THE BASIS OF VARIATION IN THE SIZE AND COMPOSITION OF GRAPE BERRIES

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

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May 2002

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STATEMENT OF AUTHORSHIP

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John D. Gray.
May, 2002.

ACKNOWLEDGEMENTS

The Australian Research Council, the Grape and Wine Research and Development Corporation and Southcorp Wines Pty Ltd are gratefully acknowledged for their financial support. I thank the following: Dr Peter Kolesik for sharing his microscopy skills; Prof. Ian Gibbins and Dr Grant Hennig from the Department of Anatomy and Histology, the Flinders University of South Australia, for access to the SGI Indigo 2 computer and 3D software; Dr Marilyn Henderson from the Centre for Electron Microscopy and Microstructural Analysis, the University of Adelaide, for assistance with the TEM analysis; Prue Henschke (C.A. Henschke and Co.) and John Harvey (Willunga), for providing the grape material; Dr Nick White from the Department of Plant Sciences, the University of Oxford, for commenting on a draft of part of the manuscript (Chapter 4); the Department of Horticulture, Viticulture and Oenology, its administration, staff and students, for providing a studentship, laboratory resources, and a stimulating work environment; Dr Bryan Coombe, Dr Peter Dry, and Prof. Peter Høj for their skilled supervision. I would like to make special mention of Dr Bryan Coombe for his enthusiasm, insight, and tutelage. Lastly, I thank my family. Without their patience, encouragement, and support this task would have been insurmountable.

ABSTRACT

The objective of this study was to explore the basis of variation in the size and composition of grape berries. The investigation focussed on selected aspects of berry development and ripening that were subject to variation. Shiraz and Chardonnay were chosen as experimental varieties because these cultivars presented a large range of variability in the field – Shiraz is susceptible to variation in colour development at veraison, whereas Chardonnay often displays variation in berry size at harvest. The extent of variation within each of the recorded berry parameters was assessed using the coefficient of variation (CV), a unitless measure of sample variability relative to the sample mean, ideally suited to comparative studies.

Chapter 1 is a literature review that documents research on selected aspects of grape berry development and ripening which are subject to variation. Berry development is explained in terms of berry set, berry growth and ovule/seed development. Berry composition is described by the relative concentration of sugars, acids, phenolics and flavour compounds in the berry tissues. Variation is discussed with respect to the Australian wine industry and the problem of supply and demand. Techniques for identifying and measuring components of variation are recommended. Experimental hypotheses are developed.

Chapter 2 describes an experiment designed to identify when variation in berry size and composition was initiated. The hypothesis was that relative levels of variation in size and composition would remain constant throughout the postflowering period of berry development. The physical properties of individual Shiraz berries were described in terms of their deformability, mass, volume, surface area and seed mass. The phenolic composition of these same individual berries was assessed. A comparison of CVs between sequential developmental stages indicated when variation in a particular physicochemical parameter was initiated. The CV in berry deformability reached a maximum at softening. The CV for berry mass was above 50% at berry set, but declined as the berries approached harvest maturity. The CVs for berry volume and berry surface area followed a similar trend. Interactions among these parameters were described by linear regressions, multiple regressions and correlation matrices. Seed mass and berry phenolics were analysed for individual berries during the growing, softening and preharvest stages. Both of these parameters were significantly correlated with berry mass, but the relationships were peculiar to each developmental stage. The CV for seed mass increased with maturity. The CV for berry phenolics was lowest during the softening stage. For most parameters, CVs had already attained high levels during the earliest growth stage (setting). The implication is that variation must have arisen at an even earlier time. This places considerable importance on the impact

that preflowering events may have on cell division in the floral primordia at budburst.

Chapter 3 describes an experiment that sought to identify the extent of variation present during the early developmental stages of berry growth. The hypothesis was that variation in berry size was already significant in the early postflowering period of berry development. Individual Chardonnay berries on two bunches from both ungirdled and girdled vines were assessed on four occasions throughout the flowering period. Individual flowers that had opened during the intervening time period were tagged. One bunch from each vine was sampled at 15 days and another at 43 days after the first flower had opened, giving a range of berry ages: bunch at 15 days comprised berry ages of 1-4, 5-7, 8-11 and 12-15 days; bunch at 43 days comprised berry ages of 29-32, 33-35, 36-39 and 40-43 days. Frequency distributions of berry mass were plotted for each age class for ungirdled and girdled vines. Distributions were negatively skewed for ungirdled vines and positively skewed for girdled vines. No "shot" berries were observed among bunches sampled from girdled vines at 43 days after flowering. Absolute and relative growth rates were typically higher for berries from girdled vines. The relationship between berry mass and seed mass was unaffected by trunk girdling. CVs for berry mass at all ages in the early bunch sample (15 days) were below 44%, and were generally lower for the girdled vines. In the later bunch sample (43 days), CVs in berry mass were all higher than those associated with the early bunch sample, and two- to three-fold lower for the girdled vines. This reduction is most likely the result of increased organic nutrition to the bunch counteracting the variation that arises from differences in hormonal stimulus to growth by the developing seed.

Chapter 4 describes a novel technique that was developed for the *in situ* measurement of cell shape and cell size using confocal laser scanning microscopy (CLSM). The technique encompassed the preparation, clearing, staining and whole mounting of large sections of berry tissue. Optical slices were collected at 1 μm intervals to a depth of 150 μm. The digital images were empirically corrected for attenuation of fluorescence intensity and axial distortion due to refractive index mismatch. Cell size and shape were determined from digital 3D reconstructions of the collected image stack. Cell volumes exhibited a 15-fold range with polysigmoidal distribution and groupings around specific size classes. The volume of individual, whole parenchyma cells within a block of grape berry mesocarp tissue could be measured *in situ* to a precision of 2 μm^3.

Chapter 5 describes an experiment that sought to resolve the relationship between fruit size and cell size in a developing grape berry. The hypothesis was that variation in cell size occurs early in the postflowering period of berry development and that this variation was responsible for the subsequent macroscopic size differences observed between berries.

Chardonnay berries belonging to the eight age classes derived in Chapter 3 were sampled from ungirdled and girdled vines. Three berries from each age class were analysed under the CLSM – one “shot”, one “chick” and one “hen”. Volumes were calculated for ten cells from the inner mesocarp tissue of each berry. Differences in cell volume were observed between berry types. Larger berries often had a larger range of cell sizes, indicating they had undergone more cell expansion during the course of their development, but the distribution of cell sizes in “shot” and “chick” berries was similar. Girdling did not affect the berry cell size distribution, but the CVs of girdled vines were ~50% higher. Cell volume for “chicks and “hens” increased between day 15 and day 43, although the increase was proportionally greater for “hens”. Variation in cell size remains relatively constant during the early postflowering development, but variation in berry mass increase regardless. This indicates the operation of different metabolic controls over these two facets of berry growth.

Chapter 6 is a general discussion and an attempt to synthesise the salient points from the preceding experimental chapters. In light of the experimental results, it addresses the validity of each of the original hypotheses on variation in berry size and composition. Concepts of developmental synchronisation among berries are viewed from the perspective of changing levels of variation between developmental stages. Increases in CVs signify a loss of synchronisation (asynchronisation) among berries. Reductions in CVs signify a gain in synchronisation (resynchronisation) among berries. The general conclusion is that averages of measurements of the population reveal nothing about the variation within that population. Calculation of variance permits this, but requires that all individuals be measured. Cell size is only one component of the berry size equation. Cell number is the other. While cell size is determined by cell expansion, cell number is linked to cell division. Future applications of techniques developed in this thesis could resolve the interplay between cell division versus cell expansion. A better understanding of these processes might ultimately minimise the impact of variability on the quantity and quality of the Australian winegrape crop.

ABBREVIATIONS

3D	three-dimensional
ABA	abscisic acid
ACC	1-amino-2-ethylaminocyclopropanol carboxylic acid
AGR	absolute growth rate
ANOVA	analysis of variance
CCD	charge-coupled device
CLSM	confocal laser scanning microscopy
CV	coefficient of variation
E-L	Eichhorn-Lorenz
FPA_{50}	formalin:propionic acid:50% ethanol 1:1:18
GA	gibberellin
GMA	glycol methacrylate
IAA	indole-3-acetic acid
MW	molecular weight
n	sample number
r^2	coefficient of determination
REML	residual mean likelihood
RGR	relative growth rate
RI	refractive index
s	standard deviation
SE	standard error
TCA	tricarboxylic acid
TEM	transmission electron microscopy
V	volume
$\bar{x}$	sample mean
ε	molar extinction coefficient
λ	wavelength
σ	sample variance