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Determining the harvest maturity of vanilla beans

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ABSTRACT

Current methods for determining the maturity of vanilla (*Vanilla planifolia* Andrews) beans are unreliable. Yellowing at the blossom end, the current index, occurs before beans accumulate maximum glucovanillin concentrations. Beans left on the vine until they turn brown have higher glucovanillin concentrations but may split and have low quality. To find a better index, changes in bean dimensions, dry matter (DM) and glucovanillin accumulation were followed over four seasons. Beans reached their maximum length and width before glucovanillin and DM accumulation began, and there was no clear point when their weight ceased to increase. Beans harvested when the blossom end turned yellow had lower glucovanillin concentrations than beans harvested later; glucovanillin accumulation continued until beans started to turn brown on the vine. Therefore, bean colour, dimensions, weights and glucovanillin content are not useful indicators of maturity, and the lack of visual changes until beans senesce explains why commercially cured beans vary in quality. DM accumulation reached a maximum before browning and was highly correlated with glucovanillin content; the central portion was the most representative of the entire bean. Therefore, optimum harvest time occurs when DM accumulation slows and should be measured in the central portion of beans. Two near infrared spectrometers using interactance geometry were trialled for non-invasive assessment of DM. Cross validation r and RMSECV values of 0.87 and 1.76, respectively, for a unit using wavelengths between 1100 and 2300 nm, and 0.82 and 1.05 for a portable unit using wavelengths between 800 and 1050 nm were obtained from the second derivative of absorbance spectra. The latter unit allows infield monitoring of maturation.

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1. Introduction

Vanilla is an expensive and popular flavouring used by food, pharmaceutical and cosmetic industries and has considerable potential as a food preservative. The annual world production of cured beans was estimated to be approximately 9000 T in 2011 (FAO, 2011). Natural, cured vanilla is a complex product, and its distinctive flavour and aroma comes mainly from the phenolic compound, vanillin, and other aromatic compounds that comprise less than 2% by weight of fresh vanilla beans. Glucovanillin is the most important storage form of vanillin; it is odourless, and mature, green pods do not generate their characteristic flavour or aroma until they are cured during which time glucovanillin is converted to vanillin.

If vanilla beans are left on the vine, they turn yellow, and then brown and eventually split in two (dehisce) from the blossom end. Overripe beans may also split during curing (Purseglove et al., 1981), which results in loss of aroma, requiring them to be sold at lower prices than whole beans. In practice, because flowering and fruit set are protracted, many beans are harvested immature resulting in a large variation in vanillin and other aroma compounds among individual cured beans (Van Dyk et al., 2010). Research by Van Dyk et al. (2010) showed that growing conditions (water supply and weather) and the physiological age of the beans at harvest are important factors affecting the quality of cured vanilla beans. However, the concentration of vanillin in the cured beans was highly variable, suggesting that levels of this phenolic are not suitable for estimating harvest maturity.

The appearance of developing vanilla beans remains unchanged for many months, and they are not considered commercially mature until 8–9 months after pollination. One of the traditional indicators of commercial maturity is the change in the colour of the blossom end of the bean from green to yellow. Sagrero-Nieves and Schwartz (1988) investigated the influence of harvest time on the concentrations of the major phenolic constituents of *V. planifolia* and found that phenolics increased as the beans matured; however, no correlation was observed between the colour of the beans and their glucovanillin content. These findings agree with earlier

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studies by Jones and Vicente (1949) and Arana (1944). Thus, colour changes do not appear to be a good indicator of harvest maturity, and a better indicator is required.

Near infrared spectroscopy (NIRS; involving the spectral range 750–2500 nm) is suited to the assessment of water and other constituents in plant material, due either to a strong spectral signature from the constituent of interest, or through a secondary relationship of the constituent of interest to another that can be assessed using NIRS. Lower cost, silicon detector-based NIRS instrumentation operating in the short wave near infrared region (750–1050 nm) has found application in agriculture. For example, Walsh et al. (2004) report the use of NIRS for assessment of dry matter (DM) content of several fruit types. In addition, Subedi et al. (2007) demonstrated the use of NIRS for on-tree measurement of the dry matter content of mango fruit and the prediction of total soluble solids after ripening. The typical root mean square error of prediction (RMSEP) reported in these studies was around 1% DM. Consequently, there may be potential for a NIRS-based measurement to be used for the judgement of vanilla bean maturity and, consequently, the production of high quality vanilla beans. This measurement would be especially valuable if it could be made in the field. The objective of this study was, therefore, to test the preceding hypothesis.

2. Materials and methods

2.1. Plant materials

Experiments following the pattern of accumulation of glucovanillin and dry matter during growth and development of the beans were conducted over four seasons.

Experiment 1 Queensland (QLD) 2009 and Experiment 2 QLD 2010: Flowers (300–350) of *Vanilla planifolia* Andrews (Asparagales: Orchidaceae) were hand-pollinated and tagged on 1st November, 2008 (Expt. 1) or 28th October, 2009 (Expt. 2) at the plantation of Daintree Vanilla and Spice, Daintree, Queensland. Beans were collected at 14 different times from 15 to 40 weeks after pollination. The beans ($n = 6$) were sent by mail to the Postharvest Laboratory, UWS, stored at 4 °C and analysed within four days of harvest.

Experiment 3 University of Western Sydney (UWS), Richmond, New South Wales 2011: About 1200 flowers were hand-pollinated and tagged on vines grown in a controlled environment greenhouse (18–28 °C, ~60% RH) during September, October and November, 2010. A sample of three beans from each pollination month was harvested at 14 different times after pollination (from 15 to 40 weeks). The beans were labelled and sent to the Central Queensland University (CQU), Rockhampton, Queensland for spectral acquisition using Fourier-transform NIRS (FTNIRS). Spectra were recorded on marked sections at the stem end, middle and blossom end. The beans were returned to UWS following which the marked sections were divided in two and used for dry weight and glucovanillin determinations.

Experiment 4 QLD 2012: Flowers were hand-pollinated in September and October, 2011 at the plantation of Daintree Vanilla and Spice. Beans were collected at three different times from 15th May 2012 to 10th July 2012. Thirty beans were harvested each time and sent to the Postharvest Laboratory, UWS. The beans were weighed, labelled and spectra were recorded on marked sections at the stem end, middle and blossom end using a hand-held NIR spectrometer (Nirvana). The beans were stored at 4 °C in sealed, plastic containers pending analysis. The marked sections were divided in two and used for dry weight and glucovanillin determinations. The time from harvesting to destructive analysis of the vanilla beans at UWS was about seven days.

2.2. Measurement of dry matter content

In Experiments 1 and 2, whole vanilla beans were cut into 20 mm pieces and dried to a constant weight in a microwave oven at full power (Panasonic, 1100 W, Australia). For Experiments 3 and 4, one half of the three sections of beans from each harvest time were used to determine dry matter using the same method.

2.3. Measurement of glucovanillin concentrations

In Experiments 1 and 2, beans were cut into pieces (~20 mm long), frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. In Experiments 3 and 4, after determining their NIR spectra (Section 2.4), the remaining half of each section of the beans was frozen and ground. Before analysis, the powder was allowed to warm to room temperature. A known weight of powdered plant tissue (~200 mg) was suspended in 10 mL distilled water, 0.5 mL of 18 M sulphuric acid was added and the powdered tissue was thoroughly mixed; the mixture was then placed in a steam bath at 60 °C for 3 h to hydrolyse the glycosides. The mixture was removed from the bath, cooled to room temperature and 2 mL of 9 M KOH was added to neutralise the mixture and to raise the solution pH to 6–7. The mixture was then transferred to a separating funnel, and 10 mL of n-pentane and dichloromethane (1:1, v/v) was added to extract the vanillin. (All reagents used were HPLC grade (Sigma–Aldrich, Australia)). The mixture was shaken, the upper organic phase was recovered, and the remaining aqueous layer was extracted three more times with 10 mL of n-pentane and dichloromethane (1:1, v/v). The combined organic phases were dried over anhydrous sodium sulphate and filtered through fibres (polyamide: polymer, 6:6, Imperial Chemical Industries Fibres Ltd UK). The filtrate was adjusted to 50 mL in a volumetric flask with n-pentane and dichloromethane (1:1, v/v). The aqueous phase was discarded.

High performance liquid chromatography (HPLC) was used to measure vanillin concentrations in the hydrolysed extracts from green beans. The HPLC system consisted of a solvent delivery system (Varian 9012) equipped with a 20 μL sample loop and a variable wavelength UV–visible detector (Varian 9050). Chromatographic separation was carried out using a 250 mm × 4.60 mm Gemini 5U C18 110A column (Phenomenex, Australia). Data were collected using a Star chromatography workstation version 6 (Varian, USA), linked to the chromatograph with a Varian Star 800 Module Interface Box. Separations were carried out at ambient temperature (~22 °C). The mobile phase consisted of 10% H_2O, 10% acetonitrile and 80% methanol. Samples were filtered through a 0.45 μm filter (Minisart) prior to injection; the injection volume was 20 μL. The separation was performed under isocratic conditions at a constant column flow rate of 1.0 mL min^{-1}. Peak absorbance was measured at 271 nm. The concentrations of extracted vanillin in the beans were calculated by a standard addition analysis produced by the addition of pure vanillin at three different quantities to the extracts of vanilla beans. Five mL aliquots of a vanilla bean extract were pipetted into four 10 mL volumetric flasks. Aliquots (0.0, 1.0, 2.0, 3.0 mL) of a standard vanillin solution (121 μg L^{-1} vanillin (Aldrich-Chemical Company, Australia) dissolved in 1:1 (v/v) n-pentane and dichloromethane) were pipetted into the 10 mL volumetric flasks and made to volume with 1:1 (v/v) n-pentane and dichloromethane. Standard addition curves were obtained ($r^2 = 0.998$), all samples were analysed in duplicate.

2.4. Spectral analysis

Concentrations of glucovanillin in water (0.0%, 0.3%, 0.6%, 1.3%, 2.5% and 5.0% (w/v)) were placed in a 2 mm transmission cell and the absorbance over 800–2300 nm was measured using a Nicholet

Anatares FTNIR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA) equipped with a transmission cell (at a resolution of 80 mm^{-1} and with averaging of 64 spectra per sample). Interactance spectra of intact beans at harvest were acquired from fruit from Experiments 3 and 4. In Experiment 3, spectra were recorded over the range of 800–2500 nm from the marked sections on each bean using the FTNIR unit referred to above, equipped with an interactance probe. The probe was placed in contact with the beans on the marked locations and duplicate spectra were acquired at a resolution of 80 mm^{-1} using 64 replicate scans per spectrum. In Experiment 4, spectra were recorded at harvest on the marked sections of beans using a hand-held, shortwave, near infrared spectrometer (Nirvana, Integrated Spectronics, Australia). The instrument takes an internal reference reading on every sample and automatically adjusts integration times for the samples to ensure (silicon photodiode) detector peak signal at 80% of saturation to maintain a high signal to noise ratio on all samples. However, while the optical front end of the detector assembly has a diameter of seven mm and is fitted with a lens ahead of the fibre optic, such that only parallel light emergent from the bean would be 'seen' by the detector, the parabolic lamp assembly has a diameter of 35 mm, wider than the bean. Consequently some light may have reached the detector directly from the source. For this reason, an accessory was designed for the optical front end of the instrument consisting of a disc with a slot 10 mm wide. The instrument was placed with the slot orientated in the long dimension of the bean, thus minimising direct entry of extraneous light into the spectrometer.

Spectral data were correlated with the dry matter content (determined gravimetrically) and total vanillin content (measured by HPLC) using partial least squares (PLS) regression, employing The UnScrambler (Camo) chemometric software. Unless otherwise stated, the software suggested the number of PLS factors (principle components) to be used, and cross validation was undertaken using a 20 member random group approach. Regression results are reported in terms of the correlation coefficient of the cross validation and the root mean square of the errors of cross-validation (RMSECV).

3. Results

3.1. Growth, total vanillin concentration and dry matter content during maturation

Experiment 1, 2009 (data not shown): The harvest weight of the beans was highly variable during the assessment period (20–35 weeks after pollination), with bean weights of varying from 10 to 15 g. However, during weeks 20–35, the dry matter percentage increased linearly ($P < 0.0001$, $r^2 = 0.98$). Total vanillin also increased linearly ($P < 0.0001$, $r^2 = 0.94$) and was highly correlated with the increase in dry matter percentage ($P < 0.0001$, $r^2 = 0.99$). No yellowing or browning of the beans was observed.

Experiment 2, QLD 2010: In this experiment, observations were conducted over a longer period (weeks 15–41 after pollination) than in 2009. During this time, the harvest weight of the beans was again highly variable with weights being from 10 to 16 g (Fig. 1A). However, bean dry matter increased from 10% to 18% during this period and dry matter was highly correlated with harvest time ($P < 0.0001$, $r^2 = 0.90$). Vanillin (% fresh weight (FW)) began to increase in beans from Week 15 after pollination and reached 1.5% (FW) by Week 40 (Fig. 1B); the increase in total vanillin could be modelled by an exponential equation. There was a strong, linear correlation between dry matter percentage and total vanillin during the assessment period ($P < 0.001$, $r^2 = 0.912$) (Fig. 2). Beans not used for the assessment of maturity turned brown after 45 weeks.

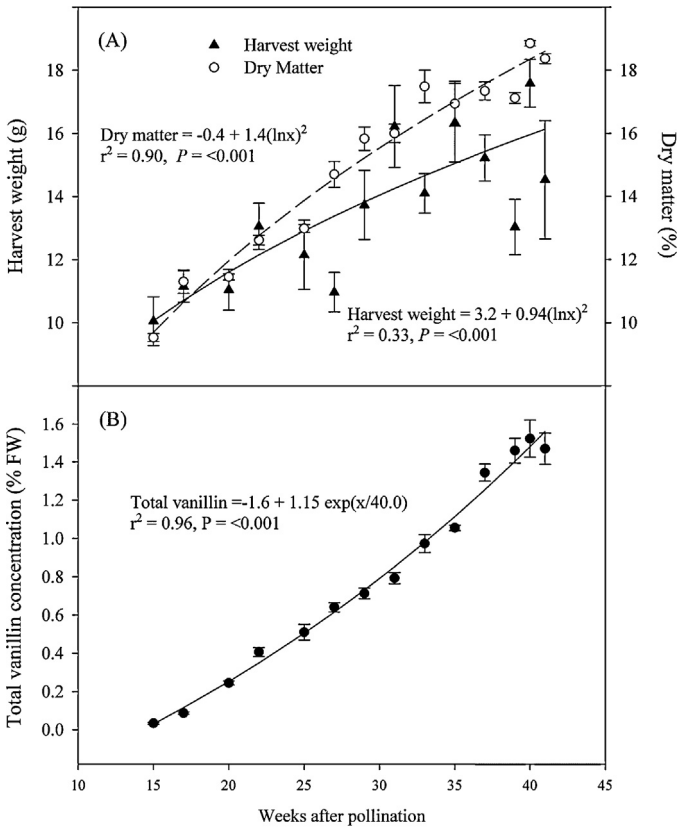

Fig. 1. Experiment 2, QLD 2010: (A) Mean bean fresh weight and dry matter as a function of time after pollination. (B) Mean total vanillin concentration extracted in pentane and dichloromethane from green beans as a function of time after pollination. Total vanillin concentrations are expressed on the basis of the fresh weight of the beans. The bars represent standard errors of the means ($n = 6$).

Experiment 3, UWS 2011: In Experiment 3, the growth of hand-pollinated beans was assessed at 11 different times after pollination in three populations of beans pollinated in the months of September, October and November. There was little difference in either the final dimensions of the beans or in the duration of growth among the beans from the three populations (Fig. 3). The beans from the populations reached a maximum length of ~180–190 mm, and longitudinal growth ceased in Week 10. With respect to width, all

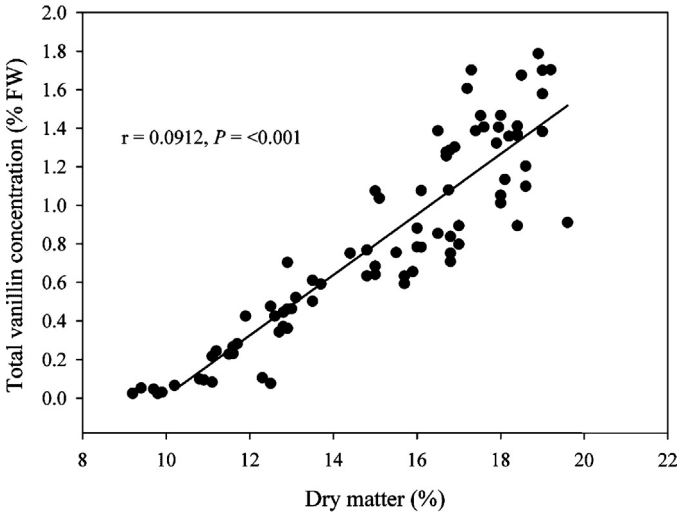

Fig. 2. Experiment 2, QLD 2010: Correlation between total vanillin concentrations and dry matter during bean development.

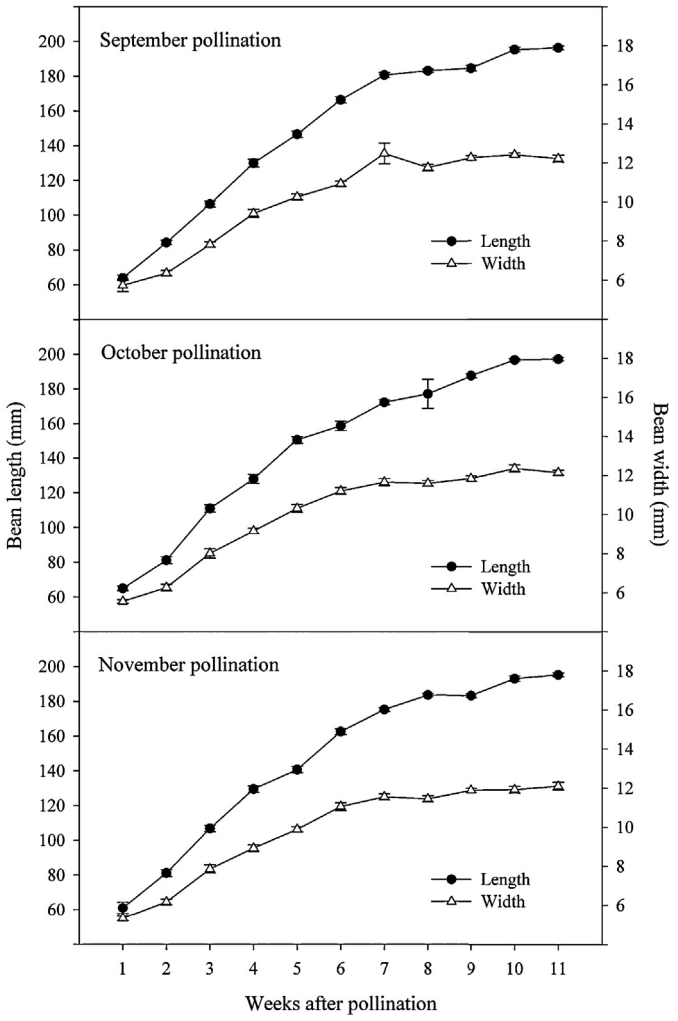

Fig. 3. Experiment 3, 2011: The length and width of developing beans from three pollination periods, grown in a controlled environment greenhouse at UWS (18–28 °C, ~60% RH). The bars represent standard errors of the means ($n = 20$).

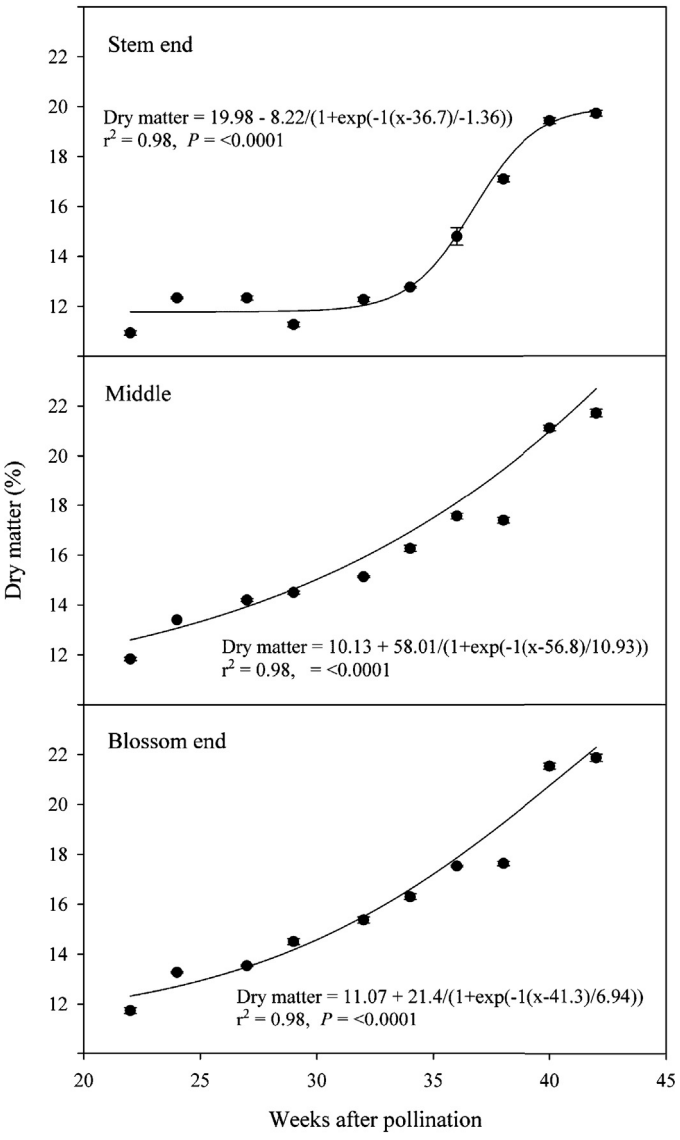

Fig. 4. Experiment 3, UWS 2011: Dry matter content in the stem end, middle and blossom end each of beans from the October pollination. The bars represent standard errors of the means ($n = 3$).

beans reached a maximum diameter of ~12 mm, and radial growth ceased in Week 7. The beans from all pollinations weighed 10–15 g at maturity. Accumulation of dry matter began after 30 weeks. The harvest weight of the beans pollinated in each month during this experiment was variable, but the average weight of beans in each population was similar (September, 13.22 ± 0.74; October, 13.61 ± 0.63; November, 13.02 ± 0.81 g (mean weights ± standard error of the mean, $n = 9$)). The blossom end of the beans from all pollinations started to turn yellow after 38 weeks; beans left on the vine for over 43 weeks turned brown. The beans from both this experiment and Experiment 2 started to turn yellow at the blossom end several weeks before they had reached their maximum dry matter content and long before maximum concentrations of glucovanillin were attained.

The dry matter content of the stem end, middle and blossom end of beans pollinated in October is shown in Fig. 4. The patterns were similar for beans pollinated in September and November (data not shown), and dry matter increase in the beans of each population could be modelled by sigmoidal equations. In the September and October populations, dry matter accumulation was clearly slowing by Week 40 at the stem end of the beans. However, in the November population, only the increase of dry matter percentage in the blossom end of the beans was slowing by this time, and dry matter was still accumulating in the other two sections until the end of the assessment period (data not shown). The total vanillin concentrations in the three sections of beans from the October population increased at an exponential rate during the assessment period (Fig. 5). Similar patterns of total vanillin increase were recorded for all of the bean sections from the September and November populations (data not shown), and vanillin was lower in section from the stem end in all populations of beans. Total vanillin concentrations and dry matter percentage were highly correlated. The highest correlations were recorded for the middle sections of the beans for all three populations (Table 1).

Table 1
Coefficients of determination (r^2) between total vanillin concentrations and dry matter from three different sections of green beans during development during Experiment 3 ($P < 0.0001$ in all cases).

Section	Pollination time		
	September	October	November
Stem end	0.83	0.90	0.84
Middle	0.90	0.98	0.92
Blossom end	0.84	0.96	0.91

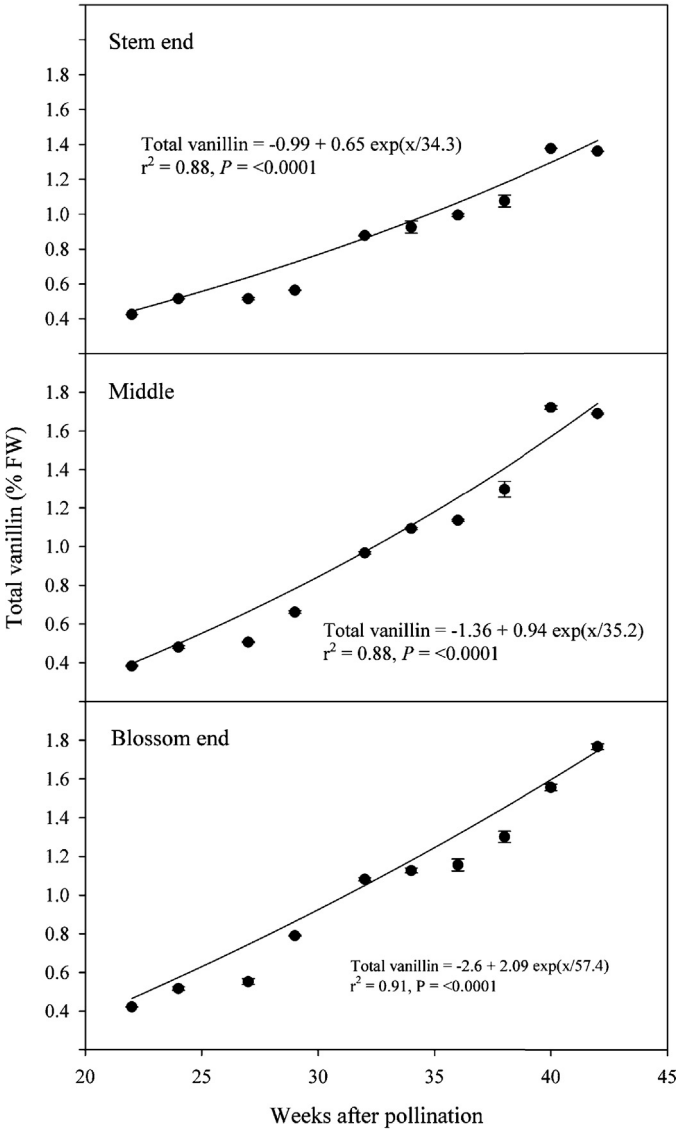

Fig. 5. Experiment 3, UWS 2011: Mean total vanillin concentrations in three sections of green beans pollinated in October as a function of time after pollination. The vanillin was extracted with pentane and dichloromethane. The blossom ends of the beans started to turn yellow 38 weeks after pollination. The bars represent standard errors of the means ($n = 3$).

Experiment 4, Qld 2012: Beans were harvested at 29, 33 and 36 weeks after pollination. The data confirmed that both dry matter and total vanillin increased during maturation and that the highest percentages were found in sections from the middle and blossom ends of the beans. There were strong correlations between dry matter and total vanillin content, between dry matter and bean age and between total vanillin and bean age (Table 2). The strongest correlation was between dry matter and total vanillin content.

Table 2
Correlation coefficients (r) for the relationships among bean age, % dry matter and total vanillin concentrations.

Factors	r
Age vs. total vanillin (% FW)	0.85
Age vs. dry matter (%)	0.90
Total vanillin (% FW) vs. dry matter (%)	0.91

Table 3
FTNIRS of pure glucovanillin solutions: PLSR calibration statistics based on absorbance and second derivative of absorbance spectra (930–2300 nm) of different concentrations of pure glucovanillin (0.0%, 0.3%, 0.6%, 1.3%, 2.5% and 5.0% w/v; mean = 1.62%, SD = 1.79% (w/v)). SD = standard deviation; RMSECV = root-mean-square error of cross-validation; SDR = standard deviation ratio (SD/RMSECV); #f = number of PLS factors; R = regression coefficient of cross validation; slope = slope of the regression line. No spectra were removed from the regression exercise as outliers.

Spectrum	#f	R	RMSECV (% w/v)	SDR	Slope
ABS	3	0.99	0.23	7.8	0.95
d2ABS	4	0.99	0.24	7.4	0.94

3.2. Near infrared spectroscopy

3.2.1. Transmission FTNIR spectroscopy of pure glucovanillin in solution

Pure, aqueous solutions of glucovanillin possessed major absorbance features at around 970, 1400 and 2200 nm due to water (O–H stretching), with no visually apparent contribution to the absorbance spectrum of glucovanillin at the concentrations employed (Fig. 6). Difference spectra (difference to pure water) revealed major features around 1000, 1600 and 2400 nm, and minor features around 2100 nm. A partial least squares regression (PLSR) 'searching window' algorithm (Guthrie et al., 2005) was used, trialling all combinations of start and stop wavelengths over the available wavelength range (800–2500 nm), with the best results obtained around the region 930–2300 nm (data not shown). The PLSR model for this wavelength range, based on either absorbance or second derivative of absorbance data, possessed an r of 0.99 and an SD/RMSECV of >7 (Table 3), indicating that this model could be used to sort this range of solutions into approximately seven groups. Results for absorbance and derivative data were equivalent, as expected, given the assumption that the Beer–Lambert Law holds for the transmission geometry used.

3.2.2. Interactance FTNIR spectroscopy of vanilla beans

FTNIR interactance spectra of intact green beans (from Experiment 3) were again dominated by features related to water (around 960, 1200, 1400 and 1900 nm), with a baseline shift obvious in the absorbance spectra with increasing wavelength that was removed by use of the second derivative (Fig. 7). A plot of the first two principal component factors of a principal component analysis using the full absorbance spectrum did not reveal any extreme outliers or groupings within the sample set (data not shown). Thus, samples of the various harvests behaved as members of a single population. On this basis, a single PLS regression model was developed.

A PLSR algorithm (Guthrie et al., 2005) was again used, trialling all combinations of start and stop wavelengths over the available wavelength range (800–2500 nm), with the best results obtained around a region with wavelengths between 1100 and 2300 nm (data not shown). There was a significant correlation between both absorbance and second derivative of absorbance spectra with dry matter, total vanillin content and bean age; however, the strengths of these relationships varied (Table 4). The correlation between pod age and spectrum was the strongest (Rcv = 0.91) and that between glucovanillin content and spectrum was the weakest. The PLSR models were slightly stronger based on second derivative spectra, compared to raw absorbance. Use of the reduced wavelength range 800–1050 nm (suited for use with low cost Si photodiode technology) resulted in only a minor decrease in calibration performance (RMSECV increase from 1.76% to 1.86% DM).

3.2.3. Interactance vis-SWNIR spectroscopy of vanilla beans

Visible-short wave (400–1100 nm) NIR interactance spectra of intact green beans (acquired using the Nirvana unit) were

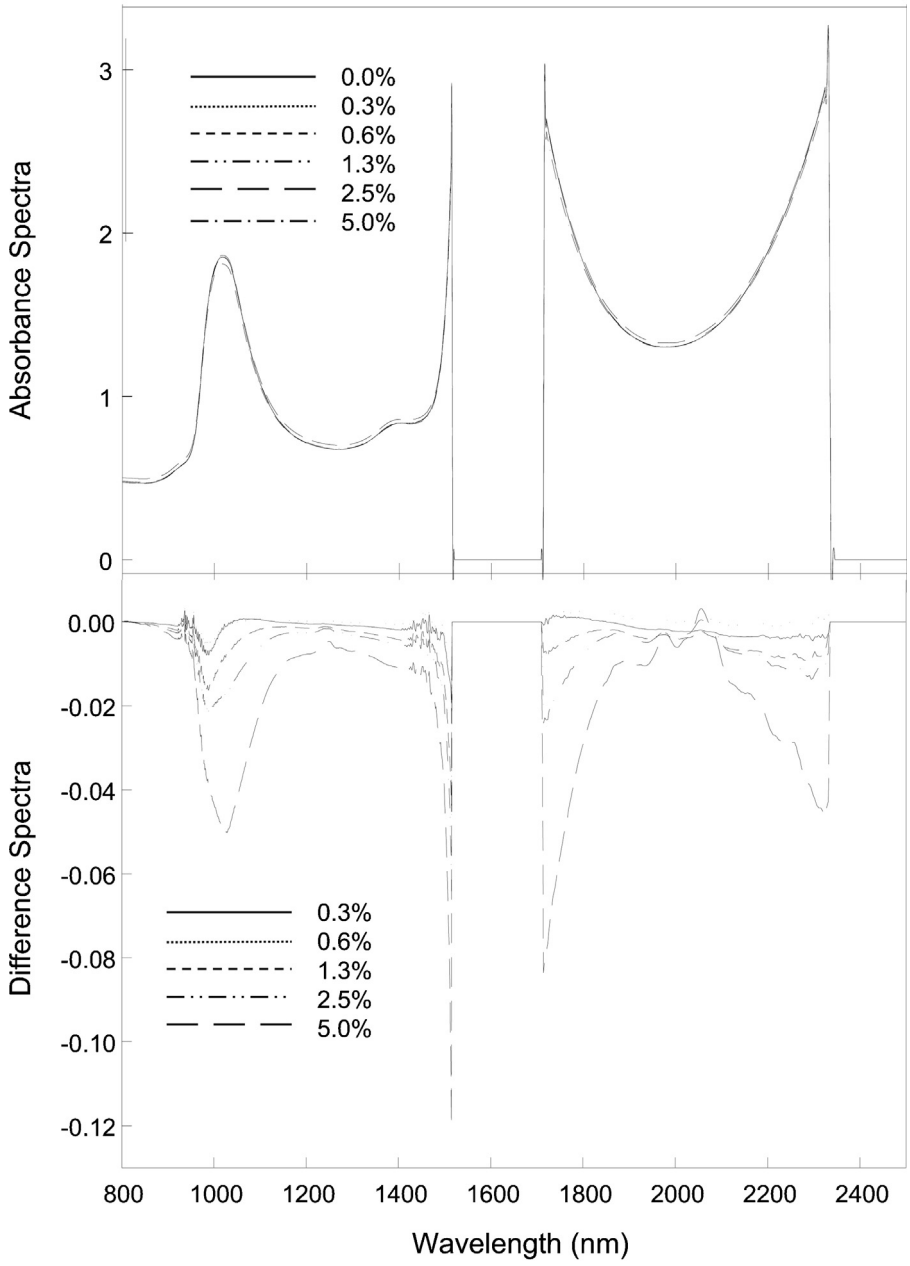

Fig. 6. Absorbance (top panel) and difference (to water) (bottom) spectra of aqueous solutions of pure glucovanillin (concentration range 0–5.0% w/v), acquired using a 2 mm pathlength cell. The spectra have been cut to excise 'saturated' regions of strong absorbance.

dominated by features related to pigmentation (e.g., chlorophyll) in the visible wavelength region and with features associated with water (around 740, 960 nm), again with a baseline shift between spectra that was removed by use of the second derivative (Fig. 8).

The plot of the first two principal component factors of a principal component analysis using the full absorbance spectrum did not reveal any extreme outliers or groupings within the sample set (data not shown). Thus, samples of beans from the last two harvests

Table 4
FTNIRS PLSR statistics for models between bean age, % dry matter and total vanillin concentrations with absorbance and second derivative of absorbance spectra of green vanilla beans ($n = 540$, from nine harvest events) using the specified spectral regions. Abbreviations as per Table 3. The upper panel is based on FTNIR spectra, the lower on Nirvana spectra.

Particular	Data	Wavelength range (nm)	Mean	SD	Number of outliers	#f	R	RMSECV	SDR	Slope
Age	ABS	1100–2300	34.14	7.72	7	15	0.90	3.38	2.3	0.82
Age	d2ABS	1100–2300	34.14	7.72	6	12	0.90	3.208	2.4	0.83
%GVAN	ABS	1100–2300	0.66	0.50	8	13	0.79	0.30	1.6	0.66
%GVAN	d2ABS	1100–2300	0.66	0.50	6	12	0.80	0.28	1.7	0.65
%DM	ABS	1100–2300	16.39	3.62	7	13	0.86	1.82	2.0	0.76
%DM	d2ABS	1100–2300	16.39	3.62	6	13	0.87	1.76	2.1	0.79
%DM	d2ABS	800–1050	16.39	3.62	23	4	0.85	1.86	1.9	0.72

Table 5
Nirvana PLSR statistics for models between % dry matter and the second derivative of absorbance spectra (727–975 nm) for two populations of green vanilla beans and prediction statistics for population 2 (Experiment 4), predicted by a model generated on population 1 (Experiment 3). RMSEP, root mean square errors of prediction; Rp, regression coefficients of prediction; other abbreviations as per Table 3.

Calibration population	Predicted Population	Mean	SD	Number of outliers	#f	Rp	RMSEP	SDR	Slope
1	–	15.6	1.7	4	4	0.82	1.05	1.05	0.68
2	–	16.4	1.7	5	5	0.79	1.00	1.00	0.55
1	2	–	–	1	4	0.64	1.26	1.26	0.67

(Experiments 3 and 4) behaved as members of a single population, and data were used to develop a single PLSR model per population (Table 5). Using a model developed on population 1, the DM in beans of population 2 was predicted with an RMSECV = 1.26% DM.

4. Discussion

This study has followed the phenology of vanilla beans as they ripen and the concomitant changes in dry matter and total vanillin content over four seasons in beans obtained from a commercial plantation and from a planting in a controlled environment greenhouse. The critical events in the development and maturation of beans observed in the greenhouse (Experiment 3) are summarised in Table 6. This experiment enabled more detailed observations during growth and development than was possible at the field plantation.

Beans from all three pollinations in Experiment 3 reached their maximum length and width between seven and ten weeks after pollination, long before vanillin and dry matter accumulation began and long before they were mature. In Experiment 3 and in our other experiments, the harvest weights of the beans were variable,

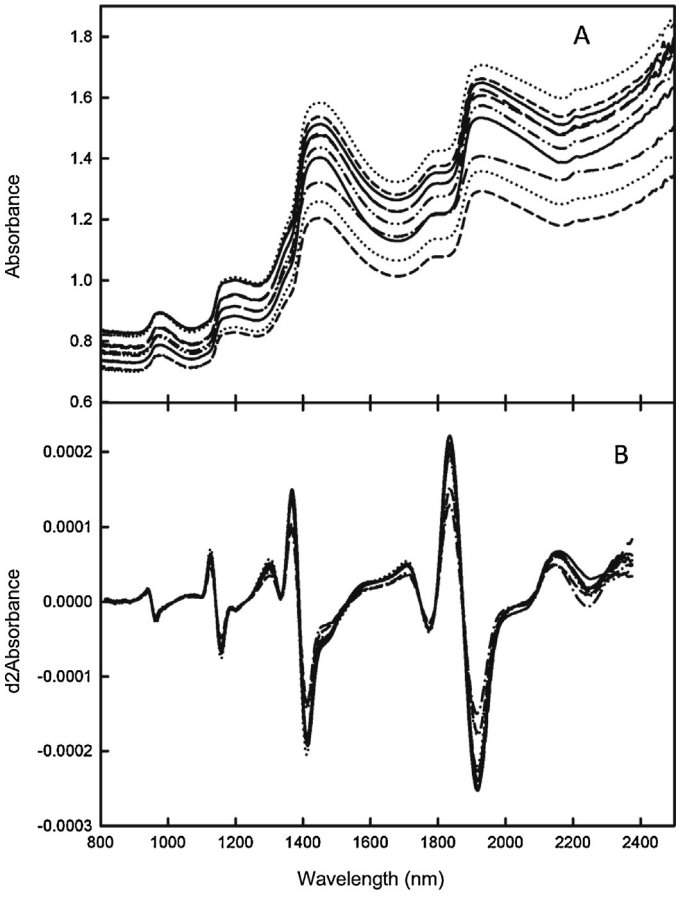

Fig. 7. FTNIR absorbance (A) and second derivative of absorbance (B) spectra of green vanilla beans.

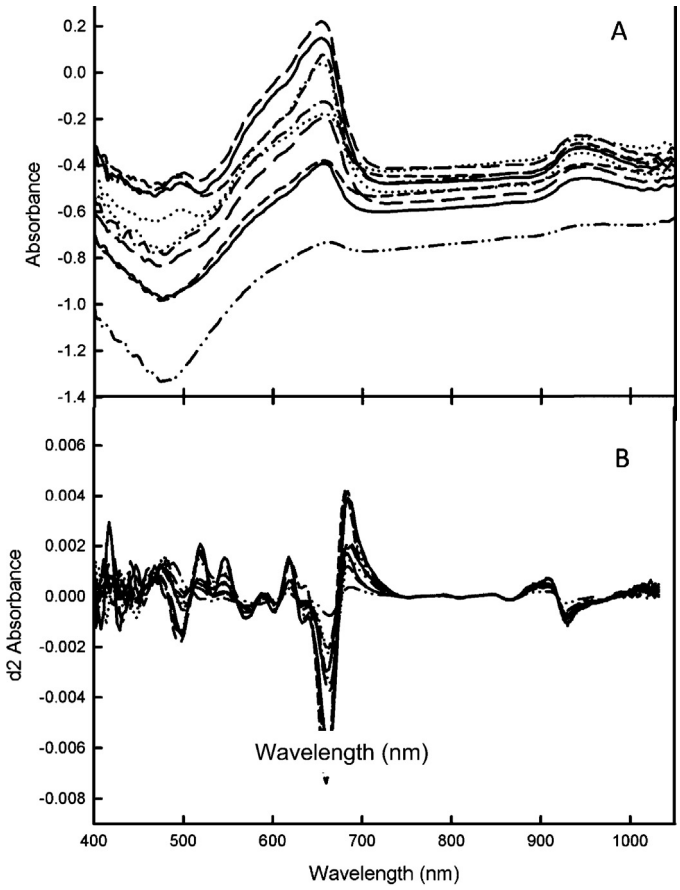

Fig. 8. SWNIR 'Nirvana' absorbance (A) and second derivative of absorbance (B) spectra of green vanilla beans.

and there was no easily determined point in their development when their weight ceased to increase or had started to decrease. Beans harvested when the blossom end started to turn yellow were not fully mature and had much lower concentrations (stem end, 0.417%; middle, 0.563%; blossom end, 0.683%) of glucovanillin than beans harvested later (Fig. 5). In Experiment 3, beans left on the vine for 43 weeks started to turn brown and, thus, were over mature. Our results are similar to those of Gregory et al. (1967), Brodelius (1994) and Havkin-Frenkel et al. (1999) who found that developing

Table 6
Time line of events associated with beans development and maturity found from Experiment 3 (Figs. 4 and 5).

Weeks after pollination	Event
7	Maximum width
10	Maximum length
20	Glucovanillin content begins to increase
30	% Dry matter begins to increase
38	Blossom end of beans start to turn yellow
40	Maximum dry matter and glucovanillin
43	Beans start to turn brown on the vine

vanilla beans reach their full size 10–15 weeks after pollination and show few changes in appearance until long after they begin to accumulate glucovanillin. The lack of visual changes in appearance until the beans started to senesce in part explains why commercially cured beans vary widely in quality (as measured by vanillin content), since growers are tempted to harvest beans while they are still immature. Also, as the crop is asynchronous, growers cannot distinguish the age of fruit until pod colour changes. In the experiments of this study, glucovanillin, measured as total vanillin, was detected in the developing beans 15–20 weeks after pollination, and accumulation continued until the beans started to senesce and turn brown on the vines. Similar observations of this pattern of glucovanillin accumulation were reported by Ranadive (1994), Tokoro et al. (1990), Kanisawa et al. (1994) and Palama et al. (2009). Therefore, as noted in Section 1, bean colour, dimensions and weights, and glucovanillin content are not useful indicators of maturity.

In our experiments, dry matter accumulation reached a maximum before browning and, in Experiment 3, reached a maximum first in the stem ends of the beans, followed closely by the middle sections and then in the blossom ends. In addition, total vanillin and dry matter were unequally distributed along the length of green beans. The stem end contained less dry matter (19%) 40 weeks after pollination than the middle or blossom ends. The concentrations of total vanillin along the length of the beans also varied from 1.1% (FM) at the stem end to a maximum of 1.7% (FM) toward the blossom end. Arana (1943) also measured the glucovanillin concentrations in the stem, middle and blossom ends and found that the proportions of total vanillin in these three regions was 20:40:40; Brillouet et al. (2010) found the proportions to be 17:43:40. In our study, the distribution of total vanillin in the three regions was 26:40:34. Consequently, the central portion was the most representative of the entire bean, and the correlation between dry matter and vanillin concentration was greatest for this portion of the bean. Therefore, optimum harvest time appears to be the point after pollination when DM accumulation slows and near maximum levels are reached and should be measured in the central portion of beans.

The use of NIRS for estimation of bean maturity was assessed in terms of its ability to estimate glucovanillin content, age and DM of intact beans. The development of an application for NIRS requires optimisation of the wavelength range, optical geometry and the spectral processing method. FTNIRS spectral data of pure solutions of glucovanillin supported an excellent regression model (RMSECV 0.23% (w/v)) at concentrations relevant to those in vanilla beans, giving confidence that glucovanillin can be detected directly by this technique. This is expected as the O–H and C–H bonds within this molecule will produce stretching absorption overtone features. This result suggests that NIRS has potential as a technology for in-field determination of bean glucovanillin content.

The interactance spectra of the vanilla beans showed a baseline shift between the spectra for individual beans. The use of a second derivative is a well-documented technique for removal of baseline shifts and offsets and, as expected, PLSR analyses were improved by use of this pre-processing technique. In practice, however, the glucovanillin content of the beans (0–1.7% FW) was strongly correlated to bean DM (12–20%) and to pod age. The *b* coefficients for PLSR models on DM and glucovanillin content were similar indicating that the glucovanillin models represent a secondary correlation of glucovanillin on DM content. A similar interpretation exists for the model on bean age. The *b* coefficient values were weighted around 910 nm and 950–970 nm, areas associated with the third overtone of C–H stretching, and O–H stretching, respectively (data not shown). Therefore, NIR spectroscopy can be used to assess harvest maturity using dry matter as a surrogate for glucovanillin.

Given the shape of the beans, the location of the tissue contributing to glucovanillin and % DM within the bean, it is logical to use a transmission or interactance optical geometry over reflectance geometry; for in-field purposes, an interactance geometry is preferred. The interactance geometries used with the FTNIRS and Nirvana units should both result in a similar effective path length of light through the tissue to approximately 15 mm depth. The front end 'slot' accessory designed for the Nirvana was successful in limiting the illuminated area to the bean.

Restricting the wavelength window of the FTNIRS spectra from 1100–2300 to the 800–1050 nm window relevant to a silicon photodiode based detector system such as the handheld Nirvana unit resulted in a marginal decrease in model quality (from *R* 0.86 to 0.85, RMSECV 1.76% to 1.86% DM). Indeed, a poorer result is expected as the FTNIR spectrum was not optimised for this region (low absorbance values). An *R* of 0.88, RMSECV 1.05% DM was achieved using the Nirvana unit (727–975 nm). Further direct comparison with the FTNIR results is compromised by the use of different populations with different SD, 3.62% and 1.7% DM for FTNIR and Nirvana sets). Nonetheless, these data indicate that a handheld instrument such as the Nirvana can be used for measurements in the 800–1050 nm region. It is, however, recommended that the robustness of the model be tested across further populations of beans.

Thus, it is feasible to monitor changes in dry matter during development of vanilla beans *in situ*, enabling a time of harvest decision at optimum maturity. A single measurement at the middle of each bean gives the best estimate of dry matter and total vanillin that is representative of the whole bean. However, since pollination is asynchronous, the whole crop will include beans at different stages of maturity. Therefore, it is suggested that sample beans should be tagged at weekly intervals during flowering and that dry matter should be monitored from 35 weeks after pollination as a guide to optimum harvest maturity.

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