and gradually decreasing through picks 4 and 5 (Figure 2A). The sharp decrease seen at pick 3 was contributed to by a severe weather event of combined high temperature and low humidity, which caused the loss of some young fruit and buds. Cumulative yield significantly increased ($P<0.01$) between the low and high treatments over the course of the season but no other significant interactions between yield and treatment were observed (Figure 2A). Berry fruit size and mass were relatively high at pick 1 and decreased significantly ($P<0.01$) throughout the season between every pick apart from between picks 1-2 and 4-5, with no interaction between mass and N treatment (Figure 2B).

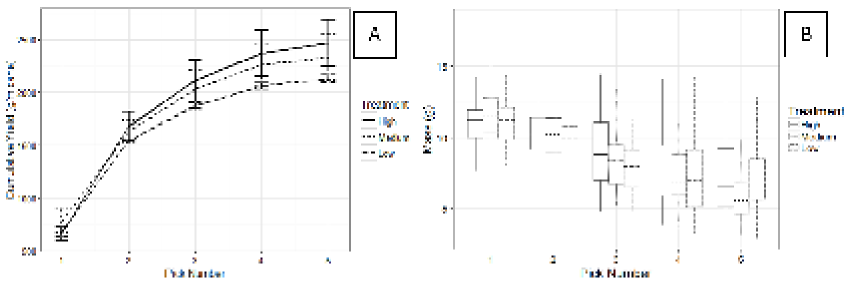


Figure 2. Cumulative yield over the course of the season for each N treatment (A) and average berry mass per pick and treatment (B).

Average berry firmness declined significantly ($P<0.05$) between the start (pick 1), middle (picks 2, 3, and 4), and end (pick 5) of the season with no significant difference between N treatments at any pick (Table 1). Though both firmness and RDD expression declined over the season, the data indicated no relationship ($P=0.15$) between these variables in individual berries. This may be partly due to the high variability seen in individual berry firmness and it is possible that with a greater sample size a significant relationship between these variables may be identified. Larger fruit tended to be firmer overall ($P<0.05$), and larger fruit was also more likely to express drupelet expression in 1-3 drupelets (Figure 1B), though this was partially explained by the higher number of drupelets per fruit as fruit increased.

There was no clear trend for in fruit chemical attributes (pH, titratable acidity and total soluble solids) between treatments, but these characteristics did vary between picks (Table 1). This may be a reflection of the high level of variability seen with ripening blackberry fruit throughout the season as the subsamples were selected for homogeneity of maturity between picks and treatments.

Table 1. Means of firmness, pH, titratable acidity, and soluble solids per pick with treatment.

Pick	Treatment (N)	Firmness (g mm^{-2})	pH	TA (% citric acid)	TSS (°Brix)
1	High	140.0 ab	3.3 abc	0.90 abcde	11.3 a
	Medium	146.6 a	3.34 abc	0.84 abcde	11.5 a
	Low	138.4 ab	2.92 c	0.77 bcde	11.3 a
2	High	132.6 abc	2.96 bc	1.05 ab	10.0 abc
	Medium	124.5 bcd	2.92 c	0.98 abcde	9.5 bc
	Low	127.6 abcd	3.11 abc	1.02 ab	10.3 abc
3	High	119.3 cd	3.30 abc	1.01 abc	10.3 abc
	Medium	126.8 bcd	3.34 abc	1.09 a	10.8 abc
	Low	113.1 cd	3.23 abc	0.99 abc	11.0 ab
4	High	118.3 cd	3.23 abc	1.06 ab	9.2 c
	Medium	119.2 cd	3.07 abc	0.99 abc	9.5 bc
	Low	119.3 cd	3.07 abc	1.00 abc	9.1 c
5	High	108.5 de	3.73 ab	0.69 de	10.5 abc
	Medium	94.9 e	3.57 abc	0.72 cde	10.7 abc
	Low	111.0 d	3.79 a	0.67 e	10.1 abc

Means followed by different letters were significantly different at $P<0.05$.

Fruit N concentration was reflective of the differing N application rates in the early and late season nutrient concentration analysis, but not in the middle season fruit or postharvest primocane leaf sample (Table 2).

Table 2. Macronutrient concentration on a dry matter basis, in fruit over the season and leaf samples postharvest.

	Nitrogen treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)
Early season fruit	High	1.17	0.17	1.30	0.22
	Medium	1.05	0.16	1.10	0.20
	Low	1.01	0.15	0.97	0.16
Mid-season fruit	High	1.15	0.15	1.08	0.18
	Medium	1.14	0.16	1.22	0.20
	Low	1.16	0.15	1.06	0.20
Late season fruit	High	1.20	0.15	0.99	0.21
	Medium	1.10	0.15	1.08	0.19
	Low	1.05	0.16	1.02	0.18
Postharvest primocane leaf	High	2.26	0.17	1.77	NA[1]
	Medium	1.98	0.19	1.82	NA
	Low	2.27	0.19	1.78	NA

[1]Calcium was not measured in postharvest primocane samples.

CONCLUSIONS

This work indicated that increasing N supply to blackberries grown in tunnel production systems can significantly increase the postharvest expression of red drupelet disorder. Increasing N from the low to high levels used in this study significantly increased cumulative yields over the season, but there was no significant difference between medium to high N treatments. There were no differences in fruit chemical quality characteristics between N fertigation levels. For the conditions and cultivar examined, RDD expression was highest at the beginning of the season and steadily declined, though it is not clear whether this is due to physiological changes within the fruit or environmental influences over the season.

Further work is required to determine the mechanisms by which RDD is causing fruit colour change as well as the mechanism by which increasing N appears to increase RDD expression.

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Literature cited

Ali, L. (2012). Pre-Harvest Factors Affecting Quality and Shelf-Life in Raspberries and Blackberries (Department of Horticulture, Swedish University of Agricultural Sciences).

Ali, L., Alsanius, B.W., Rosberg, A.K., Svensson, B., Nielsen, T., and Olsson, M.E. (2012). Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. Eur. Food Res. Technol. *234* (*1*), 33–44 https://doi.org/10.1007/s00217-011-1604-8.

Clark, J.R., and Finn, C.E. (2011). Blackberry breeding and genetics. Fruit, Vegetable and Cereal Science and Biotechnology *5*, 27–43.

Clark, J.R., and Moore, J.N. (2005). 'Oauchita' thornless blackberry. HortScience *40*, 258–260.

Goldman, I.L., Kader, A.A., and Heintz, C. (1999). Influence of production, handling, and storage on phytonutrient content of foods. Nutr. Rev. *57* (*9 Pt 2*), S46–S52 https://doi.org/10.1111/j.1753-4887.1999.tb01807.x. PubMed

Miner, G.S., Poling, E.B., Carroll, D.E., Nelson, L.A., and Campbell, C.R. (1997). Influence of fall nitrogen and spring nitrogen – potassium applications on yield and fruit quality of 'Chandler' strawberry. J. Am. Soc. Hortic. Sci. *122*, 290–295.

Nava, G., Dechen, A.R., and Nachtigall, G.R. (2007). Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil. Commun. Soil Sci. Plant Anal. *39* (*1-2*), 96–107 https://doi.org/10.1080/0010362070 1759038.

R Core Team. (2016). R: a Language and Environment for Statistical Computing (Vienna, Austria: R Foundation for Statistical Computing), http://www.R-project.org.

Salgado, A.A., and Clark, J.R. (2016). "Crispy" blackberry genotypes: a breeding innovation of the University of Arkansas blackberry breeding program. HortScience *51*, 468–471.

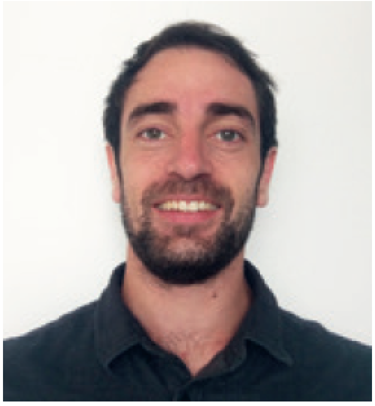
Strik, B.C., Clark, J.R., Finn, C.E., and Banados, M.P. (2007). Worldwide blackberry production. Horttechnology *17*, 205–213.

Swarts, N.D., Hardie, M., and Close, D.C. (2016). Precision nitrogen fertigation and irrigation management for improved apple quality. Acta Hortic. *1130*, 485–490 https://doi.org/10.17660/ActaHortic.2016.1130.73.

D.3. Chronica Horticulturae article

The following news article was published in Chronica Horticulturae (Vol. 57, no. 2, pp. 11), summarising the Acta Horticulturae article (Appendix D.2.).

The effects of nitrogen fertiliser application rates on red drupelet disorder (reversion) in 'Ouachita' thornless blackberries grown under tunnels



› Max Edgley

This project investigated the effects that different rates of nitrogen fertigation had on red drupelet disorder expression in commercial blackberries. Red drupelet disorder (sometimes known as red drupelet reversion or reddening) is a postharvest physiological disorder that causes fruit, which is black at harvest, to revert to a red colour following cold storage. This causes a mottled appearance on the fruit that is off-putting to the consumer and can render fruit unmarketable in some cases, causing significant financial loss to producers. In severe cases, the disorder can affect over half the crop. There is currently very little knowledge surrounding causes and contributing factors to the disorder, with no standard management practices available to reduce incidence. One factor that has previously been suggested as a contributor to high rates of the disorder is excess nitrogen fertilisation close to harvest.

Results showed that higher nitrogen fertigation rates prior to and during harvest, to canes grown under high tunnels, significantly increased rates of red drupelet disorder in fruit. The highest nitrogen application rate used in the study resulted in a 56% increase in expression of red drupelet compared with the lowest application rate throughout the course of the harvest season. This effect was highest at the beginning of the harvest season, at which time red drupelet incidence was highest, and declined as the harvest season progressed. Higher nitrogen rates also increased the total yield, but did not affect berry weight, pH, sugar content, or total acidity. This research shows that a link exists between nitrogen fertigation rates and red drupelet disorder expression, although further work is ongoing to investigate the underlying reason for this increase. These findings have the potential to guide industry standards to reduce incidence of the disorder in commercially produced blackberries.

Max Edgley won an ISHS student award for the best oral presentation at the I International Symposium on Protected Cultivation in Tropical and Temperate Climates & X International Symposium on Protected Cultivation in Mild Winter Climates in Australia in November 2016.

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