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Fast Phenomics: grapevine trait characterisation in the field



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Fast Phenomics: grapevine trait characterisation in the field

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1. Abstract

The Grapevine Rover (GRover) developed for this project is an adaptable proximal-sensing platform designed to provide rapid, high quality information on plant performance in the field. GRover integrates a light radar (LiDAR) and positioning sensors to produce precise (± 3 mm), high resolution, three-dimensional data on vineyard structure. Scans of vine rows are converted to 'point-clouds', which can then be visualised as well as analysed. In addition, the LiDAR used in this study provides data on the laser reflectance of the vine structure, allowing different tissue types, such as leaves, wood or fruit, to be distinguished. Calibration data against destructive and non-destructive (manual) measures of vine size have been developed, based on LiDAR scans at a number of sites, for wood volume, pruning weight and canopy size. The system worked well within a given vineyard with good correlations obtained between LiDAR scans and manual measures for these parameters, demonstrating a high effectiveness at distinguishing differences between individual vines. However, current limitations in the analytical tools used, limit the strength of the calibrations between sites where there can be significant differences between canopies associated with vineyard set-up and management systems and their effects on canopy architecture and size.

2. Executive summary

Plant breeding programs involve large numbers of crosses and there is an associated need to generate extensive datasets on the growth and performance of the progeny from these crosses in field environments. This project addresses this gap for grapevines. New technologies are needed to automate the process of collecting and analysing the large datasets required. Large scale measurement of many aspects of an organism's outward characteristics, or phenotype, is termed 'phenomics'. Phenomic tools developed to measure vine traits also have potential to be used at a commercial scale for vineyard management, for example, being able to assess disease incidence, the light environment around the bunch zone or detailed yield estimation. To address this gap, we developed the Grapevine Rover (GRover), a mobile and versatile phenotyping platform, which can be used to test a range of proximal sensing instruments that might be utilised to replace conventional, manual measurements, such as those used to estimate vine growth.

GRover was constructed in collaboration with the High Resolution Plant Phenomics Centre (HRPPC) part of the Australian Plant Phenomics Facility (APPF) in Canberra, Australia. GRover's frame was made from lightweight aluminium. It is powered by two electric wheels mounted on the front. There is one free-pivoting wheel in the rear. It has a collapsible mast on the front of the frame that is 3 m tall when deployed for measurements. The primary instrument currently attached to GRover's frame, is a SICK LMS-400 LiDAR (Light Radar), which is used for measuring vine size and structure. LiDAR operates in a similar way to sonar, but uses light instead of sound. A LiDAR emits rapid beams of light and detects the reflected pulses. This allows a three dimensional structure to be determined. GRover is also equipped with an LMS-400 eye-safe red laser (605 nm) that is capable of 70 degree scans, producing a two-dimensional field of view 270 times every second. The third-dimension is achieved by moving the LiDAR i.e. GRover through the vineyard. As GRover moves along the vineyard rows with the LiDAR angled towards the canopy, a wheel encoder tracks the linear distance travelled. In this way, three-dimensional point clouds of vine growth can be produced. Additionally, the LMS-400 LiDAR is capable of not only measuring the X,Y,Z coordinates of a given laser return but it also provides information about the remissivity, or reflective properties of the surface.

The LiDAR setup was used successfully to capture a variety of vine size and growth parameters at a range of different vineyard locations. GRover was able to produce scans of canopies of different management styles at any time during the growing season. Additionally, by using simple computational methods, metrics from LiDAR scan data were shown to be strongly correlated with destructive measurements of pruning weight. Work was also carried out to determine how well LiDAR scans correlated with plant growth on the basis of leaf area and trunk and cordon weight. Each trait was measured on vines grown in four different regions (Adelaide, Nuriootpa and McLaren Vale, South Australia and Irymple, Victoria).

Field trials showed that LiDAR scans involving multiple measurements over time

were highly predictive at measuring parameters of vine size within a vineyard, however calibration accuracy was limited when comparing different locations. Correlations between LiDAR scans, leaf area and pruning weight, differed significantly between some field sites. However, within a given field site, the LiDAR could be used to assess an applied treatment, such as: reduced irrigation, elevated temperature or a change in management as effectively as using destructive measurements, but with much greater speed and scale. Initial attempts to utilise the system for yield assessment in typical Australian commercial vineyards were not successful because bunches were obscured by leaves and did not appear to have different reflectance values to leaves.

The deployment of the high resolution LiDAR system in a vineyard for relatively large scale testing against vine structural parameters has successfully demonstrated its potential. Although there were issues with correlations across multiple locations, it is anticipated that improvements in data analysis and collection could be made that would ameliorate this issue. For example, a LiDAR with different properties could be used. The LMS-400 offers an excellent scanning rate and precision but is affected by sunlight during outdoor use. Alternative positioning systems could also be used and more sophisticated algorithms for analysis could potentially be developed.

Continued development of phenomic tools such as GRover require a highly collaborative team effort involving both technology and biology skills, particularly suited to a diverse organisation such as CSIRO. Further development of LiDAR technologies could yield cost effective tools for management purposes; for example tools that accurately assess canopy structural parameters such as light penetration.

3. Background

Significant advances in broad acre crops have been made in the application of genomics and phenomics in linking the genotype of a plant to its appearance, function and performance. However, phenotypic characterisation of large numbers of genotypes remains a key bottleneck in research to develop improved varieties of grapevine scions and rootstocks. A suite of advanced non-invasive technologies for field crop monitoring are now available to accelerate progress in understanding gene function and environmental responses. Utilising these in viticulture will assist breeders to develop new germplasm to support industry needs.

Marker-assisted selection (MAS) in plant breeding has become more common in recent years and is now used for single locus traits and quantitative trait loci (QTLs). Phenotyping the populations is the most laborious and technically challenging part of this process (Furbank and Tester, 2011; Tester and Langridge, 2010).

The interaction of genotype with management and the environment can also be assessed utilising these technologies in an established vineyard. Existing trials that have been set-up to compare different management strategies can be accessed for this purpose and field performance data obtained in this way can provide a benchmark for optimisation. It will also provide a platform on which vineyards of the future can be managed since it is highly probable that these will utilise the latest non-contact/remote plant and field-based technologies to monitor growth, manage irrigation, regulate vine balance, minimise crop variability and schedule harvests.

It is generally accepted that phenomics, or large scale phenotyping, is the natural complement to genomics as a pathway to rapid advances in biology (Houle et al. 2010, and references therein). Conventional field phenotyping is slow and highly labour intensive. The bottleneck in field phenotyping has driven intense interest over the past decade in applying remote sensing technologies to field crop monitoring. Field phenomics is more advanced in many respects than controlled-environment, high throughput analysis (Araus and Cairns, 2014, Furbank and Tester 2011).

Examples of important traits that can be assessed by field phenomics are growth (by digital image analysis over time) and drought and salinity tolerance. Yield is more complex and requires further advances in image analysis. One of the first effects of exposing a crop to drought or salinity is stomatal closure, which can lead to reductions in photosynthesis. Traditional measures of photosynthesis and stomatal conductance, using direct measurement of gas exchange, are time consuming. Alternative techniques such as infrared thermography or automated spot canopy temperature measurement offer great potential and have been successfully used at the young seedling stage in wheat and barley to select genotypes able to maintain stomatal conductance under osmotic stress (Sirault et al. 2009). Such techniques have great potential for low-cost, high throughput field phenotyping (Furbank and Tester 2011). Chlorophyll fluorescence is a measure of

photosynthetic function, which may vary under stresses such as drought, salinity, heat and UV. However, there are challenges in attempting to use chlorophyll fluorescence for high throughput field phenotyping. An alternative technology is leaf spectroscopy or hyperspectral reflectance spectroscopy. This is a promising technology, although its application and the interpretation of acquired data requires care (Jones and Vaughan 2010). All of these and additional technologies have potential for application in a field phenomics approach which will also deliver spin-off benefits in terms of information that can be used to streamline and improve vineyard management practices.

Wine Australia has major programs aimed at increasing the profitability of production and enhancing recognition and exports of Australian fine wines. There is a need to better characterise vineyards that consistently produce fine wines and through this understanding elevate fine wine production to another level and further enhance Australia's reputation as a leading producer of high quality wine. In addition to a potential role in field phenotype screening of breeding populations, non-destructive proximal sensing technologies may also have a role to play in fine tuning vineyard practices to achieve high-quality wine.

4. Project Aims and Performance Targets

The project aims were to utilise established grapevine populations and trial sites, at CSIRO's Irymple Farm, the SARDI Nuriootpa Research Vineyard, the University of Adelaide Waite Campus Research Vineyard or on collaborating wine company vineyards to test and refine the phenomobile GRover. The project interfaced with Wine Australia projects CSP 1303 and CSP 1304, from which base data on genotype performance were collected. GRover enabled a broader data set on multiple traits for each genotype to be collected non-invasively during the season. The project also interfaced with Wine Australia project CSP 1202 (Vine Balance) which included crop load and leaf removal treatments amenable to assessment using GRover.

The major themes included:

- Equipment fabrication

- Equipment testing and subsequent refinement for trials.

- Application to selected trials

The latter component included (a) application to grapevine hybrid populations, to assess potential for identifying genotypes with significant differences in targeted traits, potentially enabling assessment of segregation and heritability of the trait, which would be of assistance to genomics studies and breeding, (b) management treatment field trials, e.g. to potentially assist arriving at recommendations on the most appropriate practices for optimal vine balance, and (c) a field nursery site situated at CSIRO's Irymple Farm, also to asses potential for identifying genotypes

with significant differences in targeted traits. The trials selected were chosen based on the final configuration of the GRover and the performance of the installed instrumentation after testing. After the first years of establishing the frame, longer term (> 2 year) trials that were also considered for inclusion in testing were (a) phase 1 genotype assessment, providing additional information to use when making decisions on which genotypes to carry-forward to phase 2 trials, and (b) wine grape rootstock and scion trials, providing information for use when making decisions on possible commercial potential.

The goals for the first year were to prototype the frame and demonstrate the utility of the prototype. This included the preliminary design, fabrication and testing of the equipment.

The goals in years two and three were to display the utility of the prototype, through testing in a range of vineyard environments. The primary focus was to assess the prototype for non-destructive measurement of key vine growth parameters. A secondary aim was to assess the prototype to provide basic measures of crop yield.

5. Methods

The following describes key aspects of the construction of GRover, its initial testing and subsequent refinement for trials. For more detail, see Siebers et al. (2018).

5.1. Equipment fabrication

Initial goals included producing 3D scans and developing a simple computational method that uses the LiDAR to estimate a biological parameter. In this case pruning weight was measured because it is a common measurement of vine growth. It is also a tedious, labour intensive measurement that would be suitable for automation.



Figure 1. GRover's frame and components: **a)** SICK LMS-400 light radar (LiDAR), mounted 2.25 m above the ground at a 45 degree angle. The LiDAR scans at 250 Hz at a wavelength of 650 nm and a range of 3 m. **b)** Masts on which instruments can be mounted. Each mast can be moved up, down, away from or toward the frame. **c)** Each of the front wheels contained an electric motor. **d)** Batteries (48V) used to power the wheels. **e)** Instrument battery (24V). **f)** Power supply for providing different voltages to the instruments. **g)** Spring-armed wheel encoder, which tracked the linear distance travelled during scans. **h)** LiDAR, encoder and GPS/IMU spatial unit integration box. **i)** Field laptop for operating the instruments.

The frame for GRover was completed by the Hydraulic Doctors, Queanbeyan, ACT, (www.hydraulicdoctors.com.au) in May 2015. It is made of lightweight structural-

aluminium and weighs ~200 kg (Figure 1). It is 3 m long, and has a wheelbase that can be adjusted between 1.2 and 3 m to enable operation in a variety of row spacings with maximum stability. The mast can be raised for measuring taller canopies and lowered for transportation. When raised, the mast is 3.2 m tall and stabilised by an additional aluminium support beam that is stored on the frame. GRover measures 2.1 m tall when the mast is lowered. GRover's main sensor, the LMS-400 LiDAR (Figure 1a) can be mounted in virtually any position on the frame. There are three masts running perpendicular to the length of GRover's frame on which the LiDAR could be mounted. Those mounts can be raised and lowered or moved toward or away from the centre of the frame (Figure 1b). The mount-points are in positions in front of or away from the frame so that line-scan sensors can be used without any interference from the frame. The LiDAR can be rotated to scan anywhere from zero degrees, pointing straight at the ground, 90 degrees, pointed straight at the canopy, or 180 degrees, positioned underneath the canopy, aimed skyward. For all the scans presented here, the LiDAR was mounted 2.25 m above the ground, on the centre mast and angled 45 degrees, as pictured in Figure 1.

The frame was equipped with three wheels: the front two wheels have built-in electric motors and there was one free-pivoting wheel in the rear. The rear tyre allows GRover to have a zero degree turn radius, which was important for two reasons: First, looping between vineyard rows would have been difficult if the platform was less manoeuvrable. Second, since the front two wheels are powered independently, GRover could be programmed to operate autonomously in the future. The wheels were operated and driving speed was controlled by two thumb-throttle controllers mounted on GRover's rear handle bars.

5.2 Equipment testing and subsequent refinement for trials

The SICK model LMS-400-2000 LiDAR (Figure 1a; SICK AG, Waldkirch, Germany) mounted to GRover is an eye-safe red laser (650 nm) and scans a 70 degree field of view. The LMS-400 has a range of 0.7 m to 3 m and can be programmed to scan between speeds of 250 to 500 Hz. The LiDAR produces polar coordinates (distance and angle) from the resolved time of flight from the laser, which are then converted to *xyz* coordinates to generate a point cloud. Additionally, the LiDAR produces information about the reflectance of the scanned surface. The reflectance value is related to the ability of a material to reflect the LiDAR signal back to the sensor: the higher the reflectance value, the more reflective the surface.

The linear distance travelled by GRover was measured with a rotary encoder that provides linear travelled position. Viewed from the handlebars, the wheel encoder is in contact with the front left tyre (Figure 1g). A Spatial GPS/IMU (Advanced Navigation, Sydney Australia) unit was attached with double-sided tape to the top of the LiDAR (not pictured). The Spatial unit is used to record data about the angle of the LiDAR and GRover's spatial position ($\pm$2 m). The LiDAR, GPS and encoder data

are captured using the field laptop (Figure 1i) running proprietary Java software developed by the High Resolution Plant Phenomics Centre (HRPPC, Canberra, Australia; For more information about the HRPPC and their services contact: http://www.plantphenomics.org.au/). The software provides a user interface presenting: a map of the GPS location and input dialogs for the experiment name and run-number. The LiDAR data are stored in a custom binary format called Phenomics LiDAR Format (.PLF). The file format was developed by the HRPPC for efficient storage of LiDAR data.

The initial testing, fabrication and refinement of the workflow were greatly assisted and accelerated by collaboration with the HRPPC. The HRPPC provided a custom-made piece of Java software that was used to convert the .PLF and .GPS data to a standard point cloud format, the Stanford triangle format (.PLY). The integrated point cloud and encoder data, saved as a .PLY file, were visualised using the open-source software CloudCompare (2017). Point clouds were processed and cleaned using two modules within CloudCompare (Figure 2a-d). Firstly, the points were filtered by their reflective intensity, or reflectance values, using an intensity selection plugin built into CloudCompare (Figure 2b, c). All points with reflective intensities less than or equal to 1 were removed. Second, the PCL wrapper plugin was used, which employed a nearest neighbour filtering algorithm (Figure 2c, d). In detail, 10 points were used for mean point distance estimation and the standard deviation multiplier threshold was 1.00. The workflow, rationale for choosing specific thresholds and need for point cloud pre-processing are described later in this report.

After a scan was saved and visualised in CloudCompare, the point cloud was cleared of erroneous data points (Figure 2a, d). There were three main sources of error when collecting scans: 1) Debris caught in the wheel sometimes dislodged the encoder, stopping it from spinning and tracking distance. This compressed the scan into two dimensions. There is no easy computational way of solving this problem, so the encoder was monitored during scans. 2) Although the LMS-400 gives precise spatial and reflectance data at a high rate, it is designed to operate below 2000 lux and not under high light conditions. Lux values in indirect sunlight commonly range between 1000 lux on an overcast day to 130000 lux in direct sunlight (Tandon et al. 2013). High light levels are the cause of the spurious, low-intensity blue points seen between the LiDAR and the vines in Figure 2b. However, the erroneous measurements are all low reflectance values and can be removed by filtering the scan based on a set reflectance value. Points with reflectance values ≤1.0 were removed from the scan using the ‘filter points by value’ plugin in CloudCompare (Figure 2b, c). In Figure 2b, there are 1.49 million total points, and 28% of those points had reflectance values ≤1.0. The reflectance threshold was chosen qualitatively and removed spurious points without significantly affecting the biological interpretation of the scan. Green leaf material had a reflectance value between one and five. 3) As with any LiDAR scan, there was scattering at the edges of objects, where light is reflected in unpredictable ways. An example of edge scattering can be seen between the main vine cordon in Figure 2b. The

nearest neighbour statistical outlier plugin in CloudCompare removed sparse outliers based on the distance of an individual point from its neighbours. By applying these filters, point-clouds were reduced to only the scanned objects. In Figure 2c there are 1.12 million points. After the filter to remove statistical outliers is applied there are 1.04 million points in the final point cloud (Figure 2d) on which any computational analysis would be performed.

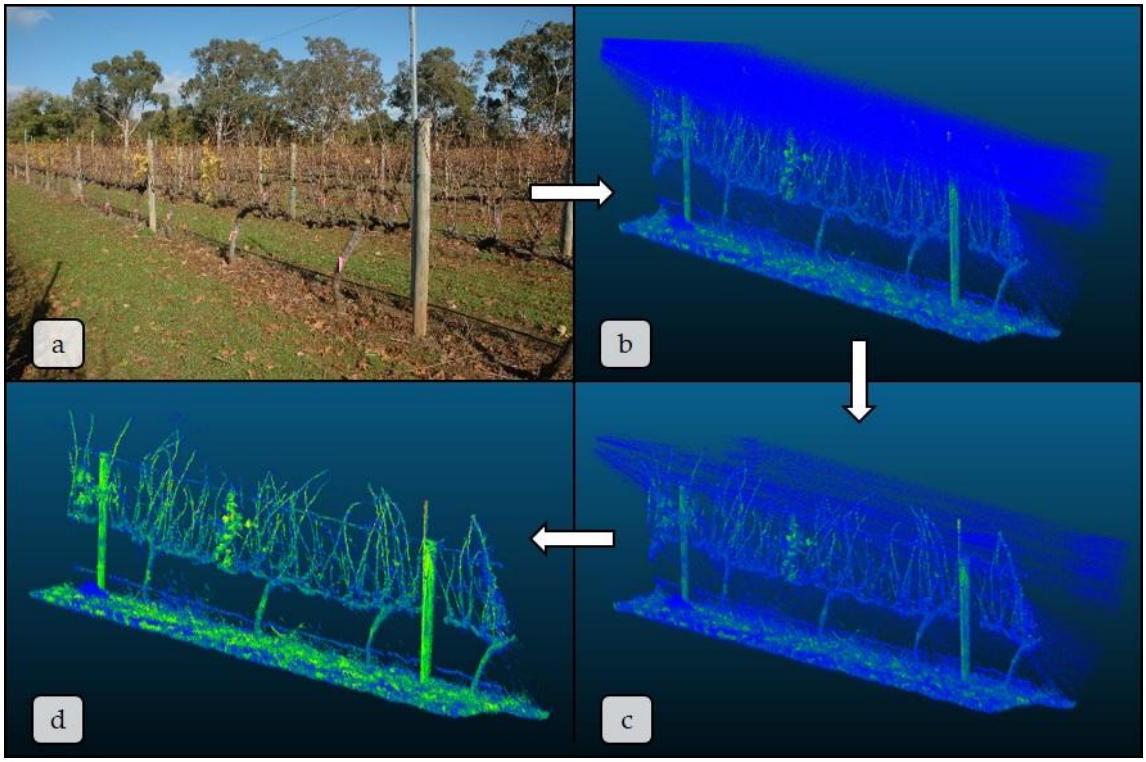


Figure 2. Following the arrows clockwise from panel **a** to **d**, this figure depicts the workflow used to process scans before computational analysis. **2a** depicts a single panel of vines in May 2015, after nearly complete senescence. **2b** shows the raw LiDAR scan of the panel before any erroneous points are removed. **2c** shows the LiDAR scan after removal of low intensity points. **2d** is the LiDAR scan after a nearest neighbour filter is applied to remove any outliers. All scans are visualised and processed using the open source program CloudCompare.

Initial testing was encouraging and reinforced the idea that GRover could operate in almost any commercial vineyard. Vines in the single panel depicted in Figure 3a were of the variety Shiraz grown at the University of Adelaide Research Vineyard at Waite Campus, Adelaide, South Australia. Vines in Figure 3c, also Shiraz, were grown at the SARDI Research Vineyard in Nuriootpa, South Australia. The smallest units scanned by the LiDAR were single, ~3.6 m panels (three vines per panel, with spacing between vines of 1.8 m) and the largest area scanned, to date, was a 500 m row containing 93 similar sized panels (data not shown). The 500 m row produced ~ 1 GB .PLY files for visualisation.

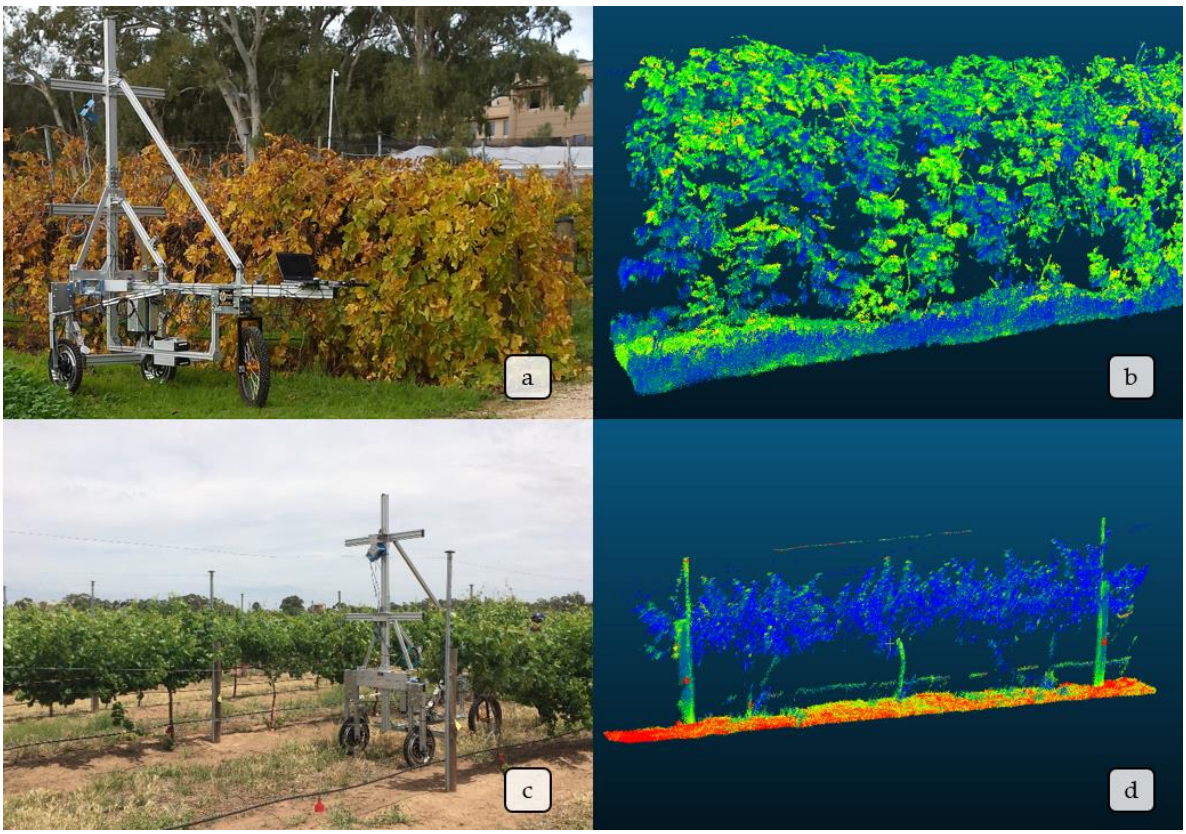


Figure 3. LMS-400 and GRover capturing parameters of vine growth of minimally pruned vines (**3a**) and of spur pruned vines trained to a two wire vertical trellis (**3c**). Right side panels are the LiDAR returns and the intensity of the returns for the minimally pruned vines (**3b**) and for the spur pruned vines on two wire vertical trellis (**3d**). Note how the intensity of the LiDAR return allows for visual distinctions between wood and leaf. The blue points, usually green leaf tissue, have the lowest reflectance values or are the least reflected. Yellow leaves (**3c**) or woody tissue (**3b, d**) are more reflective and have a higher relative (green) reflectance value. The brown soil and the identification tags on the posts in **3d** are the most reflective objects and appear red.

Initial testing on plant material was carried out during the winter growing season of 2015; the pruning weight of Shiraz vines (grown at the SARDI Research Vineyard at Nuriootpa) was compared to LiDAR scans made before and after pruning. Point clouds were processed using the workflow described earlier. Scans made before and after pruning were voxelised using the Octree function within CloudCompare (Figure 4a, b). The Octree algorithm recursively divides the point cloud into cubes by eighths, discarding cubes without any points within them (for more information see: http://docs.pointclouds.org/trunk/group__octree.html).

The voxel difference between the scans was calculated as follows:

$$V_{Before(R)} - V_{After(R)} = V_{Diff(R)} \quad (1)$$

The voxel number before pruning at recursive level 'R' ($V_{Before(R)}$) was subtracted from the number of voxels after pruning ($V_{After(R)}$) at the same level of recursion to yield the difference ($V_{Diff(R)}$). This is illustrated in Figure 4a and b, where the vines to the right have been voxelised using the octree algorithm. The green wire cubes

represent the size and number of the voxels at the given level of recursion. The difference between the before and after scans correlated well with destructive measures of pruning weight (Table 1). At the 10th level of recursion, the R^2 between pruning weight and scan difference was 0.92.

Recursion Level $_{(R)}$	**6**	**7**	**8**	**9**	**10**	**11**
R^2 ($V_{Diff(R)}$ vs. weight)	0.088	0.467	0.705	0.864	**0.916**	0.778

Table 1. This shows the R^2 of the linear correlation between the voxel difference ($VDiff_{(R)}$) and pruning weight at increasing levels of octree voxelisation (Recursion level $_{(R)}$). Levels of recursion range from six to eleven. The highest R^2 value was recursion level 10, highlighted in bold font.

The initial testing and field trial data show that simple computational procedures, i.e. voxelisation difference (eq. 1), and a laser with a high-scan-rate LiDAR can accurately and efficiently measure pruning weight. It is notable that this was possible and effective using a simple, open-source algorithm. However, this preliminary work on pruning weight needs to be expanded to a number of different varieties, at different locations and exposed to biotic and abiotic stresses to see if the correlation holds up.

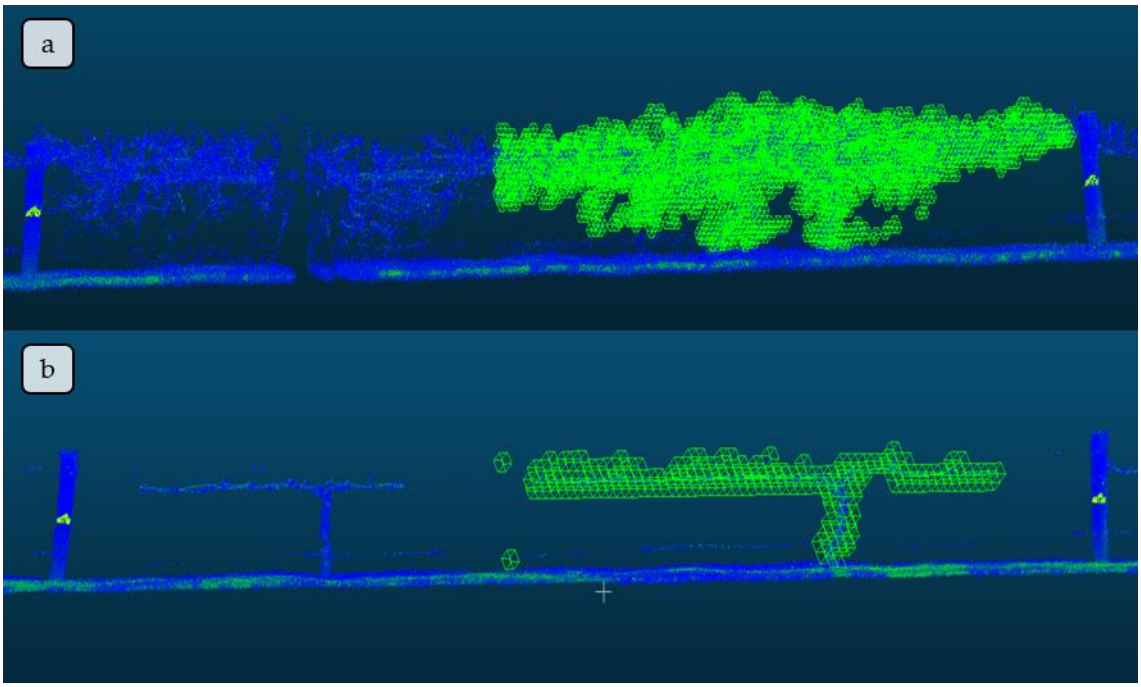


Figure 4. This Figure shows LiDAR scans made before (**a**) and after pruning (**b**). Calculations of the voxel pruning volume (V_{Diff}, equation 1) were made by subtracting the number of voxels (neon wire cubes) before and after and comparing that value to the actual, physical pruning weight. This image also illustrates what the octree algorithm looks like when applied to a point cloud.

Also, because of the ability of the LMS-400 to detect the reflectivity of a surface, the LiDAR may be able to distinguish leaves from bunches and estimate yield. While not a focus in these studies, yield forecasting is a problem for growers. Great effort has gone into modelling grapevine yields (Liu et al. 2013; Hall et al. 2010). However, yield predictions can still be off by 10 to 20 percent even when spatial variability in the vineyard is considered (Naito et al. 2017). It would be beneficial to have a single tool capable of measuring fruit and vegetative production. The balance between harvestable and non-harvestable growth, or the harvest index, is considered a pleiotropic hub and also something of interest to breeders (Roessner et al. 2006).

A range of locations and trials were used for the work. These are summarised in Table 2. Work was scheduled according to the Activities Plan as detailed in Appendix 5.

6. Results and discussion

Following the commissioning of the GRover frame and establishment of a provisional workflow for data processing, additional metrics of vine growth were compared to LiDAR scans. In the 2015 to 2017 growing seasons, LiDAR data scanned from one side of the canopy were ground-truthed with destructive measurements of cordon and trunk weight, leaf area and pruning weight. The trials were constructed to determine whether LiDAR correlations differed between field sites. Additionally, within a given field site, LiDAR scans and destructive measurements were made on plots that were subjected to controlled experimental treatments. The treatments included: differences in canopy management, reduced irrigation, and elevated temperatures. If the LMS-400 is a suitable tool for capturing vine growth features, the slope and strength of correlations between destructive measurements and LiDAR scans should not be affected by changes in location or treatment. The data presented here represent the largest effort yet undertaken in Australia to develop LiDAR technology for use in vineyards.

Material	Site	Obs.	R^2
Cordon and Trunk	Nuriootpa	51	0.77
Leaf area	Total	93	0.88
	Irymple Farm Nursery	23	0.97 [1]
	McLaren Vale Shiraz	8	0.91 [2]
	Nuriootpa Shiraz	26	0.94 [3]
	Nuriootpa Hybrids	36	0.73 [4]
Pruning weight	Total	191	0.70
	Nuriootpa Shiraz	56	0.79 [1]
	McLaren Vale Shiraz	20	0.77 [1]
	Irymple Farm Shiraz	90	0.58 [2]
	Irymple Hybrid Family	25	0.69 [3]

Table 2. This shows the locations where destructive measurements and scans were made. It also shows how well scans correlated with the destructive measurements at each site and for each type of plant material. Dummy variables were created to make multiple comparisons between slopes. Dummy variables are devices used to sort data into mutually exclusive categories. In this case data sets were compared by location (Miller and Erickson 1974). Correlations with the same slope ($p < 0.01$) share the same numerical superscript in the R^2 column.

In discussing the results shown in Table 2, with regard to the strength of the correlations, R^2 values of 0.5 to 0.7 were considered ‘weak’; 0.7 to 0.85 were considered ‘moderately-strong’; and 0.85 to 1.0 were considered ‘strong’.

LiDAR scans and manual measurements of volume correlated moderately strongly with cordon and trunk weight:

Measurements of cordon weights were taken in 2015 on Shiraz vines grown at the SARDI Research Vineyard, Nuriootpa. LiDAR scans were made on both sides of the vine before removal. Vines were cut ~5 cm from the ground and untangled from the supporting wires, then weighed. Vine trunk and cordon weights were measured in the field using a portable scale (Kingship EW-50, Taichung, Taiwan). It was difficult to find material for destructive sampling in commercial vineyards. So, unlike leaf area and pruning weight data presented later, there is only one sampling location and variety for vine cordon weights.

Measurements of cordon weight (kg) of Shiraz vines had a moderately strong correlation with the number of voxels at 10 octree recursions in the scan ($R^2 = 0.77$). Scans and destructive measurements were made on the same day. The 10^{th} recursion of the octree provided the best correlation between the LiDAR scan (data not shown).

By comparison, manual measurements of cordon and trunk volume (cm^3) correlated moderately strongly with cordon and trunk weight (kg) ($R^2 = 0.84$) and similarly with LiDAR scans. Vine volume was estimated using callipers by measuring the trunk and cordon diameter in three locations on the trunk and each cordon and summing the volume of the cylinders, as follows: The diameter of the trunk was measured in three even sections from its base to where the cordon divided to right and left side of the vine. The diameter of each cordon, right and left side of the trunk, was measured in three places. The volume was calculated by summing the volume of each connected cylinder, from the base of the plant to the ends. Although the correlations were both moderately strong when making the manual measurements of volume, it took 10-15 minutes for each plant. In that same time, GRover could capture ~200 vines.

LiDAR scans correlated strongly with destructive measures of leaf area, although the slopes of the correlations were different at each field site:

Leaf removal experiments were carried out in 2015 to 2017 to test the effects of locations on the correlation between LiDAR scans and leaf area (Figure 5, Table 2). Firstly, in 2015 to 2016, correlations were made on the variety Shiraz used for vine weight measurements at the SARDI Research Vineyard. Destructive measurements and scans were made five times during the season to determine whether or not phenological differences in the same variety of vine, growing at the same location could affect the correlation between LiDAR scans and leaf area. Secondly in 2017, destructive leaf area measurements were made at the CSIRO Irymple Farm nursery (Victoria, Australia) on three year old-vines of different rootstocks that were subjected to ambient and elevated temperatures. Thirdly, in 2017, destructive leaf

area measurements were made on Shiraz vines grown at McLaren Vale, South Australia. The canopies at the McLaren Vale site were maintained in the Vertical Shoot Position style (VSP) or as unmanaged canopies (Australian-sprawl). Lastly, a range of unique hybrid genotypes from a CSIRO marker-assisted-selection breeding program associated with Wine Australia project CSP 1303 were measured at the SARDI research facility. Each leaf area measurement collected was from a genetically unique hybrid plant.

To test whether regression slopes were statistically different between measurement locations, a dummy variable analysis was performed using SPSS. Pairwise comparisons were made between the regressions. Given the number of statistical comparisons being made between regressions, a more stringent P-value was used in order to avoid Type 1 error ($p > 0.01$).

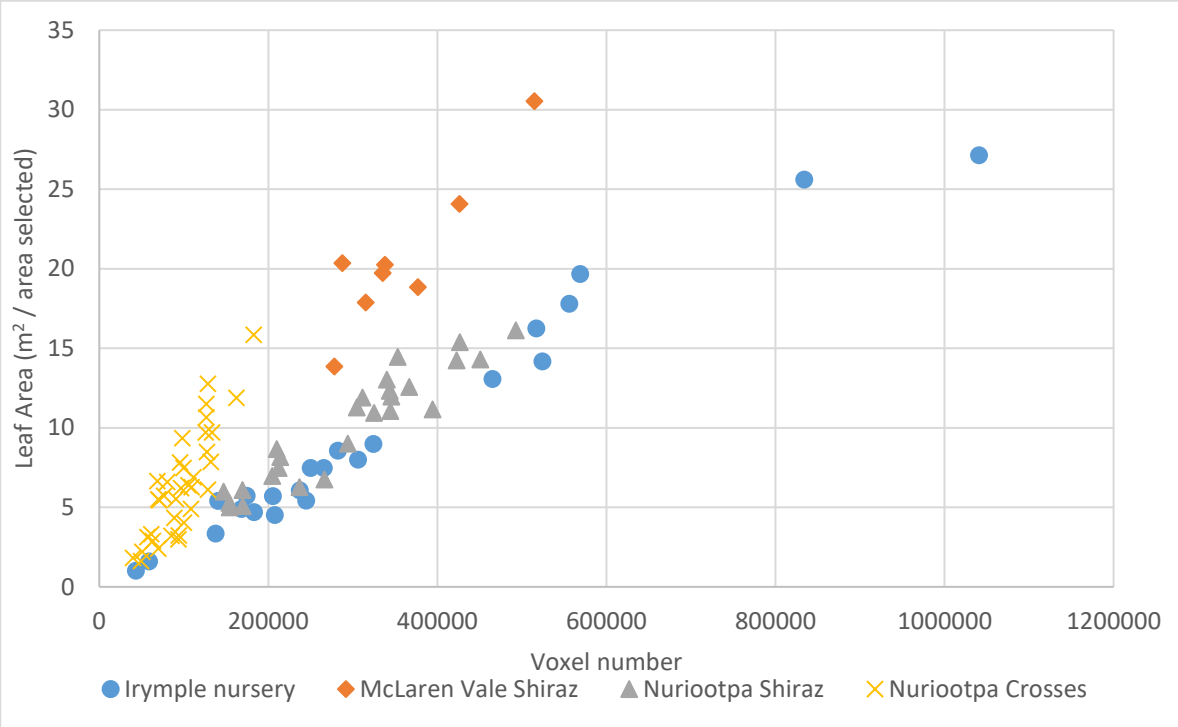


Figure 5. Shows the relationship between destructive leaf area and the number of voxels, measured at three different field sites on Shiraz and hybrid vines.

Overall, LiDAR scans made from one side of the canopy correlated strongly with destructive measurements of leaf area (R^2 over all sites = 0.88, Table 2) and even more strongly within a given field site, for example, R^2 = 0.97 at Irymple Farm Nursery (Figure 5, Table 2). However, the relationship between the number of voxels in the LiDAR scan and the leaf area changed from site to site. The weakest correlation was on the hybrid vines measured at the SARDI facility (R^2 = 0.73).

The slopes of the regressions differed from site to site. Scanning speed plays a large role in the number of returns. However, scans are made and scanning speeds vary row by row. So if changes in scanning speed were an issue, why is

there not more variability seen within a location? It is most likely due to differences in architecture between each of the vineyards. The different sites had different canopy management/architecture that influence how the LiDAR ‘sees' the canopy and explains the differences that were obtained between sites. Leaf area correlations were more strongly impacted by site than were the pruning weight correlations. This suggests that the volume-based algorithm for estimating LAI is not ideal and a different approach based on gap fraction or profile analysis should be applied instead. Moving forward, as the LiDAR is sensitive to the type of canopy management, it means that there is potential to detect differences in canopy architecture despite similar LAI, which in turn could be linked to differences in fruit quality or yield. These aspects should be investigated in future studies which could utilise the scans and field data collected from this experiment.

However, there may be other factors besides the type of algorithm used that caused site differences. To explore the possibility that larger canopies were shading leaf material on the far side of the scan, the dataset from the Nuriootpa hybrid vines was re-analysed. The Nuriootpa hybrid vines data were selected because the slope of the regression is the greatest. That is, there are relatively few returns for a given amount of leaf area. To explore this difference, instead of scanning only one side of a row, scans were made of both sides, with the overlap (returns from beyond the cordon/trellis) removed and the voxel numbers from both sides of the canopy were summed (Figure 6).

Analysing scans from both sides of the canopy had the anticipated effect. It changed the slope of the regression and there were more LiDAR returns for a given amount of leaf area. However, it made the correlation weak ($R^2 = 0.55$) and the slope of the correlation was still significantly different compared with that from any other field site (Figure 6). The weakening of the correlation was likely caused by the imperfect way plots are manually chosen. There was no way to ensure that the area of the canopy selected from the scan was exactly half. Although the re-analysis was inconclusive, it suggests that some other part of data collection or processing could be the issue. Further work could involve trying an entirely different algorithm for determining leaf area that is not volume based, or using metrics such as canopy volume instead of canopy area. For example, one could find a way to ‘fill’ the voxels in the centre of the canopy that would otherwise be obscured.

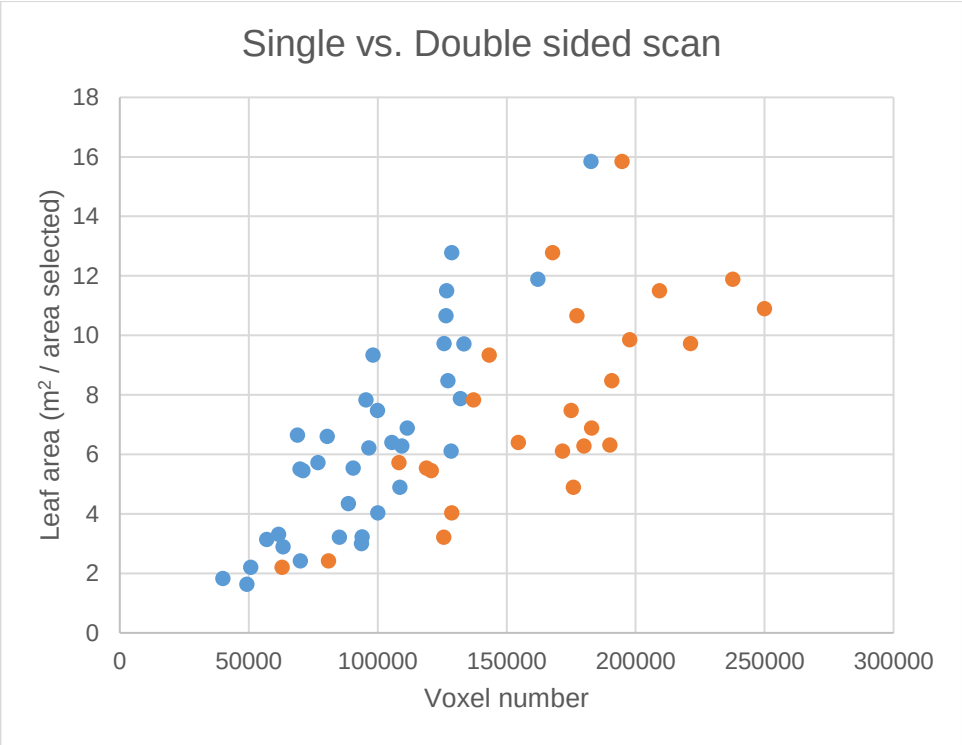


Figure 6. This shows the effects of concatenating scans, cutting them from either side and analysing them (orange circles, $R^2 = 0.55$). Or, analysing them individually ($R^2 = 0.73$). A dummy variable was also created to assess whether the slopes of the correlation were statistically the same ($p = 0.00001$).

LiDAR scans correlated well with destructive measures of pruning weight within sites but not between sites:

Pruning weights were measured at three regional field sites Nuriootpa and McLaren Vale, South Australia and Irymple, Victoria) from 2015 to 2017 each with the replicated ability to test the effect of variety, abiotic stress or management (Table 2). In every instance canes were cut above the second bud and weighed immediately using the same weighing scale that was used for leaf area. Pruning weight was measured at four locations, each with the replicated ability to test the effect of variety, abiotic stress or management. 'Nuriootpa Shiraz' vines were measured at the SARDI Nuriootpa Research Vineyard. 'Nuriootpa hybrid vines' were all different genotypes undergoing selection for resistance to powdery mildew. Second at the McLaren Vale site, all the pruning weights were collected from Shiraz vines that were all single cordon, however half of the plants measured were maintained with a VSP canopy and the other half were unmanaged (allowed to sprawl). At the CSIRO Irymple site, pruning weights were collected on Shiraz variety. There was also an irrigation reduction (water deficit) trial. Half of the Shiraz plants measured at the Irymple site were receiving ~40 percent less water compared to the control. Lastly, a hybrid population from a cross between *V. vinifera cv. Riesling* X *V. cinerea* was measured at the CSIRO Irymple site. The relationship between pruning weight and number of voxels is shown in Figure 7.

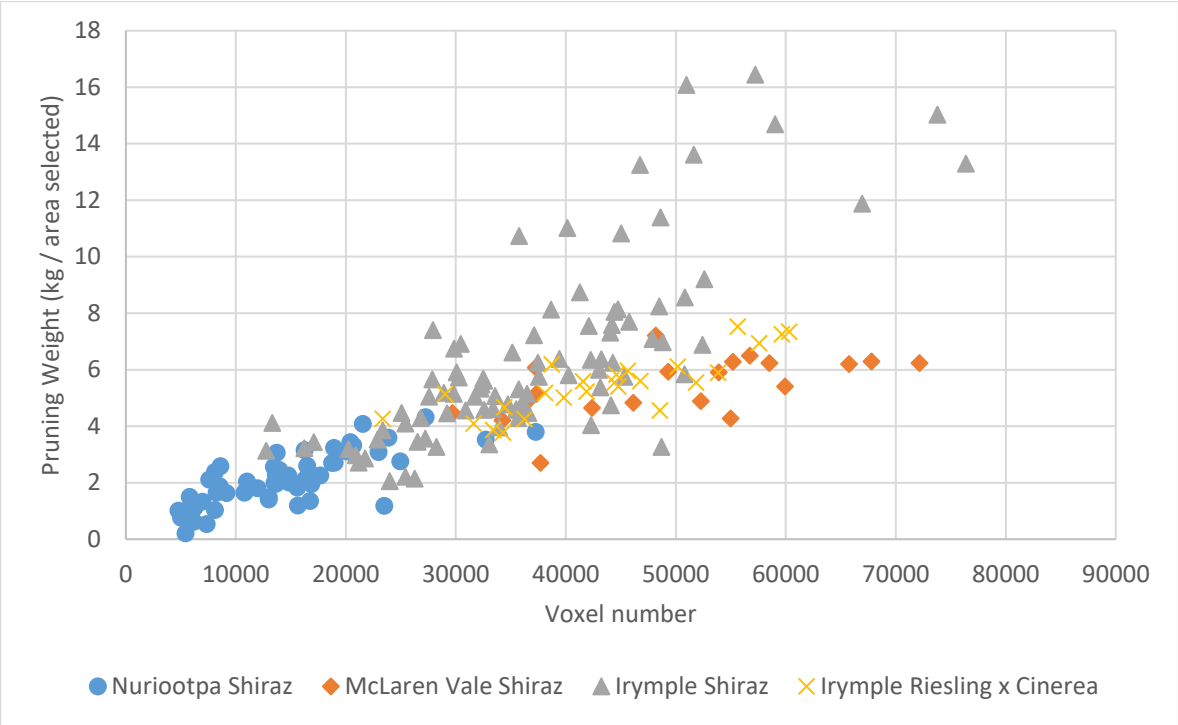


Figure 7. Shows the relationship between pruning weight and the number of voxels, measured at three regional field sites on Shiraz and hybrid vines.

Overall, LiDAR scans did not correlate as strongly with pruning weight (R^2 = 0.7, Table 2) as they did with leaf area (R^2=0.88, Table 2), and even within field sites, correlations were not as strong. Dummy variable regression analysis showed that the slopes of the Nuriootpa Shiraz vines were statistically the same as the McLaren Vale Shiraz (Table 2). All of the other slopes were statistically similar. The dataset that had a correlation furthest from the other sites was the Shiraz grown at Irymple, having only half the number of voxels for a given pruning weight compared with the other sites. Although the shading effect cannot be completely ruled out as a possible source of additional error, what about other portions of the workflow? The Irymple Shiraz dataset was made over five rows. The before and after scans were selected for analysis by hand in CloudCompare. It is possible that site variability or even variability within a site could be caused by an inability to accurately select the appropriate plant material from the scan.

To test whether plot selection was an issue that contributed to error, the results of the voxel numbers obtained in two separate analyses for the same five rows of the Irymple Shiraz pruning weight data were reanalysed and compared. The LiDAR .PLY files were renamed and chosen at random. There were few distinguishing features between rows so it would be difficult to identify what row was being reanalysed from the scan alone. The voxel number of an individual plant was reanalysed on two separate occasions. There was a strong (R^2 = 0.98) 1:1 correlation between analysis 1 and analysis 2, confirming the reproducibility of the data analysis pipeline. This removes suspicion that the plot selection contributed to poor correlation between LiDAR scans and pruning weight seen in the Irymple Shiraz dataset. Further effort

needs to be made with these data to determine why there is more noise, but it is encouraging that the error is not attributed to issues with the workflow, or likely, data collection.

Although correlations between sites were different. LiDAR was able to consistently capture within site treatment differences for both leaf area and pruning weight:

Of the multiple dummy-variable comparisons made between field sites where pruning weights and leaf area were measured, only two correlations had the same slope (Table 2) but even then the relationship between those two regressions was nearly insignificant ($p = 0.05$, data not shown). However correlations within field sites were strong. So, although every regression except one differed by location within a field site, LiDAR scans were able to detect treatment differences just as well as destructive measurements (Table 3).

At each site where a treatment was imposed, the difference between the treatments ((control – treatment / control) * 100) was calculated for each replication. The magnitude of difference measured by the destructive measurement was then compared to the difference measured by the LiDAR using a Student's t-test. In every instance, the percent change caused by the treatment was statistically the same whether it was measured using the LiDAR or by conventional destructive means (Table 3). So at the moment it is not possible to compare differences between locations but within a given location LiDAR can be a fast, accurate tool for detecting growth differences.

Type	Site	Treatment	%Diff	%Diff $_{Lidar}$	T-test (p)
Leaf	McLaren Vale	Management	-13 ± 8	-8 ± 8	0.28
	Irymple Farm Nursery	Temperature	-0.8 ± 4	8 ± 6	0.47
Prune	McLaren Vale	Management	-12.6 ± 5	-16 ± 6	0.34
	Irymple Farm	Irrigation deficit	-22.3 ± 5	-23 ± 6	0.46

Table 3. This shows the effects of a treatment on Shiraz vine growth at two sites. It compares the difference of a treatment measured by conventional, destructive means (%Diff) versus the same treatment difference measured by the LiDAR ($\%Diff_{Lidar}$). Statistical significance between the measurement methods was assessed using a student's t-test

Bunches are indistinguishable from leaves or shaded by leaves, making LiDAR an unsuitable tool for yield estimation:

The LMS-400 and its ability to measure the reflectivity of a surface made it a candidate technology for estimating yield. There was a chance that the LiDAR could distinguish bunches from leaves or woody tissue. Preliminary work on yield estimation was done in February 2016 on Shiraz, post-veraison, at Nuriootpa, to determine whether or not LiDAR is a suitable tool for yield estimation (Figure 8).

However, with leaves on the canopy, there was no range of remission values that distinguished bunches from other plant material. There was also shading of bunches by leaves, making it impossible to distinguish bunches from leaves.

We suspected that changing the location of the LiDAR on GRover's frame could improve results – for example mounting the sensor 30 cm above the ground and pointing it upwards toward the canopy. However, bunches partly or wholly obscured remained an issue, and erroneous data detected by the sensor caused by high intensity sunlight became an even greater issue.

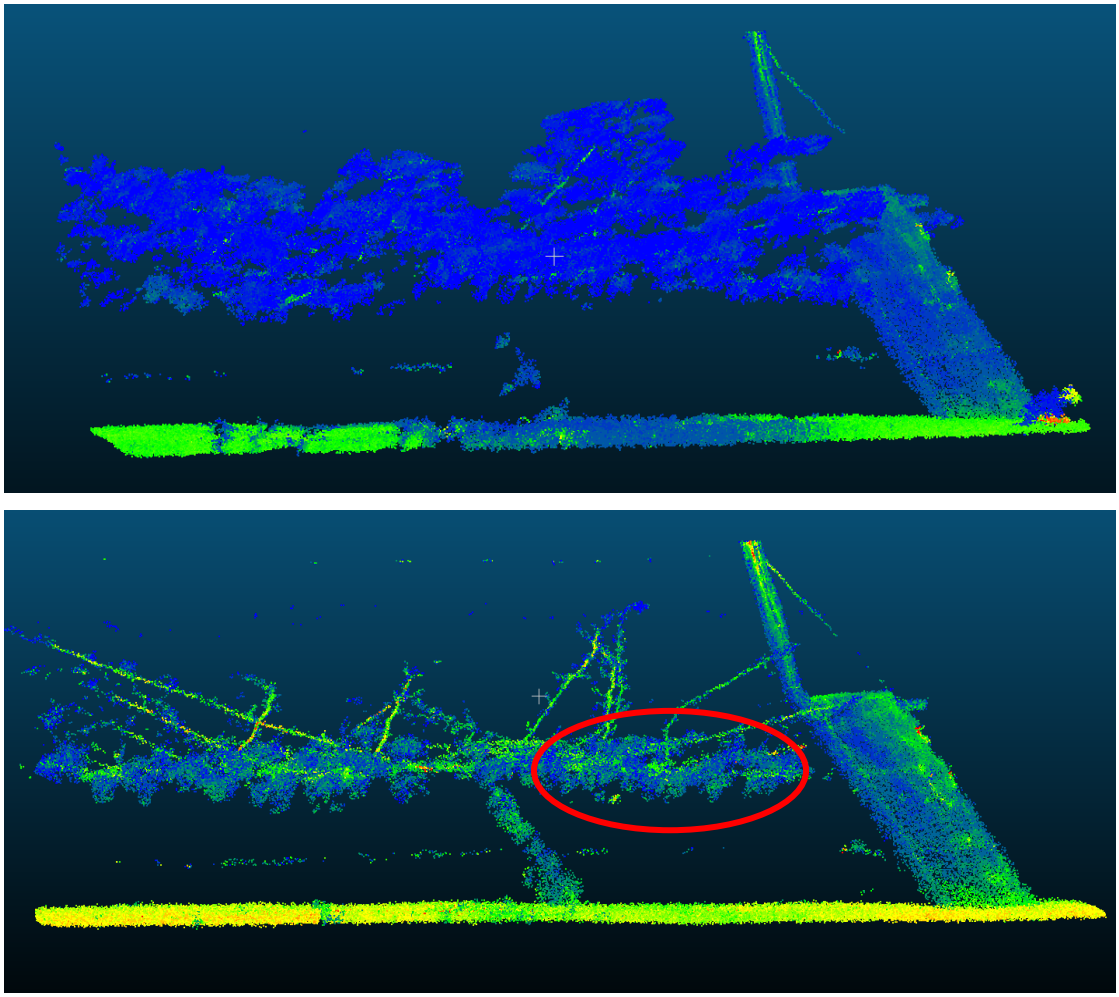


Figure 8. LiDAR scan of the same Shiraz vine at Nuriootpa, SA, before (top) and after leaf removal (bottom). The LiDAR was mounted 2.25 m above the canopy, at a 45^{o} angle. From this angle, the leaf material obscures the identification of bunches (red circle) and the trunk. Additionally, the remission values of bunches overlapped heavily with leaf material and had shapes similar to leaf clusters (bottom).

7. Outcomes and conclusions

- Project performance against planned outputs and performance targets.

The project was aided by collaborations with other groups within CSIRO. While other efforts to produce vehicles capable of taking LiDAR scans have taken 3 years or more to develop, with the help of the HRPPC, GRover was capable of capturing LiDAR data within a short time from delivery. We were able to compare two important measurements of vine growth, leaf area and pruning weight, with different kinds of controlled manipulation at four different locations. The project achieved its original objective, specifically, as a phenomics tool, GRover with our current computational capabilities can produce rapid, accurate, less labour-intensive LiDAR-based measurements of vine growth. There are still ways the methodology could be changed to improve the outcome. For instance, data capture, analysis, and the addition of new instruments could all be improved to consolidate the usefulness of GRover as a potential vineyard management tool.

Within a field site, GRover is capable of making accurate volume-based LiDAR measurements of growth that correlate well with a leaf area or pruning weight measurement. Additionally, the LMS-400 is able to detect treatment differences just as well as destructive measurements. For a plant breeder managing a number of genotypes during a field trial, GRover with its current capabilities would be significantly more efficient than the manual alternatives. Hopefully, applying a different algorithm will eliminate the observed differences between field sites. We were able to rule out two processes in the data processing pipeline as the potential sources of error. First, it was not an issue with data capture. When the LiDAR scans from both sides of the canopy were selected and added together, it did not change the slope of the correlation enough to make the regression statistically similar to any of the other locations (Figure 6). Second, plot selection was likely not the cause of location based differences. A single-blind test to reproduce the number of voxels for a given plant was successful. This suggests that the intensity and nearest neighbour functions used to clean the point cloud are reproducible. A different algorithm other than voxelisation may remove the location bias. Overall, the process of point cloud capture and cleaning was reproducible and GRover could consistently and accurately detect treatment differences within a site.

Although GRover was useful for estimating canopy growth parameters and pruning weight, it was not a useful tool for yield estimation. The problem is simple. One can't measure what the LiDAR can't see. Mounted from below or above the canopy, the LMS-400 was unable to 'see' any bunches, especially in the denser, unmanaged canopies, due to obstruction by leaves. Groups that have had success with yield estimation have been using RGB sensors on highly exposed VSP trained canopies. There are two options: (1) develop a robot that is able to 'look' at the same object from multiple angles, exposing material that may have been obscured during a single

pass. Technology like this has been tested within orchard crops but has not been tested in vineyards; or (2) finding a technology that allows for the perception of bunches but not leaves or woody materials.

This is the first time that a SICK LMS-400 LiDAR was extensively tested in a vineyard. Its high scanning rate, eye safe laser and ability to detect the reflectivity of a surface make it an appealing option for any agricultural application. However, it may be better suited for use in row crops other than vineyards. The LMS-400 is meant for indoor applications. The lux tolerance of the sensor is very low and when the LiDAR is tilted toward the canopy, the scattered light causes dense transects of low intensity, i.e. erroneous measurements (see the upper portion of the scan displayed in Figure 2b). It may be best to explore the use of a different type of LiDAR in the future. It is beneficial to have data from an LMS-400 with a dense point cloud. In future work it should be possible to artificially remove data from the existing point clouds to determine how dense a point cloud needs to be – i.e. what type of LiDAR to use – in order to maintain strong correlations.

- Practical implications of the research results for the Australian grape and wine industry.

  LiDAR measurements, if further developed, can offer growers and plant breeders quantified measurements of traits that otherwise would be difficult to determine. Canopy porosity is an example. Our initial data analysis of point clouds focused on voxelisation, or the mere number of points present in a scan of canopy leaf area. However, additional algorithms could be used to look not only at the number of returns associated with a particular canopy but where those returns occur. For example, there are many ways to structure a canopy with a leaf area index of 3. Despite having the same leaf area, the light penetration profile through the canopy could create a much different micro climate for the maturing bunches. LiDAR could be a powerful tool for growers to measure the effects of leaf-removal, quantify the changes in light availability around the bunch zone and eventually on fruit quality. Further development of point clouds could quantify the exact changes in LiDAR penetration through the canopy into the bunch zone.

- Benefits from the project in terms of economic returns to grape growers or winemakers, improvements in the quality of grapes or wine, environmental benefits or benefits to the broader community.

  Commercialisation of GRover as a management tool is not practical. GRover has fulfilled its intended purpose as a platform on which new tools can be tested. In this instance LiDAR has shown promise as a tool for measuring parameters of vine growth. The strength of relationships between LiDAR scans and vine growth was exceptionally strong in most instances. A compartmentalised LiDAR based product would need streamlining: the frame would need to be reduced, maybe to something

modular that could be attached to a tractor; and the data processing would have to be more automatic and more accessible. For example, canopy volume could be compared on a relative basis, within a vineyard, and after the scan a heat map, overlaid on, for instance Google maps, could be sent to the grower showing the area of higher or lower canopy density. From the results in this project, integration on that scale would be five years off if the only focus was on producing that product.

LiDAR has been used in other studies aimed at reducing environmental impacts, producing variable rate sprayers that could track canopy volume and significantly alter the amount of fungicide applied to a grape canopy. That work, using an LMS-400 or another LiDAR, could be adopted and applied to Australian vineyards.

8. Recommendations

- Future research directions and recommendations for further work

Recommendations centre on the use of a different LiDAR and improvements in data collection. The presence of erroneous points in the LMS-400 scans caused by high light intensity was a major hurdle for the development of any sort of semi-automated data processing. It was easy to determine by eye which points were caused by high light intensity but more difficult to find a computational tool that could reliably identify and remove those points. Consequently, the data analysis pipeline was limited by some of the physical problems associated with the LiDAR. There are limited LiDAR alternatives that provide the accuracy and speed of the LMS-400 used in this project. Moreover, the LMS-400 can detect the reflectivity of a surface, which had the advantage of providing discrimination between leaves and bunches. However, for future developments it will be important to choose one designed for outdoor use. The fast adoption of LiDAR in the car industry for safety and autonomous driving, makes this possibility very feasible for the near future as new hardware will become available for evaluation in agricultural applications.

Second, in terms of data collection and processing, it would be desirable to work toward a method that geocodes the point cloud and analysis into actual map coordinates, instead of one row at a time. This will allow for better analysis of the whole canopy and also allow application at the whole vineyard scale. Also sectioning a more continuous scan would be easier and better suited for automated data processing. There are several ways in which whole-vineyard, or just larger, continuous point clouds could be rendered. In this project, this was limited by the accuracy of the GPS/IMU device which only allowed DGPS corrections (~1m accuracy). A higher end GPS/IMU with RTK capabilities (~2cm accuracy) would allow that precise geocoding but the cost of the updated instrument would be 5-10 fold higher than that of the DGPS version. CSIRO has experts in Simultaneous Localisation and Mapping (SLAM), based on LiDAR, providing future capability to assist with the further development of procedures for data collection and analysis.

This could lead to a more cost-effective solution that is less reliant on GPS/IMU accuracy.

The continued development of GRover's frame and addition of new instruments will be a multidisciplinary effort, and CSIRO offers access to a unique talent pool. Further improvements or work will require substantial collaboration, for example, development of new measurement tools requires engineers, the planning and execution of meaningful field-work requires biologists, the development of software platforms requires programming specialists and the analysis of point clouds or other images requires imaging specialists and statisticians. CSIRO offers a flexible multidisciplinary pool of talent compared with that available at other public or private institutions in Australia.

- Research outcomes in the context of broader industry practices and priorities for further RD&E.

  Currently, there are no industry practices that employ the use of this technology, which is one of the reasons that it is being developed. However, it has scientific and practical implications. Canopy management is a vital component of vineyard management with a significant influence on crop yield, grape composition and final wine quality. This project has demonstrated the potential to capture information on key canopy characteristics non-destructively. LiDAR was able to consistently capture within-site treatment differences for both leaf area and pruning weight. Key findings from this research will be developed further through Wine Australia Project CSA 1601 'New technologies for dynamic canopy and disease management'.

- Priorities for further RD&E

  The work with LiDAR has made it clear that no one tool could be used to determine every meaningful trait of vine growth. There is potential to develop a stereo-imaging technique to produce 3-D information on canopy structure and then to compare this technique with the LiDAR techniques developed in this project (CSP 1307). Once developed, there would be potential to apply these techniques to identify key features that vary between vineyards that provide 'high value' fruit and vineyards that provide 'commodity' fruit. This may lead to management techniques to raise the value of commodity fruit.

Appendix 1: Communication

Workshop – Precision Agriculture Society of South Australia (Riverland, South Australia, 2016). The workshop was attended by over 80 growers and viticulturists. The segment relating to GRover included a scan of a vineyard row and then in the subsequent presentation, we were able to demonstrate to the audience the type of data obtained and to show on a panel-to-panel basis within the vine row, areas that were higher or lower producing. The workshop generated good feedback and demonstrated the potential for application on a larger level with a greater degree of automation. Growers were able to visualise how the tool could be applied in the future to assist Australian wine growers in making vineyard management decisions.

Crush, November 2015 – A presentation titled 'Field-phenomics in vineyards, developing the Grapevine Rover' was delivered.

Waite Snapshot, July 2015 – A presentation titled "Field Phenomics in vineyards: Developing the grapevine rover, a scientific and management tool."

Refereed Journal paper – Siebers, M.H., Edwards, E.J., Jimenez-Berni, J.A., Thomas, M.R., Salim, M. and Walker, R.R. (2018) Fast Phenomics in Vineyards: Development of GRover, the Grapevine Rover, and LiDAR for Assessing Grapevine Traits in the Field. Sensors 18, 2924; doi:10.3390/s18092924.

Industry article - An article titled 'A new scientific tool for Australian Vineyards' was published in Issue 619 (August 2015) of Grapegrower and Winemaker.

Poster – A poster titled 'Application of LiDAR to determine vine growth in the vineyard' was presented at the XVIth Australian Wine Industry Technical Conference, Adelaide, July 2016.

Appendix 2: Intellectual Property

A range of background intellectual property was contributed to the project comprising technology developed by CSIRO and by the High Resolution Plant Phenomics Centre during development of the Phenomobile Lite. This included basic design features from Phenomobile Light and software for data acquisition, processing and analysis.

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Appendix 4: Staff

Dr. Matthew Siebers (Postdoctoral Fellow)

Dr. Rob Walker (Research Scientist)

Dr. Everard Edwards (Research Scientist)

Dr. Mark Thomas (Research Scientist)

Dr. Jose Jimenez-Berni (Research Scientist)

Appendix 5: Outputs and Activities

Outputs and Activities for financial years 1-3.

FY 1	Output	Target Date	Activities
a	Project team	31/12/2014	Position advertised, applicants assessed and appointment made and detailed project planning commenced
b	Prototype in field high-throughput phenotyping equipment	31/01/2015	Preliminary design and initial fabrication of equipment for in field high-throughput phenotyping: a grapevine phenotyping device (GPD)
c	Preliminary data demonstrating the utility of the prototype GPD	30/06/2015	Season 1 evaluation of prototype GPD • At least two field trials selected for evaluation • Canopy size and structure measured by GPD and conventionally at the sites • Initial computational methodology for data capture and analysis evaluated • Data analysed and improvements to

			prototypes and all processes identified

FY 2	Output	Target Date	Activities
a	In field high-throughput phenotyping equipment	31/10/2015	Refined design and fabrication of equipment for in field high-throughput phenotyping based on season 1 data (output 2c)
b	Industry journal publication	31/10/2015	Article prepared and published in Australian and New Zealand Grapegrower and Winemaker
c	Data from season two demonstrating the utility of the prototype GPD	30/06/2016	Season 2 evaluation of GPD • At least three field trials selected for evaluation • Refinement of estimates for canopy size and structure, evaluation of LIDAR for yield and yield component estimates, and assessment of further sensor methodology for an additional trait • Refinement of data analysis method for canopy / structure estimation and further development of computational method for yield estimation • Data analysed and improvements to prototypes and all processes identified

FY 3	Output	Target Date	Activities
a	Data from season three demonstrating the utility of the prototype GPD	31/03/2017	Season three evaluation of GPD • Further refinement of estimates for canopy size and structure, evaluation of LIDAR for yield and yield component estimates, and assessment of further sensor methodology for an additional trait • Further refinement of data

			analysis method for canopy / structure estimation and further development of computational method for yield estimation • Data analysed and improvements to prototypes and all processes identified
b	Final report	22/12/2017	Final report prepared and submitted to Wine Australia

9. Appendix 6: Budget reconciliation

Statement submitted separately online via Wine Australia's Clarity Investment Management System.