

## Assessment of 'hen and chicken' disorder for marketable yield estimates of table grape using the 'Berry Analysis Tool'

K. C. DAHAL<sup>1</sup>, S. P. BHATTARAI<sup>1</sup>, A. KICHERER<sup>2</sup>, D. R. OAG<sup>3</sup> and K. B. WALSH<sup>1</sup>

<sup>1</sup>Institute for Future Farming Systems, Central Queensland University, Rockhampton, Australia

<sup>2</sup>Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

<sup>3</sup>Department of Agriculture and Fisheries, QLD Government, Australia

### Summary

**Bunch and berry size are important quality criteria for table grapes, and bunch weight is used in estimation of vine yield. The Berry Analysis Tool (BAT), a machine vision technique, was trialled for use in estimation of berry count, diameter and weight, to support estimates of 'hen and chicken' disorder and vineyard yield. Berries were spread on a plate for imaging. BAT estimates achieved a  $r^2 = 0.99$  on berry number per bunch,  $r^2 = 0.98$  on average berry minor axis length and  $r^2 = 0.99$  on bunch weight. Based on an allometric relationship between linear dimensions and weight, these attributes were used to estimate the proportion of under-size ('chicken') berries per bunch and bunch weight. The estimated bunch weight multiplied by a number of bunches per vine provides an estimation of vine yield. Use of the BAT as a decision support tool in table grape farm management is described.**

**Key words:** berry diameter; vine; image analysis; machine vision; "Millerandage".

### Introduction

Estimation of crop quality and yield prior to harvest is instrumental for optimization of vineyard management and marketing. Sub-tropical table grape production, in particular, is subject to large yield variations between seasons (DAHAL *et al.* 2014). Vineyard yield is affected by a number of current season factors, including temperature (KLEWER 1977), crop nutrition (WILLIAMS *et al.* 2007b), dose and timing of gibberellic acid application (ABU-ZAHRA and SALAMEH, 2012) and moisture availability (OJEDA *et al.* 2015), through impact on flower and berry set and growth (*i.e.* berry size and number per bunch).

CLINGELEFFER *et al.* (2001) reported bunch number/vine (assessed any time after berry set), the number of berries/bunch and berry size to explain the variation in vine yield by ~ 60, 30 and 10 %, respectively, for major wine grapes varieties. A basic vineyard yield estimation method involves count of bunch number per vine and estimation of mean bunch

weight near veraison (WOLPERT and VILAS 1992). MARTIN *et al.* (2003) developed a grape yield forecaster intended for use by Australian wine grape growers that involved assessment of "patches" (a length of vine row representative of block, crop geometry and structure) between bud burst and harvest for: (i) bunch count per vine, (ii) berry count per bunch, (iii) bunch weight at veraison, and (iv) a yield component ('bunch gain' factor, a measure of harvest efficiency) assessed at harvest. A key requirement of these methods is the assessment of a statistically adequate number of representative samples at each stage – a limiting factor given the labour requirement of manual assessment methods.

Machine vision image analysis offers the potential for rapid assessment, allowing larger sample sizes. Bunch number per unit length of row has been estimated using in field machine vision by a number of researchers. For example, LIU and WHITTY (2015) reported an image processing algorithm using color (purple berries) and texture information for segmentation and counting of bunches in canopy images, with a recall of 91.6 % (percentage of grape bunches classified correctly). Bunch overlap caused an underestimate of bunch number. Various image processing techniques have been employed for estimation of bunch size (area) or berry number per bunch or berry size using machine vision. DUNN and MARTIN (2004) used manually set RGB threshold values in counting berry pixels within vine canopy images. DIAGO *et al.* (2015) also utilized RGB in segmentation of vine canopy images to estimate leaf area and bunch size. Bunch overlap and berries of similar color to leaves limited accuracy of the estimation of bunch size. NUSKE *et al.* (2014) used both shape and visual texture to distinguish green berries from a green leaf background, achieving prediction ( $r^2 = 0.60-0.73$ ) of vine yield based on estimate of berry count per vine. Post imaging processing was required, given the need for significant time for image processing. ROSCHER *et al.* (2014) estimated mean berry diameter in field acquired images of grape bunches, with  $r^2 = 0.88$  on manual estimates.

Bunch attributes other than berry number can also be assessed using machine vision. For example, bunch compactness was assessed from indices derived from a 3D image of a bunch, with 85.3 % of test images assigned the correct rating of bunch compactness (CUBERO *et al.* 2015).

Correspondence to: K. B. WALSH, Institute for Future Farming Systems, Central Queensland University, Rockhampton, QLD 4702, Australia. E-mail: [k.walsh@cqu.edu.au](mailto:k.walsh@cqu.edu.au)

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Similarly, IVORRA *et al.* (2015) reported estimation of cluster compactness and berry size with  $r^2 > 0.80$  based on 3D descriptors obtained using stereo vision. HILL *et al.* (2014) developed image analysis software "RotBot" to assess the severity of Botrytis bunch rot in a bunch.

Another attribute of market interest is berry size uniformity. A bunch disorder known variously as 'berry asynchrony', 'hen and chicken' or 'Millerandage' is used to describe bunches with berries that have a great range of size and maturity at harvest. COLLINS and DRY (2009) defined the term 'Millerandage' as the condition when a bunch is comprised of a high proportion of seedless or "shot" berries in seeded varieties, while in seedless table grape varieties the term 'hen and chicken' is used, with 'chicken' berries defined by size (at a value determined by variety and market).

'Menindee Seedless' (syn. 'Sugraone'), is a partial seedless table grape cultivar, typically containing 1-5 seed traces per berry. Berries  $< 15$  mm diameter are commercially undesirable (e.g. the specification of an Australian retailer allows no more than 10 % of berries in a bunch to be 'chicken' sized). Berries  $< 6$  mm diameter stay green and hard after harvest. These berries are referred to as "shot" berries or Live Green Ovaries (LGOs) (COLLINS and DRY 2009). In practice, growers trim small berries ( $< 15$  mm diameter) from bunches during harvest in an attempt to meet retailer specification. These activities increase the harvest cost. A high proportion of 'chicken' berries was largely responsible for a reduction of the Australian national table grape crop by up to 30 % in 2001 - 2002 (ANONYMOUS 2002, cited in WILLIAMS 2007a). Estimation of the extent of the disorder in a crop is important as it allows grape growers to more confidently negotiate picking price, schedule labour and estimate yield. The current method of estimation using manual procedures (e.g. see <http://yvonnelorkin.com/2012/02/hens-chickens-and-frost/>) is time consuming and costly.

The Berry Analysis Tool (BAT) (KICHERER *et al.* 2013) was developed for assessment of berry morphological traits of wine grapes varieties 'Riesling' and 'Müller Thurgau', before veraison stage ("BBCH 79" growth stage, when berries have their typical shape, and are of uniform greenness). The tool was developed in context of rapid phenotyping traits measurement in grapevine breeding. Berries from a bunch are distributed over an imaging table and a machine vision system based on active learning used to assess berry number and diameter, with estimated volume. Minimum berry

diameter was defined as the minor axis of an ellipse fitted through edge pixels associated to a berry.

In the current study we assessed the BAT for estimation of table grape berry number and berry minor axis diameter, for quality assessment and yield prediction. The practical aim is a technique for use on orchards, incorporated in existing quality control practice.

## Material and Methods

**Plant material:** Bunches were collected from a table grape ('Menindee Seedless' grafted on 'Kober 5BB') vineyard in Emerald, central Queensland, Australia (23°35' S and 148°12' E) from a trial established to evaluate the effect of flowering (bloom) time gibberellic acid application on return fruitfulness. Vines were cane pruned, trained to a 1.8 m wide sloping T trellis system and spaced at 2.4 m in row and 3.4 m between rows. Inflorescences in experimental vines were marked at modified Eichhorn Lorenz stages (COOMBE 1995) before flower opening began. The number of inflorescence per vine ranged from 1 to 18. In the season 1, the age of 100 inflorescences was recorded on the day of gibberellic acid application. Age was defined in terms of the percentage of caps (calyptra) off ( $< 1$  %; 1-20 %; 21-40 %; 41-60 %, 61-80 % and  $> 80$  %). At harvest (November 14, 2015) bunches were packed separately in individual styrofoam boxes and stored at 4 °C until measurement and image capture. Bunches were also harvested in a second season (November 13, 2017) for validation of the BAT method.

**Manual measurement:** Berries were detached from the rachis of each bunch, manually counted and the minimum diameter of each berry was measured using a circle (1-37 mm diameter, 1 mm steps) template (Celco®, [www.officeworks.com.au](http://www.officeworks.com.au)), with comparison made to measurements made using a Mitutoyo digital caliper. For all bunches, berries were counted separately in each diameter class, and berries  $< 15$  mm diameter as 'chicken' and  $\geq 15$  mm diameter as "hen" were weighed separately. Berry weights were summed to estimate bunch weight without rachis.

**Image acquisition and analysis:** The method of KICHERER *et al.* (2013) was followed for image acquisition, with minor modifications (Fig. 1) A single-lens (Tamron 18-270 mm) reflex digital camera (Nikon® DSLR D7100; 24.1 MP, operated in automatic mode with flash

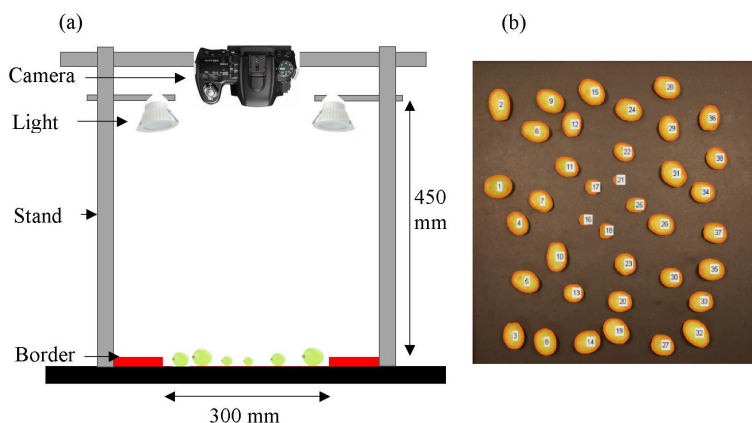


Fig. 1: Image acquisition set up (a) and BAT analysed image (b).

off) was fixed to a horizontal bar 50 cm above the image plane, giving an image resolution of 163  $\mu\text{m}/\text{pixel}$ . Two dimmable 9 Watt light-emitting diode (LED) warm white lights (Robus® RC9WDLWW) were mounted to the sides of the construction. Detached berries of each bunch were spread over a piece of black cardboard (300 x 300 x 5 mm) bounded by a red frame. Up to 90 berries could be accommodated within the frame, necessitating use of several frame images for imaging all berries from large bunches. Image(s) associated with each bunch were saved in Joint Photographic Experts Group (JPEG) format. A total of 216 images associated with 100 grape bunches were acquired (28, 38, 25, 8 and 1 bunches having 1, 2, 3, 4 and 5 images/bunch, respectively).

The BAT is a Matlab® 7.5 (MathWorks, Ismaning, Germany) based system that involves six steps: (i) detection of the construction boundary and the elimination of the red background, (ii) classification of whole image into berry and background by applying active learning (SETTLES 2010), (iii) a morphological operator (HARALICK *et al.* 1987) to remove the noise of detected objects with a radius less than 3 mm, (iv) counting of berries (for berries in contact with another berry, the detected object was eroded step-by-step with a disk-shaped structuring element of increasing size in order to separate connected berries, for use in berry count but not berry diameter), (v) estimation of single berry diameter and (vi) calculation of single berry parameters including minor and major axis length, and berry volume (KICHERER *et al.* 2013). For the current work, labelling of berries in each image was added as an additional feature in the software.

For estimation of minimum berry diameter, berries were assumed to be elliptical (with two equal minor axes,  $a_2 = a_3$ ) or round (three equal axes,  $a_1 = a_2 = a_3$ ).

Images of 28 bunches (1795 berries) from season 1 were used in comparison of BAT measured individual berry minor axis and circle template measured berry diameter. The number of berries of each diameter class (1 mm steps) measured by circle template were weighed separately to

establish the relationship between berry diameter and mean berry weight (Fig. 4). For the second season validation, images of two bunches were used in comparison of BAT and digital caliper measured berry minor axis. The manual recording of one bunch (of 140 berries, including counting of berries, measuring of minor axis per berry and weight of 'hen and chicken' berries) took about 20 min, while the automatic BAT process took about one minute per image using common PC hardware (i5, 64 bit, 32 GB RAM, 3.2 GHz).

**Bunch number, vine yield and 'hen and chicken' score:** Vine yield was calculated as a multiple of number of bunches per vine and average bunch weight of a vine. The number of total bunches per vine for 46 vines were counted four weeks after budburst and then confirmed after flowering (~ 7 weeks after budburst). Up to five bunches were harvested and weighed per vine. Each marked bunch was also visually scored (1-6 scale) one day prior to harvest for the extent of 'hen and chicken' disorder in the bunch. The visual score involved an estimation of the % of 'chicken' berries present in the bunch (score 1-6; 0-< 1 %, 1-10 %, 11-20 %, 21-30 %, 31-40 % and > 40 %, respectively).

**Data analysis:** Pearson linear correlation and linear model stepwise regression were undertaken using Unscrambler®X 10.3 (Camo, Norway) and GenStat 16<sup>th</sup> edition (Lawes Agriculture Trust 2015, Rothamsted, England), respectively. Graphical presentations were made using the ggplot2 package of the R project for statistical computing (WICKHAM, 2017).

## Results

**Berry count:** The BAT method was accurate ( $r^2 = 0.99$ ) in estimation of berry number per bunch (season 1 data; Fig. 2). Other performance metrics were assessed following NUSKE *et al.* (2014): a) True Positive Number (TPN): Berry count by the BAT underestimated actual count

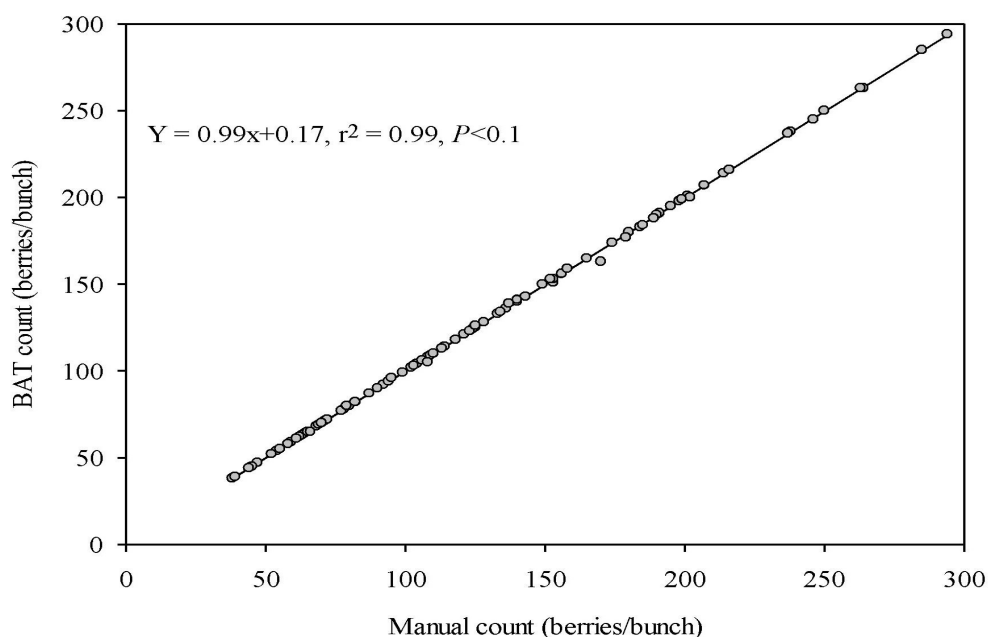


Fig. 2: BAT and manual counts berries per bunch for 100 bunches.

by 0.2 % (across 100 bunches (216 images), BAT count was 12773 true berries, manual count 12795 berries). True Positive Rate (TPR = TPN/(TPN+FNN), where FNN is false negative number) bunch ranged from 96 % to 100 %, with an average of  $99.8 \pm 0.6$  % (SD) for 100 bunches; b) False Positive Number (FPN): 9 false positives were recorded (in 8 of 216 images); c) False Negative Number (FNN): 22 berries were missed (< 0.2 %).

For the first season images, there were 10 instances where berries touched, and were thus included in berry count but not diameter assessment. In the second season images, all berries were assessed.

The BAT method employed a black background, with good results obtained for the greenish - white 'Menindee Seedless' berries. In a preliminary trial with 'Flame Seedless' (red - black) berries, TPR was 95 %, FPN was 7 %.

**Berry diameter:** Berry diameter measured using the circle template had a bias of 0.56 mm and Root Mean Square Error (RMSE) of 0.63 mm compared with the digital caliper measurements, as expected due to the 1 mm steps in circle templates, and the level of care required with use of the caliper to ensure pressure was not exerted on the berry. Due to large number of berries to be assessed, the circle template measurement was chosen as the manual reference method. To estimate method error for manual measurements, 200 berries were re-measured using the circle template within the same day, with a RMSE of 0.04 and bias of 0.02 mm noted.

BAT recorded minor and major axis lengths of objects identified as berries. BAT estimated minor axis demonstrated a coefficient of determination ( $r^2$ ) = 0.99, RMSE = 1.47 mm, bias = 1.38 mm, intercept = 0.2566 and a slope = 1.0669 for a linear regression on circle template measurement. BAT overestimated berry minor axis irrespective of berry diameter measured from circle template ranged from 6-24 mm. Hence, the BAT estimated berry minor axis

$$\left( \hat{y} = \frac{x - 0.2566}{1.0669} \right)$$

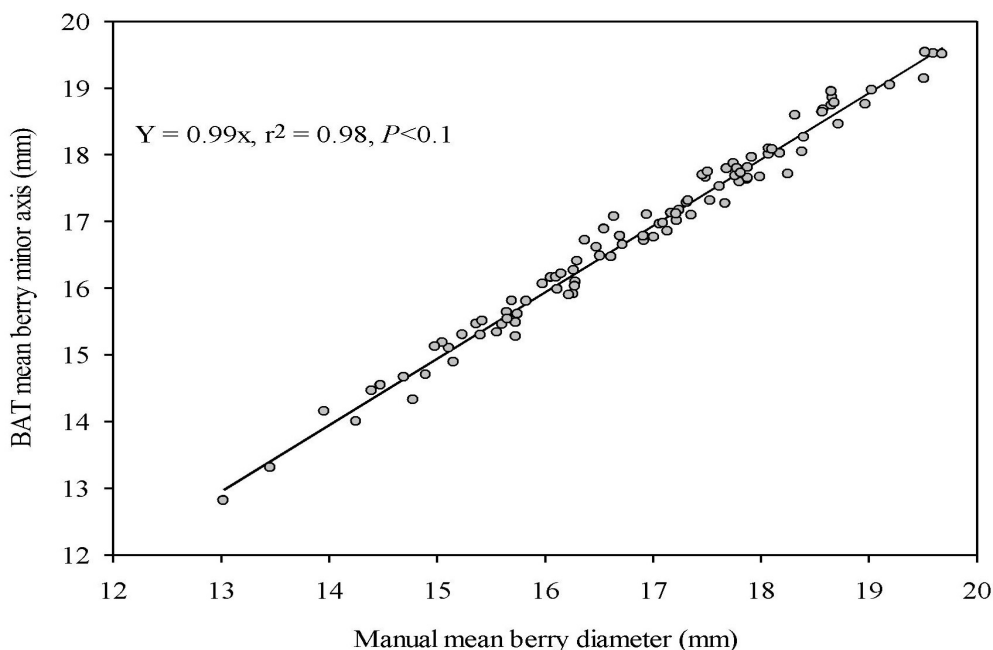


Fig. 3: BAT estimated and manual (circle template) measured berry minor axis/diameter (mm) for 100 bunches.

was adjusted for estimation of bunch weight. For berries of 100 bunches, adjusted BAT estimated minor axis demonstrated a coefficient of determination ( $r^2$ ) = 0.98, RMSE = 0.19 mm, bias = -0.06 mm and a slope = 0.99 for a linear regression on manual estimates (Fig. 3).

For the season 2 validation set, BAT measured berry diameter had a  $r^2$  = 0.99, RMSE = 0.92 mm, bias = 0.77 mm and a slope = 1.07 against digital caliper measured berry diameter (data not shown).

**Berry and bunch weight:** Berry number per bunch alone explained 78.9 and 77.7 % of total variation in bunch weight for manual and BAT estimated berry number, respectively. The remaining variation is attributable to variation in berry weight. Berry weight was positively correlated with circle template berry diameter (Fig. 4;  $r^2$  = 0.99). Berry weight was described by a power relationship ( $x^{3.1043}$ ) on berry diameter as berries were near spherical (slightly ellipsoid) in shape. The measured mean bunch weight (weight of total berries without rachis) and coefficient of variation (CV) of 100 bunches (12795 berries) was 542 g and 45 %.

Individual berry weights estimated from adjusted BAT minor axis were summed to give calculated bunch weight.

$$Y_{\text{bunch}} (\text{g}) = \left[ \sum_{i=1}^n y_{\text{berry}^*i} \right] \quad (\text{equation 1})$$

where  $n$  is the number of berries in a bunch. The calculated bunch weight demonstrated a coefficient of determination ( $r^2$ ) = 0.99, bias = 10 g, RMSE = 27 g per bunch (Fig. 5) against the gravimetric measurement of bunch weight (without rachis). For the season 2 validation set, use of the relationship, described in Fig. 3 yielded an accurate estimate of individual berry weight ( $r^2$  = 0.98, bias = 0.16 g, RMSE = 0.37 g, slope = 1).

Measured bunch weight varied from 123 to 1176 g, reflecting variation in number of berries per bunch (38 to 294 per bunch) and the average berry weight (2.34 to 6.69 g). Berry number per bunch explained 78 % of total variation

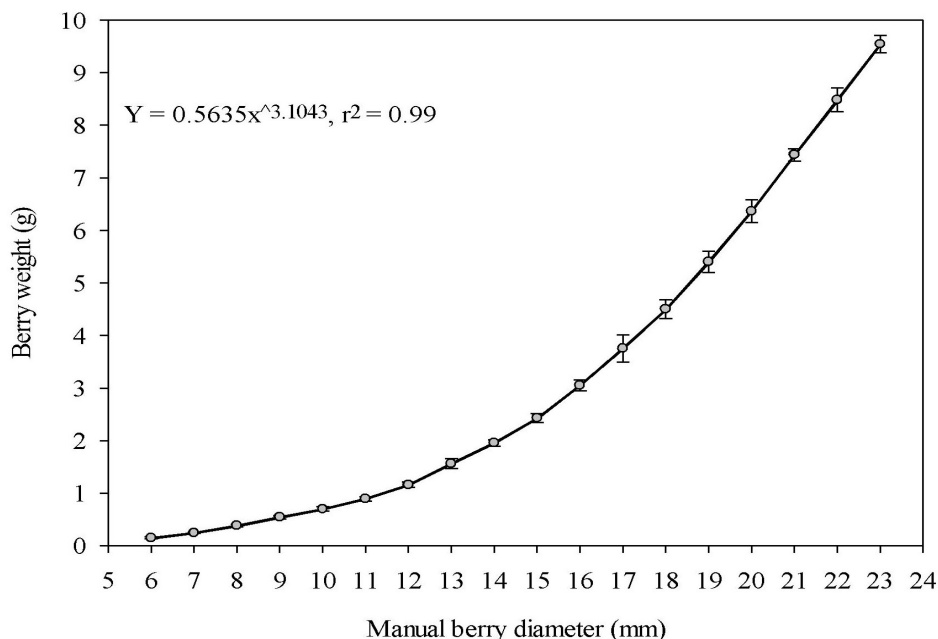


Fig. 4: Relationship between mean berry weight (g) and circle template measured berry diameter (mm).

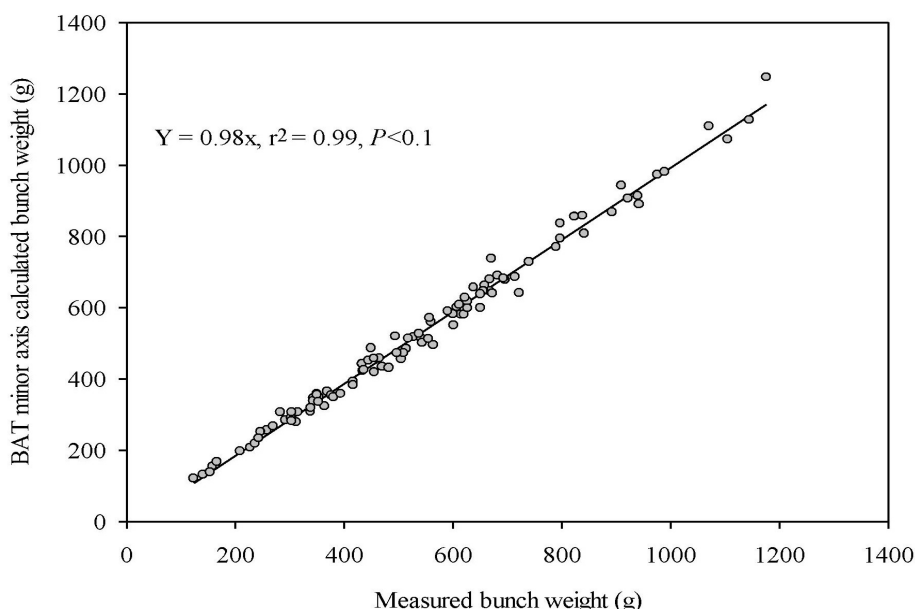


Fig. 5: Calculated bunch weights based on adjusted BAT berry minor axis plotted against gravimetric measured bunch weight (g) for 100 bunches.

in bunch weight and 17 % was explained by average berry weight estimated using the adjusted BAT minor axis (Tab. 1).

**Quantification of 'hen and chicken' disorder:** The linear correlation between visual score value for 'chicken' berry frequency and the assessment of percent of 'chicken' berries (diameter < 15 mm) based on diameter measurements by circle template was described by  $r^2 = 0.73$ , RMSE = 26.3 % (data not shown). This level of error invalidates the use of visual score for the quantification of 'hen and chicken' disorder. Of all berries from 100 bunches, 27 % were 'chicken' berries, accounting for 7 % of total weight (Tab. 2). The estimate of the percentage weight of each bunch in 'chicken' berries from BAT supported a linear regression with  $r^2 = 0.95$ , RMSE = 1.33 % and bias = 0.74 % against gravimetric weight measurement.

For a case study in use of the BAT tool in assessment of 'chicken' berry frequency, BAT assessment was made of bunches produced from an experiment in which the time of gibberellic acid application was varied. Increase in inflorescence age on the day of gibberellic acid application was associated with decreased extent of 'hen and chicken' disorder (Fig. 6), while mean berry diameter of a bunch was increased.

## Discussion

**Berry count:** KICHERER *et al.* (2013) recorded 100 % accuracy on BAT berry count for comparatively smaller and uniform coloured green immature berries. The active learning procedure applied in BAT should ac-

Table 1

Explained variation in estimation of 100 bunch weight from manual or BAT estimated berry number and weight

Bunch attributes	Variation explained (%) in bunch weight	
	Manual	BAT
Berry number per bunch	78.9**	77.7**
Mean berry weight/bunch	15.5**	17.1**

\*\* denotes a significance contribution ( $p < 0.01$ ) of the predictor to the variation in bunch weight.

commodate change in image conditions, and in berry size, position and colour, and the morphological operator should distinguish connected berries. Thus, in the current study with mature 'Menindee Seedless' bunches, BAT count was accurate (99.8 %) despite presence of widely varied berry sizes (minor axis diameter of 6-28 mm), and despite berries sometimes touching on the display plate. BAT failed to count only < 0.2 % berries and only < 0.1 % of the berry count was due to foreign objects. In summary, the technique is sufficiently robust to allow use within an on-farm yield assessment program, coupled to estimates of bunch number per vine or length of row.

However, less accurate results were obtained with 'Flame Seedless' (red-black) berries, further work is required to optimize the method for use with colored berries.

**Berry diameter and weight:** Berries sometimes failed to lie with the major axis horizontal, resulting in a BAT underestimate of the major axis length (data not shown). This issue was readily dealt with by shaking the display plate.

Connected berries distort the BAT berry minor axis measurement. Images of connected berries were eroded step-by-step to allow segmentation of the berry, invalidating axis diameter measurement. To avoid berry contact, greater spacing could be used on the display plate.

Table 2

Percent (number and weight) of 'chicken' berries in 100 bunches measured manually and estimated using adjusted BAT berry minor axis

'Chicken' berries	Percent (mean $\pm$ SD)	
	Manual	BAT
Number	26.5 $\pm$ 12.7	26.9 $\pm$ 12.8
Weight	7.0 $\pm$ 4.5	7.8 $\pm$ 5.0

The accuracy of the BAT measured berry minor axis was a function of effective image pixel dimension (163  $\mu\text{m}$ /pixel) and accuracy of the fit of an ellipsoid to the berry image. KICHERER *et al.* (2013) noted an average BAT overestimation of minor axis length by  $\sim 3\%$  (0.3 mm on berries of diameter  $\sim 10$  mm) compared to manual assessment with digital caliper, while ROSCHER *et al.* (2014) reported up to 12 % over estimate for harvest stage grapes. In that case the berries were comparatively round, uniform and smaller in size compared to berries used in the current study (17 mm mean minor axis length), for which the overestimate of minor axis was 8 % relative to circle template measured berry diameter. However, as the overestimate was consistent in this study, it can be corrected.

In the current study, the overestimate of minor axis diameter will be, at least in part, due to the difference in distance from camera to image scale (on baseplate; 450 mm) and camera to berry (with a berry of 17 mm minor axis, 441.5 mm). Being closer to the camera, the berry diameter will be overestimated using the baseplate scale (by 2 % on a 17 mm diameter berry, based on simple trigonometry). This error can be adjusted based on an initial estimate of minor axis length. Bunch weight based on the initial BAT measure of berry minor axis was 23 % higher than actual weight due to overestimation of BAT calculated berry minor axis, but use of adjusted BAT calculated berry minor axis

$$\left(\hat{y} = \frac{x - 0.2566}{1.0669}\right)$$

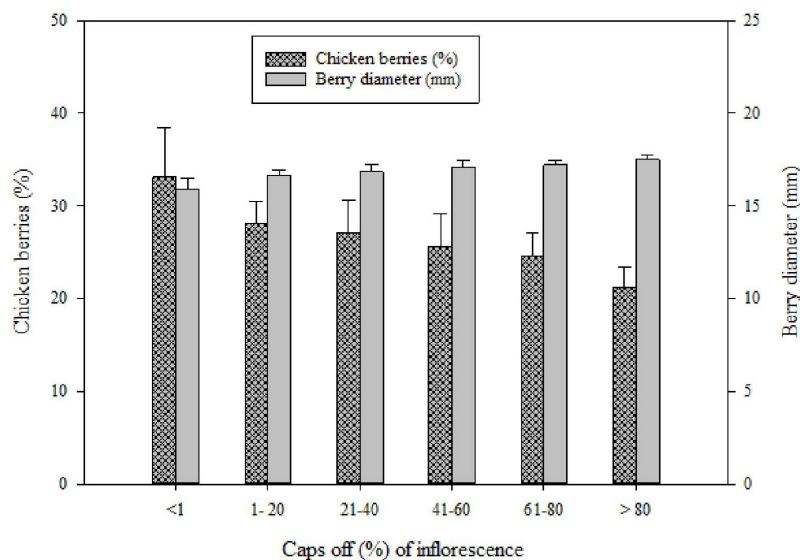


Fig. 6: Percent 'chicken' berries and mean berry diameter of bunch against age of the inflorescence on the day of GA3 application (mean  $\pm$  se).

improved the results to < 2 %. The estimate of 'chicken' berry number and weight using adjusted BAT minor axis was a close estimate of the manual measurement. Therefore, manual assessment of 'hen and chicken' disorder can be replaced with the BAT.

**Sample number:** The number (n) of samples required for a reliable estimate of bunch number per vine, mean berry weight and berry number per bunch depends on the desired level of accuracy (e) and the level of variability (standard deviation, SD) for the attribute of interest

$$n = \left( t \cdot \frac{SD}{e} \right)^2 \quad (\text{equation 2})$$

where t is the t statistic for a given probability. Bunch number per vine accounted for 77.7 % of total variation in vine yield (n = 46 vines, mean = 7.6, SD = 4.23, range 1-18 bunches per vine). Accepting a 10 % error and 95 % confidence level, for this population a minimum of 118 vines need to be assessed for a reliable estimate of bunch number per vine for block/vineyard yield estimation. The high level of variation in bunches per vine in this population was due to the range of gibberellic acid treatments applied to the experimental vines. For more consistently bearing vines, less sampling effort is required. WOLPERT and VILAS (1992) suggested assessment of 64 vines for reliable estimates of bunch number per vine, accepting a 10 % error level, at 95 % confidence level.

Berry number per bunch and the average berry weight of a bunch accounted for 79 % and 16 % of the variation in bunch weight, respectively, comparable to the results of CLINGELEFFER *et al.* (2001) for wine grapes (75 % and 25 % of bunch weight variation attributed to berry number and size, respectively). For the population of the current study (a gibberellin trial with larger than usual variation in berry size and berry number), accepting a 10 % error and 95 % confidence level, a minimum of 90 and 16 bunches need to be sampled for reliable estimation (equation 2) of berry number per bunch (mean  $\pm$  SD = 128  $\pm$  62) and mean berry weight (mean  $\pm$  SD = 4.38  $\pm$  0.88 g), respectively.

**Use on farm:** On farm quantification of yield and of 'hen and chicken' disorder is useful to the table grape grower for estimation of labour requirement for bunch trimming, marketable berry yield and effective market planning. A machine vision tool allows for faster assessment of berry number and size than a manual method. We extend the protocol of MARTIN *et al.* (2003) to involve use of the BAT system on farm, to achieve marketable berry yield estimation.

- Identify representative patches of vines per block, following MARTIN *et al.* (2003).
- Count bunch number per vine or segment of rows in patches after any bunch or shoot thinning operations have occurred.
- A representative number of bunches could be harvested at time intervals during crop maturation, with the BAT used to estimate fruit size in replacement of current manual sizing estimates, feeding data to models of time of maturation.
- Harvest a representative number of bunches (n) from a block before harvest, using a yield gain factor to adjust for growth between sampling and commercial harvest,

assessing berries of each bunch with the BAT system to estimate bunch weight and extent of 'hen and chicken' disorder.

- Integrate the BAT measures of bunch weight and undersize berries into a farm management system to record estimated yield and 'hen and chicken' disorder per block.

## Conclusions

The machine vision based BAT has application to table grape management, for estimation of the extent of 'hen and chicken' disorder and to improving estimates of vineyard yield by enabling rapid assessment of berry size per bunch. The BAT software could be modified to prompt the user on required sample number, based on a recursive estimation using equation 2.

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