Hyperspectral imaging of botrytis in grapes

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INTRODUCTION

Although it may be desirable in certain wine styles, infection of winegrapes with *Botrytis cinerea* generally creates a serious loss of quality and can promote growth of other spoilage organisms. Botrytis tends to be an ongoing problem in cool, wet climates but in adverse weather conditions it can also spread rapidly in regions that normally have a dry climate. An example of a challenging vintage was 2017, when many of the high-production inland areas experienced late summer rains at a time when the grapes were very susceptible to botrytis infection. Most wineries inspect fruit in the field before harvesting, but when the conditions are very conducive to botrytis growth the infection can expand explosively in a matter of days. What may have been a patch that had minimal botrytis infection on inspection could be out of specification when harvested a few days later.

Unlike powdery mildew, which forms a diffuse infection, botrytis infects the whole berry and is easier to assess visually in bunches, particularly with white grapes. However, botrytis can be hard to detect in mechanically harvested grapes unless the infection is severe. Botrytis is also more difficult to detect visually in red grapes. An objective method for botrytis assessment at the weighbridge is therefore required to allow effective screening of fruit on intake. The aim of this project is to evaluate hyperspectral imaging for this purpose, as a rapid method for the real-time analysis of botrytis with minimal requirements for sample preparation.

Visible near-infrared (Vis-NIR) and mid-infrared (MIR) spectroscopy have gained favour in food and agriculture as rapid methods of analysis, including in the wine industry (Dambergs *et al.* 2004, 2015; Cozzolino and Dambergs 2009). It has been demonstrated that Vis-NIR and MIR have potential for botrytis analysis (Hill *et al.* 2013, 2014; Porep *et al*. 2015) but the error is too large to be used at the low infection levels specified by most wineries, particularly considering that the assessment may result in fruit rejection and therefore serious economic loss.

A recent variant of Vis-NIR spectroscopy is hyperspectral imaging (Sun 2010). A normal photographic image is an RGB image; that is, each pixel only contains information from red, green and blue wavelengths (650, 532 and 473nm). A hyperspectral camera collects information from a large number of wavelengths, and this information is stored as a spectrum in each pixel of a digital image. Hyperspectral cameras may collect visible light information (400-700nm) but usually include some NIR wavelengths (700-2500nm). One of the most commonly used wavelength ranges is 400-1000nm. All organic materials have a distinct NIR absorbance fingerprint, based on the vibration of hydrogen attached to carbon, nitrogen and oxygen. The NIR spectral profiles of organic materials can be used to identify and quantify those materials. The spectral information in a hyperspectral image creates a third dimension within the image: X and Y for the spatial component and Z for the spectral information in each pixel; this is what is known as a spectral hypercube.

The spectral information can be used to identify materials and create false colour overlays on the original spatial image, to visualise the distribution of the materials and allow quantification. This technology was initially used mainly with satellites, fixed wing aircraft and more recently with drones, for imaging of agricultural crops and mineral deposits. More recently the technology has also been applied to land-based imaging in the field and in production environments. With the help of relatively recent developments of advanced cameras, software, computer storage and computing power, this technology has become more affordable and accessible and offers great potential for use in agriculture.

Hyperspectral imaging of powdery mildew in white grapes has been demonstrated recently using tandem cameras covering the full Vis-NIR range (400-2500nm) but the 400-1000nm range showed the largest spectral variation related to powdery mildew infection (Knauer *et al*. 2017). A simplified form of imaging using RGB images has been successful in identifying botrytis in grapes and has been developed as a phone app (RotBot). However, RotBot will only work on white grapes and fruit must be photographed on a specific background (Hill *et al*. 2014). The aim of the current project is to develop hyperspectral methods to quantify botrytis in both red and white grapes.

MATERIALS AND METHODS

Botrytis samples

Botrytis-infected samples were collected from the field in the 2017 vintage, and clean bunches were also inoculated with botrytis spores from cultures provided by the PIRSA-SARDI Horticulture Pathology Laboratory. Bunches were sprayed with an aqueous botrytis spore suspension (approximately 10^6 spores/mL). Individual plucked berries were infected by immersing in a spore suspension for 15 minutes or, if on bunches, were infected by injecting berries with a spore suspension. Inoculated grapes were incubated at room temperature in a humidity chamber exposed to diurnal lighting.

Hyperspectral imaging

Hyperspectral images were collected under halogen lighting with a Specim FX10 camera provided by Adept Turnkey (Figure 1). This camera collects images using a wavelength range of 400-1000nm and operates in a line-scan mode; that is, a row of pixels is collected with each frame, so either the sample or the camera must be moved to create the full image. The camera collects many frames for each image and the resolution of the image is defined by the width of the line-scan (1024 pixels) and the length of the linear movement. In this case the samples were moved either on a linear stage or a conveyor belt, and the frames were then stitched together to create an image.

Image analysis

Images were analysed using Scyven software provided by CSIRO's Data61 (Habili and Oorloff 2015). Images imported into Scyven were analysed with a kernel fitting algorithm, support vector machines (SVM), and false colour images were created to identify materials. The Scyven false colour images were analysed with ImageJ Fiji to achieve quantification based on the area of pixels for the various materials identified.

RESULTS AND DISCUSSION

Imaging of botrytis-infected berries

Individual berries offer the simplest system as proof of principle for hyperspectral imaging of botrytis in grapes. Figure 2 shows an RGB image and false colour overlays of material identified using an SVM algorithm. This is an unsupervised method, which means the algorithm decides the spectral profiles that best discriminate various materials in the samples in a 'material discovery' process. Despite this unsupervised approach, uninfected berries were clearly discriminated from infected berries. The grape variety (Merlot or Colombard) was also clearly discriminated. The background material (in this case a polymer conveyor belt) could also be discriminated, but shadows on the background were identified as a different material, illustrating a potential problem with the methodology: lighting is critical to obtaining reproducible spectral signatures.

Quantifying image data

The false colour pixels from the image shown in Figure 2 were extracted from the remainder of the image using Fiji and quantified to determine the relative area of infected and clean berries for the two varieties. As is often the case, the infected berries were smaller, likely due to dehydration (Figure 3). Using multiple software packages makes this a somewhat tedious process, but software is available that can both control the instrument and analyse the images in real time, based on training data.

Imaging whole bunches

Whole bunches present more of a challenge with hyperspectral imaging,

Figure 1. Specim FX10 Vis-NIR hyperspectral camera with linear stage.

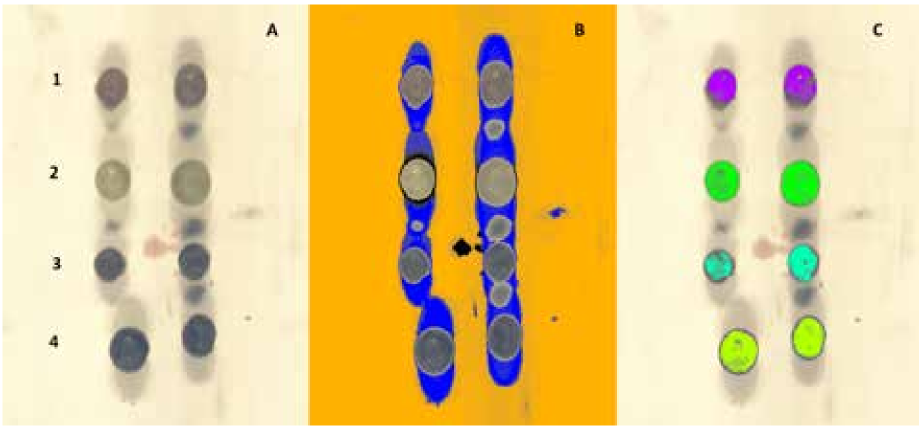

Figure 2. RGB image (A) of infected (1) and non-infected (2) Colombard berries and infected (3) and non-infected (4) Merlot berries. False colour overlay (B) of hyperspectral identification of the background material (orange) and shadows (blue). False colour overlay (C) showing hyperspectral discrimination of the grapes by variety and infection status.

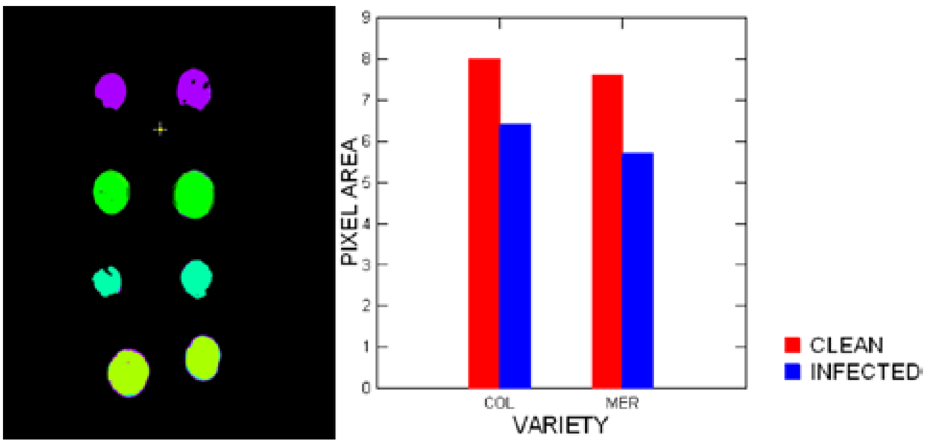

Figure 3. False colour components extracted from the image shown in Figure 2 and material quantified based on pixel counts.

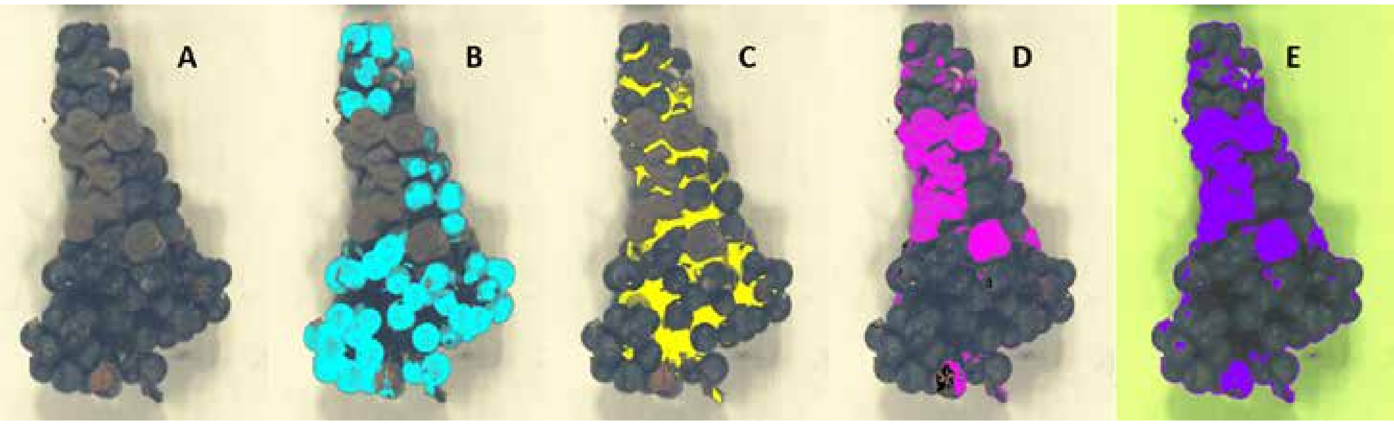

Figure 4. RGB image of a Botrytis-infected Merlot bunch (A); false colour overlay of hyperspectral analysis (400-1000nm) showing uninfected berries (B), shadowed internal spaces (C) and infected berries (D). False colour overlay of multispectral analysis (three wavelengths) highlighting infected berries (E).

as a greater depth of field is required and shadowing becomes more of an issue. This is illustrated in Figure 4. Uninfected berries (image B) were clearly discriminated from infected berries (image D) but the shadowed internal spaces were identified as different material (C). Bunch scanning also presents problems in that internal infected berries will be obscured, as with classical visual inspection of intact bunches. From this aspect, mechanically harvested grapes may be more effectively imaged as samples can be spread out in a single layer under ideal lighting conditions; however, collecting a representative sample from a mechanically harvested load is a critical issue. An important consideration with lighting is the wavelength profile of the light source, which ideally should be evenly spread over the range of the imaging detector (Sun 2010). For example, LED light sources tend to have distinctly defined wavelength peaks and are generally not suitable. In the case of the detector used in this study, a halogen light source is ideal, but has the disadvantage that it generates a lot of heat, so care must be taken to avoid overheating the samples.

Multispectral imaging

Creating hyperspectral images over large wavelength ranges has only become technically feasible with the advent of modern computing power and data storage capacity. The image files generated are very large and require computers with fast processors, a large amount of RAM and a large amount of fast access data storage capacity. The full spectral data are required in the exploratory phase of a spectral imaging project, but from these data a smaller number of wavelengths can be selected to achieve the desired result. This is commonly known as multispectral imaging. Fewer wavelengths allow a more cost-effective solution for both the camera and computer, and allow faster processing to enable a real-time solution. An example of multispectral imaging is shown in Figure 4. Using spectral weightings from image analysis over a 400-1000nm wavelength range, three wavelengths were chosen to achieve a result similar to that from the full wavelength range (Figure 4E). In this case the multispectral method performed slightly better at identifying small patches of infected berries and was not affected by shadows in the darker internal parts of the bunch, so may be less affected by lighting conditions.

CONCLUSION

This work demonstrates proof of principle for hyperspectral imaging of botrytis in grapes. Unlike other more common spectroscopic methods, hyperspectral imaging is sensitive enough to detect very low levels of botrytis as the infected berries are not diluted out by the signal of the bulk of the sample. Also, any prediction information can be readily confirmed as the spatial image is retained with the data. The next step in this project is to develop methods that are sufficiently robust to be applied in a production environment, with real-time analysis. The development of more cost-effective multispectral systems is likely to bring this technology within reach of more wine producers, particularly if a simple turn-key system can be developed.

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