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Environmental and management factors contributing to variability in flesh colour of a red kiwifruit cultivar in New Zealand

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ABSTRACT

Consistency of flesh colouration is important for commercial acceptance of red-fleshed *Actinidia chinensis* cultivars, but colour variation between and within orchards is widely reported. Physiological and environmental factors reported to influence this variability include parent cane and shoot type, ploidy of pollen parent, and high temperatures, specifically cumulative time above 25 °C over the growing season. However, the impacts of temperature in a temperate climate (e.g. New Zealand) on colour variability have not been explored. Light, a commonly reported influence on anthocyanin expression in other plants, does not directly influence red kiwifruit colour but the indirect effects of light exposure are unknown. This study explored the colour variability found in eight New Zealand red kiwifruit orchards spread across five regions and examined correlations of environmental conditions (temperature and light exposure) with fruit colour. Two harvests were collected from each orchard in 2014, with two further harvests being conducted in one orchard in 2015. It was found that the variation between orchards and regions was larger than within orchards but no clear regional patterns existed. A significant relationship existed between softer fruit and stronger red colouration, measured by hue angle and outer pericarp colour score. A significant relationship also existed between increased flesh colour and higher number of leaf layers, lower estimated light exposure, and paler skin colour, with skin colour score having the largest effect on flesh colour. No significant correlations between temperature in March and colour expression were found, but three out of the four lower colour orchards had significantly more hours above 25 °C during March. Collectively, these data suggest that full colour development may be promoted directly by carbohydrate supply from a denser canopy and is probably negatively affected by excessive fruit temperatures that may be more common under poorly-developed canopies or in areas with high daily maximum temperatures.

1. Introduction

The kiwifruit genus *Actinidia* holds plants with considerable variation in fruit shape, size, taste, and colour, but the first internationally traded cultivar was green-fleshed 'Hayward', developed in New Zealand. Subsequently, yellow-fleshed kiwifruit cultivars were developed and became a significant component of the New Zealand kiwifruit industry. Genetic potential also existed in the *Actinidia* genus for the development of red-fleshed cultivars, and in recent years this has begun to be realized, starting with the commercialisation of Chinese-bred red-centred cultivar 'Hongyang' in the late 1990s (Wang et al., 2003). Red cultivars have yet to become a significant component of the New Zealand kiwifruit industry. However, achieving this may be desirable, as cultivars with red colouration show potential to achieve higher prices than gold fruit (Jaeger and Harker, 2005) and could create further interest in kiwifruit consumption.

One of the factors limiting commercial production potential of red kiwifruit in New Zealand is inconsistency of colouration. Red colouration of existing cultivars has frequently been reported to be inconsistent or less than expected, whether grown in New Zealand or elsewhere. This variation often occurs between orchards and regions, but has also been noted within vines (Wang, 2011; Zhong et al., 2007). However, the relative amount of variation occurring at each level has not been quantified. As variation is often the source of risk for commercial operators, it is important that the amount and location of this variability be identified.

The pigmentation of red-fleshed kiwifruit is produced by anthocyanins. In *A. chinensis* red-fleshed cultivars, these anthocyanins have been identified as cyanidin 3-galactoside and cyanidin 3-(xylosyl)-galactoside (Montefiori et al., 2009). Anthocyanin content in plants is

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determined by genetic potential and subsequent regulation of this potential by physiological and environmental factors. While anthocyanin content in some plants, such as blueberries, is mainly genetically determined, with the same cultivar grown in significantly different climatic regions producing similar levels of colouration (Finn et al., 2003), the variation in red kiwifruit colouration within a cultivar shows that factors other than genetics are clearly important for its anthocyanin accumulation.

Physiological factors indicated to generally increase anthocyanin accumulation include nitrogen and phosphorus deficiency and sucrose availability (Chen et al., 2013; Keller and Hrazdina, 1998; Koshita et al., 2011; Lillo et al., 2008). In red kiwifruit, cane and shoot type and ploidy of pollen parent have also been shown to have significant effects on fruit colour. In work on a cultivar with strong outer pericarp colour expression in a New Zealand orchard, fruit from long shoots off small diameter canes had significantly more colour than fruit from short shoots off large diameter canes (Nardozza et al., 2015). Pollination of diploid red-fleshed *A. chinensis* with tetraploid or hexaploid pollen has been shown to result in significantly lower anthocyanin concentrations than pollination with diploid pollen in red-fleshed *A. chinensis* seedlings and a red-centred *A. chinensis* cultivar, 'Hort 22D' (Seal et al., 2013; Seal et al., 2016). These factors go some way towards explaining variation occurring within and between orchards, as plant vigour, pruning strategy, and pollen parent may vary.

However, environmental conditions have been shown to play a significant role in determining anthocyanin concentration in many types of plants, including red kiwifruit. A temperature and anthocyanin concentration relationship has been demonstrated with a wide variety of plants, including roses (Dela et al., 2003), model crops for monocots and dicots such as maize and *Arabidopsis* (Christie et al., 1994; Leyva et al., 1995), apples (Lin-Wang et al., 2011; Xie et al., 2012), grapes (Mori et al., 2007; Spayd et al., 2002), and sweet potatoes (Villavicencio et al., 2007) with high temperatures decreasing anthocyanin concentration and low temperatures increasing it. Links have also been made between temperature and anthocyanin content in red kiwifruit: in multiple studies in China, higher altitude fruit with lower summer temperatures had higher fruit colour (Man et al., 2015; Zhong et al., 2007). However, it has yet to be shown if temperature variation within and between regions in New Zealand is sufficient to create colour variation in red kiwifruit and how large this effect is.

Light exposure is also a significant influence on anthocyanin expression in many types of fruit. Red-skinned apples have been shown to require light exposure for anthocyanin synthesis (Saure, 1990; Takos et al., 2006), with red-skinned pears and some cultivars of grapes also showing a similar requirement (Zhang et al., 2012; Zheng et al., 2013). However, the synthesis of anthocyanins in other plants, including some grape cultivars (Zheng et al., 2013) and strawberries (Kawanobu et al., 2011) is light-sensitive but not dependent, while in crops such as blood oranges, sweet potatoes, and potatoes, anthocyanin synthesis is constitutively activated (Lee, 2002; Lewis et al., 1999; Shi et al., 1992). It is apparent that anthocyanin expression in red kiwifruit, with brown skin, is regulated differently to anthocyanin expression on the surface of a fruit and its regulation is likely to be more similar to that in plants where anthocyanin production is unresponsive to light levels. Indeed, bagging or shading red kiwifruit has been shown not to reduce red colouration (Nardozza et al., 2015). Despite this, the light environment of the plant and fruit may still have an impact on fruit colour e.g. through carbohydrate supply (Hou et al., 2010). Additionally, direct light received by a plant organ can increase its temperature by up to 15 °C above the air temperature (Woolf and Ferguson, 2000), which may produce a flow-on effect of reduced anthocyanin concentration through transcriptional regulation. These indirect and temperature-mediated light effects have yet to be examined on red kiwifruit. While it is known that shading does not decrease red kiwifruit colouration, the impacts of direct light exposure are not known.

Here, we undertook a survey of colour expression of a red kiwifruit cultivar grown in five regions of New Zealand and the corresponding environmental factors in the 2013/2014 growing season, with additional work on one orchard in 2015. We quantified the levels (region, orchard, vine, and position) at which colour variation of red kiwifruit occurred and explored the impacts of indirect light effects.

Table 1
Location (region and coordinates) of eight New Zealand red kiwifruit blocks selected for study of flesh colour variation in 2014 and 2015.

Orchard	Region	Coordinates	Altitude (m)
1	Northland, Far North	−35.244, 173.941	120
2	Northland, Far North	−35.237, 173.941	110
3	Bay of Plenty, West	−37.670, 175.962	120
4	Bay of Plenty, East	−38.040, 177.227	90
5	Bay of Plenty, East	−38.000, 177.311	60
6	Gisborne	−38.623, 177.878	20
7	Gisborne	−38.537, 177.873	30
8	Tasman	−41.063, 172.965	40

2. Materials and methods

2.1. Study sites and plant material

For this study, eight blocks of a trial Zespri red-fleshed kiwifruit cultivar, with potential for strong colour expression throughout the fruit flesh (hereafter referred to as 'red'), were selected. The blocks gave a range of geographical locations and climates within New Zealand, with two located in Northland, one in Western Bay of Plenty, two in Eastern Bay of Plenty, two in Gisborne, and one in Tasman; they cover only a narrow range in altitude (Table 1). The same trial cultivar was grafted onto 'Bruno' rootstock in each block, in 2010 (five sites), 2011 (Orchard 5 and Orchard 2) or 2012 (Orchard 1). The vines had been pollinated with combinations of 'M33′, 'M91′, 'CK3′, 'CK2′, and 'Chieftain' male cultivars. Pollination for Orchards 1, 2, 6, 7, and 8 was by bees, with Orchards 1 and 2 using 'M33′ and 'M91′ male cultivars, Orchard 6 using 'M33′, 'M91′, and 'CK2′, and Orchards 7 and 8 using 'M91′ and 'CK2′. Orchards 3, 4, and 5 had no male vines and were spray pollinated with 'Chieftain' pollen. Girdling had been used in Orchard 1, 2, 3, and 7, and a bud-break enhancer, hydrogen cyanamide (Hi-Cane), was used in Orchard 4, 6, and 7.

2.2. Sampling method and in-orchard measurements

Two samples of 90 fruit each were collected from each orchard in the 2013/2014 season. The first harvest (hereafter called '2014 commercial maturity harvest' or 'harvest one') occurred near commercial maturity (12 March for Orchards 6 and 7; 13 March for Orchards 4 and 5; 20 March for Orchard 1; 21 March for Orchard 2; and 1 April for Orchard 8), with the timing determined by the time of commercial maturity in each region. For harvest one, 10 vines distributed across a 0.25 ha block were selected from each orchard and nine fruit were picked from set positions on each vine, resulting in a 90 fruit sample for each orchard. The nine positions refer to the position of the cane along the main leader and of the fruit along the cane, with positions one to three comprising proximal, medial, and distal locations, respectively, along a cane arising on the main leader close to the trunk; positions four to six comprising proximal, medial, and distal locations along a cane in a medial position along the main leader; and positions seven to nine comprising proximal, medial, and distal locations along a cane in a distal position along the main leader. These positions are commonly used when collecting kiwifruit for maturity testing to provide a representative sample of dry matter and SSC. Vine and position were recorded for each fruit and light exposure assessed as no direct light exposure, partial exposure, or mainly exposed, based on the amount of direct light reaching fruit at time of harvest.