Canopeo, a new mobile device application with potential to measure seed colour change in canola

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Abstract
Seed colour change (SCC) is commonly used to determine windrow timing in canola, it is however often regarded as being subjective and time consuming. The aim of this research was to compare SCC measurements using a standardised visual assessment procedure with Canopeo, a new mobile device application commonly used to measure fractional green canopy cover. Windrow timing experiments were conducted at Tamworth in northern NSW, in 2017 and 2018, with SCC measurements undertaken at 2-3 day intervals from the commencement of SCC on the primary stem up until 100% SCC on branches. Pictures of seed at each windrow timing were analysed by Canopeo and then compared to a standardised visual assessment measurement. There was a linear relationship (R^2 = 0.93 to 0.95) between SCC measured using the mobile device application Canopeo and the detailed visual assessment method. Canopeo was also between 10 and 20 times faster than the visual assessment procedure. These results indicate that Canopeo can be used to take pictures to measure SCC and may be a viable alternative for determining SCC and hence windrow timing in canola crops. Importantly, Canopeo offers the potential for a rapid standardised objective measurement for determining SCC, thereby alleviating issues around subjectivity commonly associated with visual assessment methods and as such, is considered a useful new technique for the determination of SCC in canola.

Key Words
Canola, Canopeo, seed colour change, windrow timing

Introduction
In Australia, windrow timing of canola (*Brassica napus* L.) is commonly determined by the percentage of seed colour change (SCC) within a crop. SCC reflects metabolic activity within the seed, and is an indicator of late stages of physiological development involving oil synthesis and dry matter accumulation, and is strongly correlated with seed moisture content (Hertel, 2012, 2013). Elias and Copeland (2001) observed that SCC was a reliable indicator of physiological maturity (PM) of seed in canola. The current recommendation for windrowing, is when 40-60% of seeds collected from pods (siliques) on both the main stem (primary racemes) and branches (secondary racemes) have changed colour from green to red, brown or black (Anon, 2017). The problem with determining SCC is that it has traditionally been a subjective measurement based on visual assessments. The introduction of clearly defined definitions and pictorial references for determining SCC has helped to reduce subjectivity but, depending on the methodology adopted, this can be time consuming and is open to interpretation.

In the last decade, there have been rapid improvements in the quality of images produced by affordable mobile devices, and the associated development of software packages to analyse digital images of plants, for a variety of applications. The development of mobile apps capable of differentiating green and non-green images for example, may provide a more rapid, robust quantitative measurement for determining SCC in canola.

Canopeo is an automatic colour threshold (ACT) image analysis tool developed in the Matlab programming language (Mathworks, Inc., Natick MA) that analyses pixels based on the red to green (R/G) and blue to green (B/G) colour ratios and an excess green index (Shepherd et al. 2018). Developed as a high-speed tool for measuring fractional green canopy cover (FGCC) by the Oklahoma State University App Center, Canopeo is a free app for iOS and Android devices (Patrignani and Ochsner, 2015). Importantly, Canopeo's pixel categorisation technique using red to green and blue to green colour value ratios has been shown to be effective in separating green vegetation from non-green backgrounds (Wang and Naber, 2018). The resultant output is an image where colour pixels are converted into black and white, with the white pixels corresponding to the percentage green area and the black pixels equating to the non-green.

The objective of this research was to compare SCC measurements, using a standardised visual assessment procedure with the Canopeo app, from canola windrow timing experiments conducted in 2017 and 2018 at Tamworth in northern NSW. The relationship between thousand seed weight TSW and windrow timing (WT) was also discussed.

Methods

Experiments were conducted at Tamworth Agricultural Institute in 2017 and 2018. The experiments were designed as replicated split plot design, with windrow timing as the main plot and variety randomised within windrow timings. Individual experiments were analysed spatially using Genstat 18th edition (VSN International, 2015). Experiments were sown on the 5th May in 2017 and 8th May in 2018, and were managed using best management practices to limit biotic stresses and nutritional constraints. The hybrid canola variety Pioneer® 44Y90 (CL) and the open pollinated triazine tolerant (TT) ATR Bonito, with similar flowering times but different maturity ratings were sown in both experiments.

Windrow timings were conducted at 2-3 day intervals (i.e. Monday, Wednesday and Friday) from the commencement of SCC on the primary stem up until 100% SCC on branches. For consistency across experiments, SCC was defined as when 'a minimum of two-thirds of the surface area of an individual seed changed colour from green to brown, red or black'. Actual SCC was determined using a representative 200 seed sub-sample, taken from 30 pods from the middle third of the primary stem and randomly from across the branches of individual plants.

The mobile device application Canopeo (Oklahoma State University, Stillwater, OK) was used to determine the percentage green area and non-green area (i.e. SCC) by difference. Pictures were taken using the Canopeo app on an iPad (Apple). Three pictures per windrow timing sample, used to visually assess SCC, were taken with an iPad held ~20-25 mm above a 45 mm diameter circular container that was placed on a green background, with seed obtained from the 30 pod sample (main stem or branches) covering the entire surface area of the container (Figure 1).

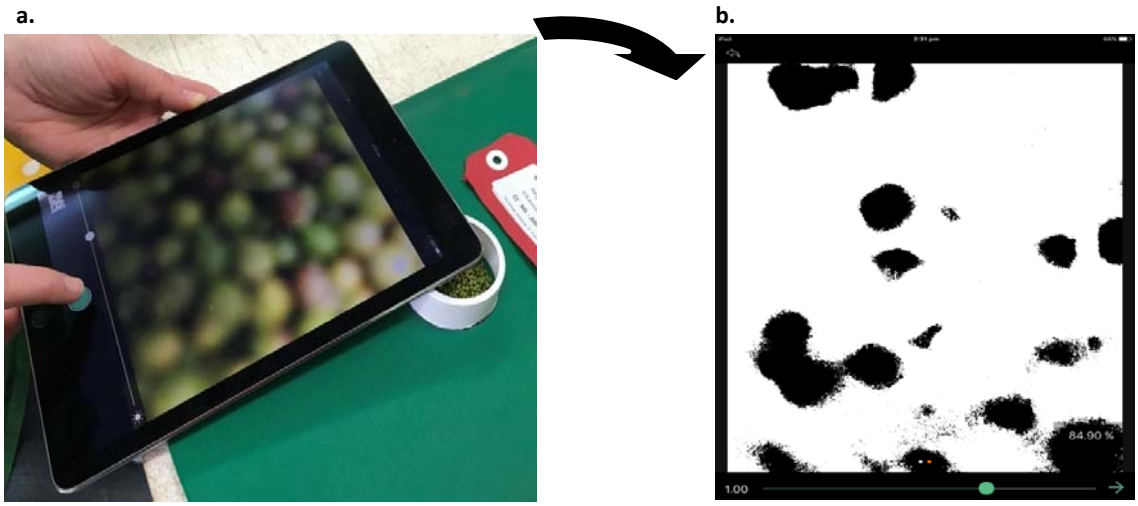

Figure 1. (a) Photos of canola seeds being processed using the Canopeo app on an iPad at each windrow timing. The resultant output is an image (b) where colour pixels are converted into black and white, the white pixels corresponding to the percentage green seed.

Six plants sampled from the middle rows, three from each end of a plot, were threshed to determine the proportion of yield from branch and stems, with seed moisture percentage and thousand seed weight (TSW) at actual SCC and SCC for yield components (stem vs. branches) recorded.

Results and discussion

Despite SCC being the most common method for determining windrow timing in Australia, the issue confronting producers, is how to determine SCC in the field, quickly and accurately. Preliminary research conducted by Hertel (2013) noted that there was considerable misunderstanding within the Australian canola industry as to what constituted optimum SCC as it related to windrow timing. The accurate determination of SCC is important, when you consider the potential for substantial yield and quality penalties associated with

incorrect windrow timings. Although clearly defined definitions around what constitutes SCC and new industry guidelines have been adopted (Anon, 2017), the current methods of visual assessment are time consuming and open to interpretation.

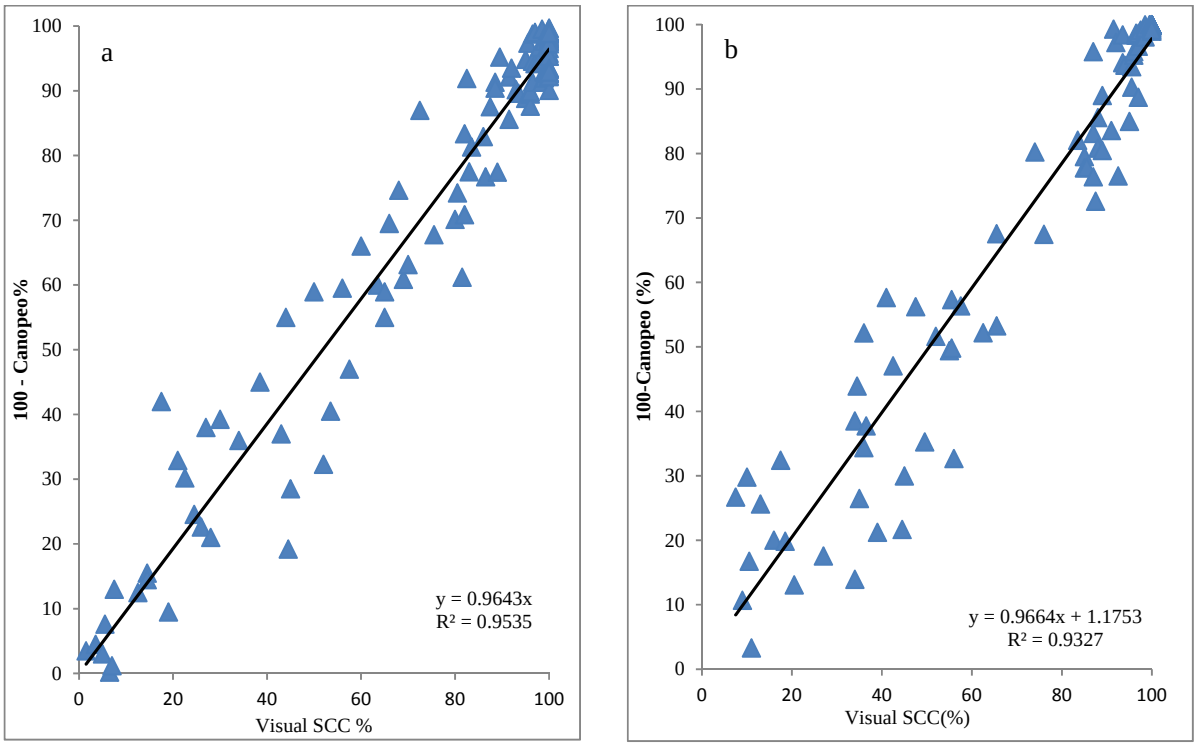

Figure 2. Relationships between percentage SCC based on visual assessment and percentage SCC, based on pictures in Canopeo at Tamworth in 2017 (a) and 2018 (b).

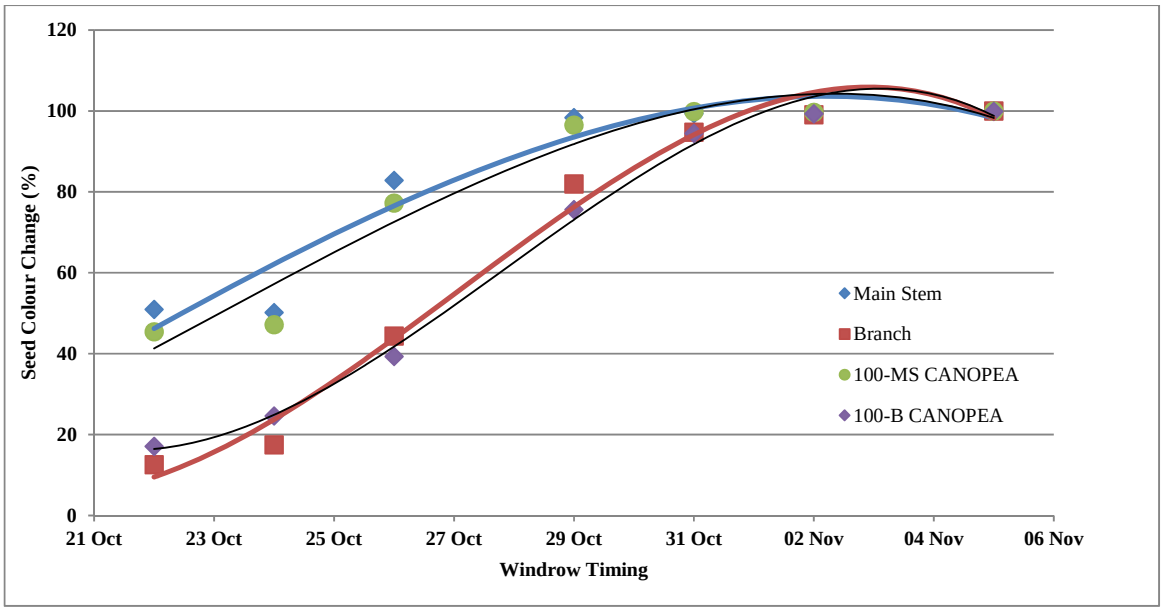

Figure 3. Predicted visual and Canopeo seed colour change (%) on the primary stem vs. branches over time, as related to windrow timing, Tamworth 2018.

Results for percentage SCC based on pictures analysed with Canopeo were found to be linearly related to percentage SCC determined by visual assessment in both 2017 (R^2 = 0.95) and 2018 (R^2 = 0.93) (Figures 2). Canopeo was between 10 and 20 times faster than the visual assessment procedure, taking less than 1 minute to take 3 photos per sample compared to up 10 – 20 minutes for a detailed visual assessment. These results

suggest that Canopeo may be used to take pictures of seed samples to determine SCC and hence WT, as a more objective and faster method of measuring SCC, as opposed to the commonly used subjective visual assessment process.

Importantly, Canopeo offers the potential for developing a robust and rapid standardised objective measurement for determining SCC, helping to alleviate subjectivity and hence human error around existing visual assessment procedures. Furthermore, because of its speed and repeatability Canopeo enables producers and contractors the flexibility to monitor SCC over time and/or from multiple sites within a crop; this flexibility is important, when you consider the rate at which SCC can occur. Results for 2018 illustrate how rapidly SCC change can occur within a canola crop (Figure 3), with previous research showing the potential for yield losses of >50% and declines in oil concentration of up 6.5% points as a result of incorrect windrow timing due to suboptimal SCC within the crop (Graham et al. 2017).

Conclusion

There was a strong linear relationship (R^2 = 0.93 to 0.95) between SCC measured using the mobile device application Canopeo and a detailed visual assessment method. The results from this study indicate that Canopeo may be a viable alternative for measuring SCC in canola crops. Moreover, because of its speed and repeatability, it enables producers the ability to determine SCC accurately over time. Canopeo offers the potential to develop a new standardised objective measurement technique for assessing SCC in canola crops, alleviating concerns around subjectivity and human error, commonly associated with current visual assessment methods.

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