



Short communication

Alternatives to phosphonates for fruit colouration



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ABSTRACT

Red colouration is a prime consideration for the consumer when purchasing any fruit, whether tomato, pepper, strawberry or apple. Phosphonates, previously used to enhance fruit colouration e.g. in apple, have now been classified by the EU as plant protection agents, which implies scrutiny of treated fruit to residues.

Therefore, two phosphonate-free, potentially colour enhancing agents, i.e. WuxalP45 and Sunred, were examined on apple cv. 'Braeburn' at Klein-Altendorf as to their effect on fruit quality i.e. colour, starch degradation (ripe-ness), sugar (taste) and firmness (storability) in comparison with reflective mulches, spread in the alleyways 5–6 weeks before harvest. In 2014, a year with good fruit colouration, WuxalP45 ($2 \times 5 \text{ L/ha}$) showed no effect on fruit colouration, firmness or class I or fruit size distribution relative to the control. Sunred, a new bio-stimulant of PAL activity, containing phenyl-alanine, monosaccharides, oxylipins, and methionine as ethylene precursor improved colouration by ca. 2% when expressed as fruit with >50% colouration and 12.2% of fruit >75% red colouration without affecting fruit firmness but accelerated ripening.

Both inorganic mulches increased light reflection to the fruit from 1 to 2 to $45\text{--}55 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$, which improved colouration particularly of the down-facing side of the apple fruit to values of $a = 25.7$ (Daybright) and 34.1 (Extenday) relative to green peel of the control fruit (grass; $a = 1.0$) with a concomitant reduction from hue = 87 (green) in the control to hue = 46 with Daybright and 36 (red) with Extenday. Similarly, both reflective mulches increased the portion of fruit with >50% red colouration by 44% (Daybright) and 55% (Extenday) without affecting fruit quality. The results indicate utilisation of reflective materials as most efficient option in the future, followed by Sunred, when phosphonates are no longer available for fruit colouration; this work appears to be the first work in search for alternatives to phosphonates for fruit colouration in horticulture.

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1. Introduction

Red colouration and size determine the attractiveness of any fruit from tomato to red pepper, strawberry, apricot, nectarine and kaki to apple. This consumer appeal is due to the formation of anthocyanin during maturation (Overbeck et al., 2013; Blanke 2015).

Phosphorus based compounds can promote both fruit quality issues, (i) this red colouration i.e. anthocyanin biosynthesis in fruit by stimulation of the PAL enzyme (Arakawa et al., 1985; Lancaster, 1992) with enhanced expression of MYB 10 gene (Wang Lin et al., 2011) and (ii) fruit firmness for longer storability (Funke and Blanke, 2006; Solomakhin and Blanke, 2007).

In April 2014, the EU divided phosphorus compounds used in agriculture into phosphates or phosphonates (salts of phosphoric acid). The new terminology 'phosphonates' was introduced and they were classified as plant protection agents due to their effective phytosanitary property (EFSA, 2013); this subjected fruit treated with colour-enhancing agents containing phosphonates to the scrutiny of residue (MRLs) legislation (EU, 2014); phosphonate residue levels are temporarily revised (increased) for an interim period until December 2015 to allow these changes in the market to take place (EFSA, 2014). As a result, foliar fertilisers for horticulture based on phosphonates are currently withdrawn from the market and new phosphonate-free foliar fertilisers have emerged.

Hence, we selected a representative of each group, a phosphorus based foliar fertilizer and a phosphorus-free biostimulant. The objective of the present study was to examine the efficacy of two phosphonate-free foliar fertilisers on promoting the red colouration required by the fruit market and consumer in comparison to

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Table 1
Weather conditions at spray application.

| | | | | | | | |
|---------------|---------|---------|----------|--------------|-------------|----------|------------|
| 16 Sept. | 18:00 h | Sunred | 3.5 L/ha | 1000 L/ha | 21 °C | 73% | 1.0 m/s |
| | 18:30 h | WuxalP | 5.0 L/ha | 1000 L/ha | 21 °C | 74% | 1.1 m/s |
| 29 Sept. | 17:00 h | Sunred | 3.5 L/ha | 1000 L/ha | 20.0 °C | 72% | 1.5 m/s |
| | 17:30 h | WuxalP | 5.0 L/ha | 1000 L/ha | 19.4 °C | 72% | 1.4 m/s |
| Calendar date | Time | Produce | Dose | Spray volume | Temperature | Humidity | Wind speed |

white, interwoven plastic mulches, which induce colouration by reflecting incident light.

2. Materials and methods

2.1. Trees and treatments

Three rows of four-year-old cv. 'Braeburn' trees on M9 rootstock at a spacing of 1.0 m × 3.5 m were planted in N–S orientation at Campus Klein-Altendorf of the University of Bonn, Germany. These relatively young apple trees were trained to slender spindles (Supplement 1) with an open crown and hence good light penetration into the canopy. The location with a latitude of 50.5°N and 9.8 °C annual average temperature has a fertile loess-based luvisol soil.

2.2. Biostimulants and colour enhancing foliar fertilisers

The phosphonate-free foliar fertilisers WuxalP45 (5L/ha, Aglukon, Düsseldorf, Germany) and the 'bio-stimulant' 'Sunred' (3.5 L/ha; Biolchem, Bologna, Italy) were applied under optimum conditions (high humidity, no wind, low radiation, warm temperature) on 16 September and on 27 September 2014 (Table 1), i.e. 4 and 2 weeks before anticipated harvest, following the manufacturer's recommendation.

2.3. Biostimulant—mode of action

Sunred™ is a new generation of products, designated as a 'bio-stimulant', with four alleged modes of action, (i) stimulation of the PAL enzyme activity and anthocyanin pathway by containing the substrate phenylalanine, (ii) monosaccharides, (iii) oxylipins for pigment enhancement and (iv) methionine as precursor of the ethylene pathway to promote red colouration but without premature softening (Biolchem. Co. Italy).

2.4. Reflective mulches

For a comparison with phosphonate-free foliar fertilisers, the two reflective mulches were spread on 1 September 2014 i.e. ca. six weeks before anticipated harvest in mid October (Table 2). Three m wide strips of Extenday or Daybright, which had both been used several times before (e.g. Meinhold et al., 2011; Solomakhin and Blanke 2007), were spread on both sides of the tree rows on the grass alleys leaving a ca. 1 m wide uncovered tree strip with open soil underneath the apple trees. Fruit samples were analysed from

the centre row of the three with reflective mulches either side; adjacent untreated apple trees of the same row served as control (Colour Supplement).

2.5. Light reflection

Light reflection was measured with a portable EGM-4 (PPSystems, Amesbury, MA, USA) and attached HTR sensor as previously described (Solomakhin and Blanke, 2007). Light measurements were taken in 1 m height perpendicular to and in the centre of the reflective cloth or grass strips (as control) with the light sensor facing upward for the incoming and facing downward for the reflected light (Funke and Blanke, 2005; Blanke, 2015).

2.6. Individual fruit colouration measurement

To study the efficacy of the mulches on light reflection, the down-facing side was marked with a circle by pen on attached fruit in the lower half of the canopy. Per treatment, 15 apple fruits were individually harvested on 10 October 2014, colouration of 45 fruits was measured inside the marked circle using Lab values in the CIE colour scale with a X22 (Xrite, Leverkusen, Germany) spectrometer and Lab values converted into hue values (McGuire, 1992) as described previously in detail (Funke and Blanke, 2006).

2.7. Fruit quality assessment

Starch degradation was measured by iodine staining of fruit halves and firmness by a Bareiss penetrometer incorporated into an ART System (UProducts, FRG).

2.8. Statistics and guard trees

Blocks with reflective mulches were adjacent to each other and blocks with chemical colour-enhancing chemicals were next to each other to avoid interactions with the spatial effects of the reflective mulches. Two guard trees were employed between the treatments to avoid any interactions between treatments and excluded from sampling for analysis.

Each of the five treatments consisted of 12 trees viz replicates resulting in 11 degrees of freedom. Fifteen replicates viz fruit from the lower Northwestern side of the tree were employed for the individual colour measurements resulting in 14 degrees of freedom. Data were processed for statistical means and SDs expressed at the $p=0.05$ (5%) level.

3. Results and discussion

3.1. Autumnal weather pattern

Previous work had shown that anthocyanin formation commences ca 4–6 weeks prior to anticipated harvest (Arakawa et al., 1985; Lancaster, 1992). The expression of MYB 1, the gene responsible for temperature-stimulation of the anthocyanin biosynthesis in apple, is stimulated by cold nights and large temperature differentials (Wang Lin et al., 2011). In September 2014, the coldest nights were early with a minimum of 5.4 °C on 4–5 September and 3.7 °C on 22–23 September 2014 with maxima of 15–25 °C daytime

Table 2
Experimental design and timetable at Campus Klein-Altendorf.

| | |
|---|-------------------|
| Spreading reflective mulches | 1 September 2014 |
| Light reflection | 30 September 2014 |
| First spray | 16 September 2014 |
| Second spray of WuxalP/Sunred | 27 September 2014 |
| Marking down-side of apple fruit on the trees | 7 October 2014 |
| Fruit harvest | 10 October 2014 |
| Individual colour measurement | 10 October 2014 |
| ART fruit quality assessment | 14 October 2014 |
| Mulch removal | 15 October 2014 |
| Grass mulching in alleyways | 28 August 2014 |
| Treatment | Date |

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