Hyperspectral imaging and multivariate data analysis as a sensitive determinant of cut flower age

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I. INTRODUCTION

The vase life of cut flowers is an important parameter influencing the value of individual species or cultivars. This vase life is affected by growing, packing and transport conditions and yet the subsequent effects of such treatments cannot be quantified. The variation in vase life can cause difficulties for wholesalers and retailers when a guaranteed vase life is offered - a practice common in the EU and a growing trend in the US [1]. However, the only vase life testing possible for the retailer occurs concurrent with that by the consumer and failure to achieve the guaranteed vase life causes consumer complaints and discourages further purchases. A non-invasive diagnostic test that can establish the quality of cut flowers and their potential vase life is needed.

During development and senescence there are numerous changes in physiology and gene expression [2], any one of which might form the basis of a test to distinguish the developmental stage of the tissue. However, such assessments are likely to be relatively time consuming, technically demanding and expensive. To be practical, tests employed need to be cheap, rapid and simple, the most appropriate being visual inspection, however, it is often impossible to distinguish visual differences between isolated petals of differing ages and thus impossible to predict just how much longer a flower may last if there are no morphological differences associated with the inflorescence. Even if differences are apparent to the naked eye, visual assessment is inevitably subjective and difficult to standardize. Developing an imaging technique that can distinguish different stages of petal development is the first step in providing the floriculture industry with a useful diagnostic tool.

II MATERIALS & METHODS

Alstroemeria peruviana cv Samora stems were harvested and transported upright in water. Various developmental stages [3] were then imaged. Stages were **1:** outer sepals fully pigmented (about 2 d before flower opening, day-2), **2:** flowers fully open with sepals reflexed (day 0) **3:** the top three anthers dehisced (day +2), **4:** the bottom three anthers dehisced (day +4), **5:** separation of the stigmatic lobes (day +6), **6:** the petals showing discoloration and inrolling (day +8). **7:** abscission of the perianth (day +10) (Fig. 1).

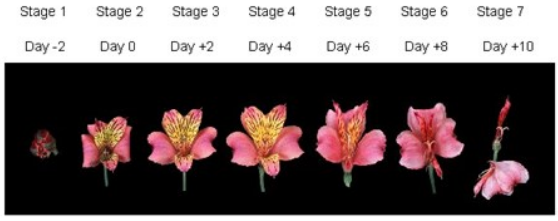

Fig. 1. Floral senescence was divided into 7 stages from closed but to tepal abscission

Isolated upper petals were mounted onto matt black sugar paper using spray-mount glue. Fibre optic lighting was used, and a panel of Spectralon (Labsphere Ltd., Poynton, Cheshire UK) was used to determine light intensity of the fibre optic lighting and for correction of reflectance spectra prior to data analysis. Hyperspectral images were collected using a SenSys 1401E CCD camera (Roper Scientific Inc., Tucson, AZ 85706, USA) with a CRI VariSpec liquid crystal tunable filter (Cambridge Research and Instrumentation, Woburn, MA 01801, USA) fitted with a hot mirror that reflected over 99% of radiation above 800nm. The camera and filter were controlled by MetaMorph software (Universal Imaging Corporation, Downingtown, PA 19355, USA). Images (12 bit, 830 x 550 pixels) were collected at 1 nm intervals between 500 and 700 nm using an exposure of 50 ms.

Data pre-processing and analysis was with MatLab software (The Mathworks, Natick, Massachusetts, USA) with the addition of the Image Processing Toolbox. Image areas representing petals were extracted from each montage image; an extraction routine based on fitting three closely spaced contours to the spectral

image at grey scale values between the darker areas of the petal edge and the background and then selecting the contour best representing the edge of the petal was used. Because other small areas of the petal had similar contours the longest contour was automatically selected and used to produce a mask. The mask was then applied to the hyperspectral data set for each petal and the mean and standard deviation of petal reflectance per pixel was calculated at each of the 201 wavelengths used. Principal Components Analysis (PCA) was used to interpret the reflectance spectra across the whole wavelength range. The mean or the mean and standard deviation of the reflectance of each petal at each wavelength were used as inputs to a PCA. The plots of the major principal components coded by developmental stage were then examined to see if they separated petal developmental stages.

III RESULTS & DISCUSSION

Hyperspectral imaging of petals at various developmental stages yielded extensive data sets. Although four biological replicates were used the number of individual spectra for each biological sample exceeded 150,000. Each development stage was therefore represented by approximately 600,000 spectra, with a total dataset of over 4 million spectra. By summarizing the vast number of spectra using the mean reflectance and the standard deviation of that reflectance, it was possible to discriminate between the various floral developmental stages (for example Fig. 2).

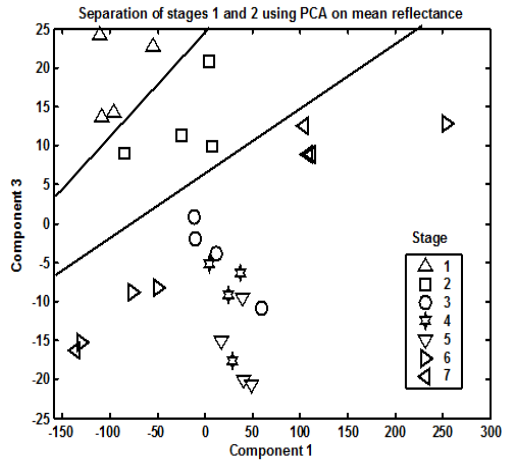

Fig. 2. Principal components analysis applied to the mean reflectance data alone was sufficient to give good discrimination between flower stages 1, 2, and the later stages. Stages 3, 4 and 5 also formed a distinct cluster separate from stages 6 and 7.

Use of the mean distinguished the early developmental stages from the later stages and using the mean and SD distinguished the older stages from the younger stages, thus indicating the potential of these imaging routines to distinguish the various stages of floral development and senescence in this variety. We were also able to discriminate between similar stages of flowers that had been given different post-harvest treatments (data not shown).

IV CONCLUSIONS

Our results suggest that a more extensive project could establish simple, non-destructive methods that could detect flowers that may have been mistreated (and thus likely to have a reduced vase life). Such a tool could rapidly become established as the only viable method available to detect poor quality flowers with a potentially reduced vase life. Of course these data relate to just one variety of *Alstroemeria*; to be successful algorithms suited to all varieties of a given species would be required and tests need to be carried out using the same algorithms throughout the year to ensure that they are robust enough to work year-round and with different growers.

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