



**Effects of pre-flowering conditions of temperature and light on flower
and berry development in model grapevines**

by

Ali Ebadi

*(M. Sc. Horticulture)
University of Tehran, Tehran, IRAN*

*Department of Horticulture, Viticulture and Oenology
Waite Agriculture Research Institute
The University of Adelaide
South Australia*

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60413

To my family

TABLE OF CONTENTS

Summary	i
Declaration	v
Acknowledgements	vi
List of Tables	vii
List of Figures	ix
CHAPTER 1: Introduction	1
CHAPTER 2: Literature review	
2.1 The reproductive organs of the grapevine	4
2.1.1 The inflorescence	4
<i>Inflorescence initiation</i>	4
<i>Inflorescence structure</i>	5
2.1.2 The flowers	6
2.1.3 Organogenesis of flower parts	7
2.1.3.1 The androecium	8
2.1.2.2 The gynoecium	10
<i>Normal development of the ovule</i>	11
<i>Abnormal development of the ovule</i>	13
2.1.4 Flowering	14
2.1.4.1 Anthesis	14
<i>Opening of the calyptra</i>	14
<i>Opening of the anthers</i>	15
<i>Stigma receptivity</i>	15

2.1.4.2	The mode of pollination	16
2.1.4.3	Pollen germination and pollen tube growth	17
2.1.4.4	Temperature effects on pollen activity and pistil receptivity	18
2.1.4.5	Pollen tube inhibition	19
2.1.4.6	The process of fertilisation	19
2.1.5	Post-flowering development of the ovule	20
2.1.5.1	Functional seeds	20
	<i>Nucellus</i>	20
	<i>Integuments</i>	20
	<i>Endosperm</i>	21
	<i>Embryo</i>	22
2.1.5.2	Non-functional seeds	23
	<i>Stenospermy</i>	23
	<i>Empty-seededness</i>	24
2.1.6	Berry development	24
2.1.6.1	Berry development in relation to seed development	25
2.2	Fruit-set	26
2.2.1	Effects of environmental factors	27
2.2.1.1	Temperature	27
2.2.1.2	Light	28
2.2.1.3	Water stress	29
2.2.1.4	Mineral nutrition	30
2.2.2.	Vine growth and fruit-set	32
2.2.2.1.	Interactions with vegetative growth	32
2.2.2.2.	Plant growth regulators	32
	<i>Growth promoters</i>	33
	<i>Growth retardants</i>	36

2.3. Model experimental plants	39
CHAPTER 3: General materials and methods	
3.1 Controlled environment	41
<i>Growth rooms</i>	41
<i>Growth cabinets</i>	41
3.2 Producing small fruiting plants	42
3.2.1 Water and nutrient supply	43
3.2.2 Timing of watering	44
3.3 Microscopic examination	44
<i>Light microscopy</i>	44
<i>Experiments</i>	45
3.4 Statistical methods	46
CHAPTER 4: Effect of short-term temperature and shading treatments on fruit-set and berry number in <i>Vitis vinifera</i>, cvs Chardonnay and Shiraz	
4.1 Introduction	52
4.2 Materials and methods	53
<i>Experiment 1</i>	53
<i>Experiment 2</i>	53
4.3 Results	55
<i>Experiment 1</i>	55
<i>Flower numbers and time of flowering</i>	55
<i>Fruit-set and berries per bunch</i>	57
<i>Experiment 2</i>	60
<i>Flower numbers</i>	60

<i>Fruit-set and berries per bunch</i>	60
4.4 Discussion	61
4.5 Conclusions	64
CHAPTER 5: Ultrastructure of the flowers of grapevine cultivars	
 Chardonnay and Shiraz	
5.1 Introduction	65
5.2 Materials and methods	65
5.3 Results	66
<i>Pistil</i>	66
<i>Pollen grain</i>	66
5.4 Discussion	67
5.5 Conclusions	74
CHAPTER 6: Effect of short-term exposure to lowered temperature on	
 pollen germination and pollen tube growth in grapevines of <i>Vitis</i>	
 <i>vinifera</i>, cvs Chardonnay and Shiraz	
6.1 Introduction	75
6.2 Materials and methods	75
<i>Evaluation of culture media for in vitro pollination tests</i>	75
<i>Germination of pollen from vines grown at different temperatures</i>	77
<i>Pollen germination on stigma, and pollen tube growth in the pistil</i>	78
6.3 Results	79
<i>Effect of temperature treatments of vines on pollen germination</i>	79
<i>Pollen germination on stigma and pollen tube growth in pistil</i>	79
6.4 Discussion	82
6.5 Conclusions	85

**CHAPTER 7: Effect of low temperature near anthesis on ovule
development in *Vitis vinifera* L., cvs Chardonnay and Shiraz**

7.1 Introduction	86
7.2 Materials and Methods	86
7.3 Results	87
<i>Measurements of ovule volume</i>	87
7.4 Discussion	93
7.5 Conclusions	96

CHAPTER 8: Seed development and abortion in cv. Chardonnay

8.1 Introduction	97
<i>Terminology</i>	99
8.2 Material and Methods	99
8.3 Results	101
8.4 Discussion	111
8.5 Conclusion	113

**CHAPTER 9: Effect of variations in temperature regimes and shading
on seed and berry development of *Vitis vinifera***

9.1 Introduction	115
9.2 Materials and methods	115
9.3 Results	116
<i>Seed complement per berry</i>	116
<i>Per cent one-seeded berries</i>	116
<i>Seed number per berry</i>	120
<i>Seed weight</i>	123
<i>Pericarp weight per berry</i>	126

<i>Relationship between seed content and berry development</i>	126
9.4 Discussion	129
9.5 Conclusions	133
CHAPTER 10: General discussion	
<i>Pollen and pollination (Chs 4 to 6)</i>	135
<i>Ovule abnormalities at flowering (Ch 7)</i>	137
<i>Seed development abnormalities (Ch 8)</i>	138
<i>Relationship of seed development to berry set and berry growth (Chs 4, 9)</i>	139
<i>The timing of low temperature treatments</i>	140
<i>The difference between cultivars</i>	141
References	143
Appendix	168
Publications	177

Effects of pre-flowering conditions of temperature and light on flower and berry development in model grapevines

Summary

Successful grape varieties produce regular crops of saleable products. Regularity of fruit production results from suitable combinations of three components of reproductive growth: (a) number of flowers per m² land surface (numbers of inflorescences and flowers per inflorescence), (b) percentage of fruits set per 100 flowers (degree of fruit setting), and (c) the harvest weight of berries that set. In temperate regions, these successive events cover a total period of about 16 months; the approximate duration of each is 12, 0.4 and 4 months. Each of these phases is subject to variable development, particularly the first two. Degree of fruit-set fluctuates between seasons, for example in such *Vitis vinifera* varieties as Chardonnay, Merlot, Muscat Gordo Blanco and Grenache. Years of poor setting ('coulure') are usually attributed to adverse weather conditions during the flowering period, especially to low temperatures. Temperature may affect fruit-set in grapevines through its effect on the development of the flowers up to flower opening and through its effect on pollination, be it on the germination of the pollen and the growth of the pollen tube, or on the differentiation and post-fertilisation growth of the ovule. These aspects were the subject of this research.

Small container-grown Chardonnay and Shiraz vines were held under controlled conditions at 25°/20°C day/night temperatures but transferred for one week to 17°/14°C or 12°/9°C at four stages of growth between budburst and flowering. In Chardonnay, half the vines were given supplementary pollination with pollen produced under favourable conditions. Per cent fruit-set and berry number per bunch of Chardonnay were reduced by about one third to one half by 12°/9°C, applied just before and at the early stage of flowering (E-L stages 17 and 20). At these stages, and with this low-temperature treatment, supplementary pollination had significant positive effects. In examination of seeds, distinction was made between seeds that floated in water (floaters) and those that sank (sinkers); sinker seeds are filled with endosperm and embryo while floaters have

pockets of air due to degradation of endosperm, nucellus and embryo i.e. empty-seededness. Total seed number per berry was not affected by treatment, but the proportion of sinker seeds decreased in the treatments which had reduced set. There was a positive relation between the number of functional seeds per berry and the number of berries per bunch. In Shiraz, the differences between the various treatment means were small and mostly not significant. It is concluded that the reduction in fruit-set of Chardonnay due to cool temperatures near flowering is due to detrimental effects on the development of both pollen and ovules.

Light incidence is another climatic factor that has been reported to have effects on fruit-set in grapevines. Cloudy weather during flowering has been associated with poor fruit-set; this is assumed to be due to its effect in reducing photosynthesis by causing low light incidence. An experiment similar to the previous one was set up. Small Chardonnay and Shiraz vines were grown under controlled conditions at 25°/20°C day/night temperatures but transferred for one week to 17°/14°C or 12°/9°C at two stages (E-L stages 15 and 17) to growth cabinets in which light intensity was reduced by 8%, 45% and 70% (respectively 375, 220 and 120 $\mu\text{E m}^{-2}\text{s}^{-1}$). The results of this experiment confirmed the previous findings on the effect of temperature on fruit-set in Chardonnay and Shiraz and seed number per berry in Chardonnay (Shiraz was not examined for seed characters). There was a tendency for fruit-set to decrease with increasing shade. Shading did not reduce the total seed number per berry but it reduced significantly the proportion of sinker seeds per berry. Per cent berries with one or two sinker seeds were reduced whereas the proportion of berries with one floater seed was increased by cold but not by shading. These effects were more pronounced at E-L stage 17. Vines treated with 12°/9°C had lower pericarp and seed weights compared to those treated with 17°/14°C or 25°/20°C. Applying cold had similar effects on pericarp and seed weights when applied at E-L 15 and 17 but shading treatments had no effect on either weight. Pericarp and seed weights were positively and linearly correlated, the regression for berries on vines exposed at pre-flowering to 25°/20°C and 17°/14°C having a steeper slope than that for berries on vines exposed to 12°/9°C. It is concluded that the effects of one week of light reduction on fruit-set, seed

weight and pericarp weight of Chardonnay were small, while those of temperature reduction were large.

To study ovule development, small Chardonnay and Shiraz vines were grown in pots under controlled conditions at 25°/20°C day/night temperature until flowering or were transferred to 12°/9°C two days before the beginning of flowering and then returned to 25°/20°C after one week. The ovules of flowers at three positions on three branches per inflorescence were excised on the day after they had opened. The ovules of 'king' (terminal) flowers tended to be larger than those of the other flowers. The ovules exposed to the lower temperatures tended to be smaller and less advanced in development, even when otherwise normal, especially on Chardonnay. Over half of the ovules of Chardonnay exposed to the low temperatures were abnormal (with embryo sacs either absent or abnormal, and some also having a degenerated nucellus), while 35% of the Shiraz ovules were without normal embryo sacs. To study pollination and pollen tube growth, vines similar to those of the ovule study were exposed to 12°/9°C two days before or on the day of flowering. In Chardonnay pistils exposed to low temperature, a reduction occurred in the number of pollen tubes present in the lower ovary on days 2 and 4 after flowering, from about four in control to almost nil. The reduction in Shiraz was smaller. In the absence of cool temperatures, pollen tubes penetrated on average less than one ovule per ovary in Chardonnay and about one ovule per ovary in Shiraz. It is concluded that temperature sensitivity to fruit-set is a varietal characteristic, expressing itself in quantitative differences in the damage imparted to the structure of the ovules and the function of the pollen.

Seed development was investigated by bright field microscopy in early and late Chardonnay inflorescences at 4, 14, 28 and 42 days after flower opening and at maturity. The percentage of ovules penetrated by a pollen tube and the percentage of fertilised ovules with a zygote and free nuclear endosperm were low. Ovules without an embryo sac, or those with normal but unfertilised embryo sacs, aborted shortly after flowering, but abortion also occurred after normal fertilisation. The time course of seed development was as follows: Fertilised ovules were identified by the presence of free nuclear endosperm and

of a zygote by day four after flower opening. Cellularisation of the endosperm commenced at 28 d after flower opening. The first division of the zygote occurred between 14 and 28 days after flower opening and coincided with cell wall formation in the endosperm. The proembryo grew slowly from 14 d to 42 d but the rate of growth accelerated thereafter to maturity. The endosperm completed most of its growth by 42 d. Degeneration of the zygote and endosperm was observed as soon as 14 d after flower opening. The proportion of such ovules increased by 28 d after flower opening and accounted for 60% of the largest ovules in the ovary. There was also abortion during later stages giving empty-seededness (floater seeds) at maturity. Floater seeds looked normal externally but some had no embryo and/or endosperm inside the integuments while others had a few degenerating embryo cells with degenerated endosperm, or no embryo but some endosperm which had started to degenerate. In addition there were seeds in which there was no sign of embryo or endosperm, and the remaining nucellus cells were degenerating. The results indicate that empty-seededness is a consequence initially of embryo degeneration in the developing fertilised seed while integument development continues. The incidence of empty seeds was 31% in bunches which flowered early in the season and were exposed to low temperature, and 10% in those bunches which flowered later under warmer conditions. It can be concluded that, in Chardonnay, ovule abortion at an early stage can lead to either flower abscission or to small growth of berries ('chickens') having seed traces, but that later abortion leads to floater seeds which provide sufficient stimulus for berries to develop to maturity, attaining a size larger than 'chickens' but smaller than berries with fully formed seeds.

DECLARATION

I HEREBY DECLARE that the thesis presented here has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously published or written, except where due reference is made in the text.

I am willing to have this copy of my thesis available for loan and photocopying, when deposited in the university library.

ALI EBADI

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LIST OF TABLES

Table 1.1 Annual cycle of the grapevine, cycle of development of the reproductive system, with chapter numbers where descriptions of the various events can be found.

Table 3.1 List of experiments

Table 4.1 Flower number of Chardonnay and Shiraz vines.

Table 4.2 Per cent fruit-set and berry number per bunch of Chardonnay and Shiraz vines.

Table 6.1 Per cent germination of Chardonnay pollen on the three culture media after 6 h and 24 h.

Table 6.2 Per cent germination of pollen from Chardonnay and Shiraz.

Table 6.3 Per cent germination of pollen from Chardonnay and Shiraz.

Table 6.4 The course of pollination.

Table 7.1 Volume of the average, largest (Max) and smallest (Min) ovule (in $\text{mm}^3 \times 10^{-3}$) per ovary, and of the ratio Max/Min, of Chardonnay and Shiraz flowers.

Table 7.2 Number of occurrences of types of ovules.

Table 7.3 Relationship between ovule size and ovule normality.

Table 8.1 Terminology of seeds.

Table 8.2 The structure of Chardonnay ovules and seeds, observed from four days after anthesis to maturity.

Table 8.3 Dimensions of Chardonnay ovule and seed structures, measured from four days after anthesis to maturity.

Table 9.1 Per cent berries with one seed.

Table 9.2 Number of total seeds per berry.

Table 9.3 Number of sinker and floater seeds per berry.

Appendix Table 1 Means of the treatments for per cent fruit-set and number of berries per bunch of Chardonnay and Shiraz vines.

Appendix Table 2 Effect of treatments on per cent fruit-set and number of berries per bunch of Chardonnay and Shiraz.

Appendix Table 3 Per cent berries with one seed (A) and per cent berries with one floater seed (B).

Appendix Table 4 Percentage of Chardonnay berries with varying seed complement.

Appendix Table 5 Number of total seeds per berry.

Appendix Table 6 Number of sinker seeds (A) and floater seeds (B) per berry.

Appendix Table 7 Mean fresh weight (mg) per seed.

Appendix Table 8 Mean pericarp fresh weight (mg).

Appendix Table 9 Per cent sinker seeds per total seeds.

LIST OF FIGURES

- Fig. 3.1** Chardonnay shoot at E-L stage 12, deleafed up to the first inflorescence and tipped at that node. Insert: budburst.
- Fig. 3.2** Chardonnay inflorescence at E-L stage 15, flowers in compact groups.
- Fig. 3.3** Chardonnay inflorescence at E-L stage 16, pedicel and rachis lengthening; lateral shoot elongating and at 4-leaf stage.
- Fig. 3.4** Chardonnay inflorescence at E-L stage 17, single flowers separating; shoot tipped to 4 leaves.
- Fig. 3.5** Shiraz inflorescence at E-L stage 15, flowers in compact groups.
- Fig. 3.6** Shiraz inflorescence at E-L stage 16, pedicel and rachis lengthening; lateral shoot elongating and at 4-leaf stage.
- Fig. 3.7** Shiraz inflorescence at E-L stage 17, single flowers separating; shoot tipped to 4 leaves.
- Fig. 3.8** Plants growing in the growth room, 25°/20°C, showing the light system; two sets of 5 cool white fluorescent tubes supplemented with high pressure sodium lamps and high pressure Metal Halide lamps in each set.
- Fig. 3.9** Model plants at flowering time, showing paper cones to collect calyptrae.
- Fig. 3.10** Pre-rooting of cuttings in a hot box, located in a 4°C room.
- Fig. 3.11** Chardonnay bunch after fruit set.
- Fig. 4.1** A. Berry number per bunch Exp. 1; B. Per cent fruit-set Exp. 1; C. Berry number per bunch Exp. 2 D. Per cent fruit-set Exp. 2 -Pollination
+Pollination

- Fig. 5.1** Calyptra of Chardonnay, showing the demarcation of five petals at the top of the flower. Bar represents 500 μm .
- Fig. 5.2** Chardonnay flower on the day of anthesis, showing anthers (a) close to the pistil. Bar represents 1 mm.
- Fig. 5.3** Stigma and style of Chardonnay one day after anthesis, showing no indication of stigma collapse. Bar represents 100 μm .
- Fig. 5.4** Chardonnay flower one day after anthesis, showing functional stigma (s), nectaries (n) and receptacle (r). Bar represents 500 μm .
- Fig. 5.5** Chardonnay stigma one day after anthesis, showing many dehydrated pollen grains. Bar represents 100 μm .
- Fig. 5.6** Pollen grains on the Chardonnay stigma one day after anthesis, showing hydration of one pollen grain (arrow) compared with others which are dehydrated. Bar represents 20 μm .
- Fig. 5.7** Germinated pollen grain on the stigma of Chardonnay one day after anthesis, showing the pollen tube. Bar represents 10 μm .
- Fig. 5.8** Ellipsoidal pollen grain of Chardonnay, showing many perforations on the exine and one of three furrows. Bar represents 5 μm .
- Fig. 5.9** Ellipsoidal pollen grain of Chardonnay, showing few perforations on the exine and one of three furrows. Bar represents 5 μm .
- Fig. 5.10** Pollen grain of Chardonnay showing all three furrows from the top. The Bar represents 5 μm .
- Fig. 5.11** Patterning of exine of Chardonnay pollen grain, showing small perforations. Bar represents 2 μm .

Fig. 5.12 Calyptra of Shiraz, showing demarcation of petals at the top of the flower. Bar represents 500 μm .

Fig. 5.13 Shiraz flower one day after anthesis, showing collapsed stigma (s), nectaries (n) and receptacle (r). Bar represents 500 μm .

Fig. 5.14 Collapsed stigma of Shiraz pistil one day after anthesis. Bar represents 100 μm .

Fig. 5.15 Stigma and style of Shiraz flower on the day of anthesis. Bar represents 100 μm .

Fig. 5.16 Papillae tissue of the stigma of Shiraz with hydrated pollen grains on the day of anthesis. Bar represents 50 μm .

Fig. 5.17 Pollen grains on the stigma of Shiraz one day after anthesis, showing hydration of most pollen grains and a large collapsed area resulting from degeneration of stigma cells. Bar represents 100 μm .

Fig. 5.18 Pollen grains on the stigma of the Shiraz pistil one day after anthesis, showing hydration of most pollen grains and degeneration of stigma papillae . Bar represents 20 μm .

Fig. 5.19 Ellipsoidal pollen grain of Shiraz, showing many large pores on the exine and one of three furrows. Bar represents 5 μm .

Fig. 5.20 Patterning of exine of Shiraz pollen grain, showing larger pores than in Chardonnay. Bar represents 1 μm .

Figs. 6.1 Pollen grains germinated *in vitro* after 12 h incubation at 25°C. Bar represents 50 μm .

Figs. 6.2 Germinated pollen grains *in vitro* after 12 h incubation at 25°C. Pollen grains have three germination apertures and pollen tubes are swollen adjacent to the pore. Bar represents 50 μm .

Fig. 6.3 Fluorescence micrograph of a squash preparation of a pistil of a Chardonnay Control vine 1 d after anthesis, showing pollen tubes growing from the stigma through the pistil to the ovules. Bar represents 1 mm.

Fig.6.4 Fluorescence micrograph of a squash preparation of a pistil of a Chardonnay Control vine 1 d after anthesis, showing one or two pollen tubes penetrating ovules. Bar represents 20 μm .

Fig. 7.1 Scheme of a grapevine inflorescence and of a branch showing the position of king (\blacktriangle), central-lateral (\bullet) and lateral-lateral (O) flowers (May 1987).

Fig. 7.2 Longitudinal section of an ovule of a Chardonnay Control vine 1 d after anthesis, showing normal development with outer integument (oi), inner integument (ii) which forms the micropyle (m), nucellus (n) and embryo sac (es). Bar represents 20 μm .

Fig. 7.3 Longitudinal section of an ovule of Shiraz Control vine one day after anthesis, showing micropylar half of embryo sac containing synergids (s) with well developed filiform apparatus (f), egg cell (e) and polar fusion nucleus (pn). Bar represents 3 μm .

Fig. 7.4 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing chalazal end of embryo sac containing three antipodal cells (a). Bar represents 3 μm .

Fig. 7.5 Longitudinal section of a fertilised ovule of a Chardonnay Control vine 1 d after anthesis showing cytoplasm (c) and two nuclei (n) of free nuclear endosperm. Bar represents 3 μm .

Fig. 7.6 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after the anthesis), showing absence of embryo sac and reduced overall size. Bar represents 20 μm .

Fig. 7.7 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing small, incomplete embryo sac. Bar represents 20 µm.

Fig. 7.8 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing cross wall (arrow) in the middle of the embryo sac. Bar represents 20 µm.

Fig. 7.9 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing deteriorating nucellus. Bar represents 20 µm.

Fig. 8.2 Pollen tube (arrow head) penetrating embryo sac (es) within ovule on day 4. Nucellus (n), integument (i). Bar represents 20 µm.

Fig. 8.3 Normal fertilised seed on day 14, showing embryo sac with zygote (z), one nucleus (arrow head) and cytoplasm (ct) of the free nuclear endosperm (fe). Bar represents 10 µm.

Fig. 8.4 Fertilised seed on day 28, showing proembryo (pe), the endosperm becoming cellular, showing formation of cell walls (cw). Bar represents 10 µm.

Fig. 8.5 Fertilised seed on day 42, showing globular multicellular proembryo (pe) with a short suspensor (s) and cellular endosperm (ce). Bar represents 20 µm.

Fig. 8.6 Normal seed on day 98. Embryo (e) with vascular tissue (vt) surrounded by cellular endosperm (ce). Bar represents 100 µm.

Fig. 8.7 Ovule without embryo sac on day 28, showing degenerated nucellus (n) within large integument (i). Bar represents 50 µm.

Fig. 8.8 Ovule on day 14 with normal but unfertilised egg apparatus (ea). Bar represents 10 µm.

- Fig. 8.9** Seed trace on day 28 with normal but unfertilised embryo sac (es) and proliferated nucellus (n), integument (i). Bar represents 50 μm .
- Fig. 8.10.** Fertilised seed on day 28, showing undivided zygote (z), free nuclear endosperm (fe) having degenerated. Bar represents 5 μm .
- Fig. 8.11** Seed on day 42 with degenerating free nuclear endosperm (fe) and without zygote or proembryo. Bar represents 10 μm .
- Fig. 8.12** Seed on day 28, with no embryo, very small cellular endosperm (ce) and proliferated nucellus (n) beginning to degenerate. Bar represents 50 μm .
- Fig. 8.13** Floater seed on day 98 showing well developed testa (t), with invaginations into the seed lumen, but no embryo or endosperm and with fragments of degenerating nucellus tissue (n). Bar represents 480 μm .
- Fig. 8.14.** Mature sinker seed, halved longitudinally, with testa (t), cellular endosperm (ce) and embryo (em), and invaginations of the testa (arrow head). Bar represents 1 mm.
- Fig. 8.15** Mature floater seed, halved longitudinally, without embryo and showing empty space with some remnants of cellular endosperm (ce). Bar represents 1 mm.
- Fig. 8.16.** Line diagrams drawn to scale to interpret the seed development shown by the data in table 8.3 and in figs 8.2-8.13. For clarity, the invaginations of the testas as seen in fig. 8.14 have been omitted. The five columns show schematically development examined at the five sampling days. The four rows describe the development patterns as follows:
- ovule – no embryo sac, no integument growth and nucellus generated at all stages;
 - trace – embryo sac present but unfertilised or, in some cases, fertilised, as evidenced by endosperm formation, with degeneration setting in at the free nuclear stage; no zygote; testa development incomplete; degree of sizing and hardening of the testa, and timing of the degeneration of the nucellus varying;

- floater – at early stage, embryo sacs present, full testa development but nucellus, endosperm and proembryo degenerate later:
- sinker – normal development of testa, nucellus and embryo.

Fig. 9.1 A. Per cent berries with one seed Exp. 1-4; B. Per cent berries with one floater seed Exp. 1-4; C. Per cent berries with one seed Exp. 2-4; D. Per cent berries with one floater seed Exp. 2-4.

Fig. 9.2 Percentage of Chardonnay berries with varying seed complement; s=sinker; f=floater

Fig. 9.3 A. Number of sinker seeds per berry Exp. 1-4; B. Number of floater seeds per berry Exp. 1-4; C. Number of sinker seeds per berry Exp. 2-4; D. Number of floater seeds per berry Exp. 2-4

Fig. 9.4 Weight per seed of Chardonnay berries with varying seed complement; s=sinker; f=floater

Fig. 9.5 Seed (A) and pericarp (B) weight per Chardonnay berry with varying seed complement

Fig. 9.6 Comparison of the means of the treatments for number of berries per bunch and per cent sinker seeds of Chardonnay and Shiraz vines.

Fig. 9.7 Regression of pericarp weight against total seed weight per berry for Chardonnay. ■ 25°/20°C; ▲ 17°/14°C; ● 12°/9°C

List of publications

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Chapter 1

Introduction

The grape is ^{a major} ~~the most important~~ fruit crop ^{throughout} ~~in the~~ world, as it is also in Australia. Its wide extent in Australia is due to the availability of environments that suit the culture of grape cultivars that are popular commercially. The predominant cultivar is cv Sultana, reflecting its all-purpose utility. In addition there are many white- and black-berried grape cultivars that are grown because they yield well and have produce of good quality.

The winegrape cultivar Chardonnay is noteworthy in this context because of the upward surge in the area planted to it in Australia—Chardonnay production increased from 4000 tonnes in 1983 to 63000 tonnes in 1994—reflecting a recognition of its attractive flavours and the large increase in demand for it. Many of these new plantings have been in regions with cool climate. Unfortunately, it has been found that the productivity of this cultivar has been variable, in some cases disastrously low. Much of the yield reduction has resulted from poor fruit-set or from a preponderance of small berries. The significance of these two components of yield are set in perspective by the Table 1.1. showing the key stages in the lengthy reproductive development of the grape berry as it occurs within the seasonal cycle of vine growth. Various phases of the development of the reproductive system of these two cultivars have been dealt with in this investigation. They are also indicated in the schema shown by the number of the chapters in which they are described.

Research on fruit-set and early fruit growth, with which are coupled ovule and seed development, has not provided practical solutions to problems described above regarding yield in Chardonnay. Like all investigations of reproductive development, study of fruit-set and fruit growth presents many difficulties in research method. Some recent field work by May (1992) pointed to the potent effects of cold temperature during early flower development. This lead, plus the possibility of using small fruiting potted vines in controlled environment conditions, offered the opportunity for an intense investigation of

the effect of cold temperature on development of the male and female gametophyte, a topic of basic research that needs more study.

Table 1.1 Annual cycle of the grapevine, cycle of development of the reproductive system, with chapter numbers where descriptions of the various events can be found.

Annual cycle of growth	Development of the reproductive system	Chapter No.
Flowering	Anlagen initiation	
	Inflorescence primary branching	
	Inflorescence secondary branching	
Harvest	Rest	
Leaf fall		
Winter rest	Inflorescence tertiary branching	
Bud burst	Flower initiation	
Shoot growth	Flower development	
	Androecium	5,6,8
	Gynoecium	5,7
Flowering	Pollination	5,6
	Fertilisation	6,7,8
Fruit set	Fruit set	4
	Seed growth	8, 9
	Berry growth	9
Veraison		
Harvest		

To provide a point of comparison, most experiments were repeated on the cultivar Shiraz—the most widely planted black grape in Australia—which seems not to be as sensitive to cold temperatures as is Chardonnay.

Aims of the study

The aim of this study is to investigate the effect of variations in the temperature regime and light intensity during various stages of floral development on fruit-set components including pollen development and pollen germination, pollen tube growth, ovule development before and after flower opening up to berry ripening time and its relation to berry growth.

Chapter 2

Literature Review

This literature review deals with the formation and subsequent development of the inflorescences, their flowers and subsequent fruits of the grapevine *Vitis vinifera* L. and with environmental conditions which impinge on these processes.

2.1 The reproductive organs of the grapevine

2.1.1 The inflorescence

Inflorescence initiation

The formation of inflorescences and flowers in the grapevine involves three well-defined stages: formation of anlagen (derived from the German word Anlagen, which means 'initials'), formation of inflorescence primordia, and formation of flowers (Barnard 1932, Barnard and Thomas 1933, Carolus 1971). This process extends over a period of some 15 months, from about the time of flowering onward, when the anlagen are formed, during late spring and summer, when the inflorescence primordia develop and temporarily cease growth, until the time of next season's budburst, when the individual flowers start to differentiate.

Anlagen arise as club-shaped meristematic protuberances which arise on the apices of shoot primordia enclosed in buds. The most proximal anlage on the primordial shoot of Muscat of Alexandria starts forming concurrently with the fifth or sixth most proximal leaf on the bud axis (Buttrose 1970a, b). Anlagen are "uncommitted primordia" which may develop to form several different structures: inflorescence primordia, tendril primordia, shoot primordia or mixed primordia comprising some or all of these three types. Anlagen become inflorescences by division and formation of several globular branch primordia

(Scholefield and Ward 1975). According to Barnard and Thomas (1932) for cv. Sultana, the differentiation of anlagen into inflorescence primordia takes place during late spring, summer, and autumn, while anlagen which are initiated after winter rest become tendril primordia. Barnard and Thomas (1932) also found that the anlagen which are initiated in late summer or autumn and have not developed sufficiently before winter to acquire a definite growth habit become tendril primordia during the following spring whereas anlagen which had just begun to acquire the inflorescence mode of growth in autumn develop into 'transition' forms when growth recommences. Thus, the number of anlagen which differentiate as inflorescence primordia is determined by the amount of their development during summer and autumn.

May (1987) stated that there are three types of inflorescences: (a) those formed in the 'latent' or 'dormant' bud which is situated on the basal node of the axillary shoot and on which next season's crop is based, (b) those formed in the 'accessory buds' (the basal buds of the primordial shoot inside the latent bud) whose formation varies with cultivar and which develop infrequently into bunches and (c) those formed on the axillary, 'lateral' shoot. The initials mentioned under (a), and those of (b) in buds which burst, become bunches during the following season, but those mentioned under (c) mature during the season of their initiation and form the 'second crop'.

May and Cellier (1973), following early studies on cv. Sultana (Barnard and Thomas 1932), found for a number of varieties that the number and size of inflorescence initials per bud increases along the shoot up to about the 10th node and then decreases again. These differences between node positions vary in magnitude according to variety. They may be modified by changes in shoot development, e.g. in Sultana due to shoot direction (May 1966) or by changed environmental conditions (Buttrose 1974).

Inflorescence structure

An inflorescence consists of an inner arm, its main part which is always present, and an outer arm which is often reduced to a tendril or may even be lacking altogether. May (1987) proposed that the two arms are likely to be two inflorescences of a rudimentary

shoot which, in rare cases, is fully developed. The inner arm is the proximal inflorescence of such a shoot. The outer arm is the distal inflorescence; on cursory inspection it appears to be the proximal branch of the inflorescence (often called the 'wing'). The number of its flowers can vary from a few to almost equal that of the inner arm and the frequency of occurrence of the outer arm and its size are important varietal and seasonal characteristics of fruitfulness.

Branching of the inner and outer arms gives rise to branches of the second and third order, each of which is subtended by a bract. The degree of branching of the inner arm gradually decreases in an acropetal direction and this gives the inflorescence a conical shape (Pratt 1971). According to Troll (1964, cited by May 1987) the inflorescences finally consist of a 'main axis' (rachis) carrying side branches called 'paraclades'. These are themselves copies of the main axis and terminate in so-called 'coflorescences' and may carry second order paraclades. Flowers are situated singly or in the form of a 'dichasium', a group of three flowers with two placed laterally at the base of the central one. The distribution of the flowers over the various parts of the inflorescence is likely to have important effects on their subsequent development both before and after anthesis.

2.1.2 The flowers

Differentiation of the flowers of the inflorescences occurs as the buds are opening in spring (Barnard and Thomas 1932, Snyder 1933, Winkler and Shemsettin 1937, Alleweldt and Itler 1969, Agaoglu 1971, Srinivasan and Mullins 1978). In the southern hemisphere, much growth and branching of the inflorescence occurs during the post-dormancy period from mid-August to bud burst, but it is highly probable that the extent of the growth of an inflorescence during this period is largely dependent upon the stage of development reached when it entered its stage of dormancy during the preceding season (Sartorius 1926, Barnard and Thomas 1932, Antcliff and Webster 1955).

In contrast to these earlier findings, Pouget (1981) showed with small Cabernet Sauvignon and Merlot vines that the differentiation and number of flower initials could be modified by environmental conditions (mainly by temperature) during a period which commences some days before budburst and which ends when the third-inserted leaf-opposed organ above the base of the shoot, inflorescence or tendril, appeared on the shoot. More flowers were present on inflorescences of small vines held at 12°C than at 25°C. Ezzili (1993) however showed that, when plants were exposed to 12°C, about 5% of flowers showed incomplete development.

2.1.3 Organogenesis of flower parts

The formation of the different floral parts of the grapevine flowers occurs in the order calyx, corolla, stamens and pistil (Barnard and Thomas 1933, Winkler and Shemsettin 1937, Scholefield and Ward 1975, Srinivasan and Mullins 1981), and within 20 days after appearance of the inflorescence at budburst these parts are fully developed (Swanepoel and Archer 1988). Most ancient grapevines in the wild were dioecious but most commercial cultivars of today are hermaphrodite, and remain so as they are vegetatively propagated. Hermaphrodite flowers contain functional male and female sexual organs. The less common imperfect flowers are of two types: pistillate (female) flowers have a well developed and functional pistil but their stamens are more or less reflexed and the pollen is generally sterile. In contrast, the stamens of staminate (male) flowers are functional but the pistil is reduced and not functional (Pratt 1971, Winkler et al. 1974).

The hermaphrodite flower of *V. vinifera* consists of five sepals which stop growing at an early stage of flower development. The five green petals of the corolla are joined at the tip to form the calyptra or cap. This fully encloses the pistil and stamens until the petals detach themselves at their base from the receptacle when the flowers open. Each of the five stamens has a filament and a two-lobed anther, each with two pollen sacs. Nectaries, placed on the receptacle between the stamen filaments, which are not functional in most varieties of *V. vinifera* or their products are not attractive to insects. The pistil

comprises the stigma, style and ovary which has normally two locules, each with two ovules (Winkler et al. 1974). Ovaries with three locules and up to seven ovules are not uncommon, e.g. in cvs Pinot noir and Chardonnay (P. May, personal communication) and even four locules containing eight seeds have been described in cv. Ribier (Olmo 1946).

2.1.3.1 The androecium

The archesporium cells of each of the four pollen sacs of the anther produce a primary parietal layer and a primary sporogenous layer. The former produces the wall of the anther and the latter the pollen mother cells (Davis 1966), as is the case with angiosperms in general (Maheshwari 1950).

The pollen grains are formed by meiosis in the pollen mother cells which produces tetrads of four nuclei. Each develops into a pollen grain and the four pollen grains of each tetrad are released into the anther locule from the cell wall of the pollen mother cell (Pratt 1971).

According to various descriptions (Sartorius 1926, Pratt 1971), the uninucleate pollen grains undergo mitosis and are binucleate at the time of anthesis. They have a thin intine and a thick exine which has three longitudinal furrows, each with a germ pore.

The pollen grains of *V. vinifera* are small, 20-30 μm in diameter. The shape is ellipsoid to oblong with flattened ends when shed from the anthers but become round when imbibing water or stigmatic secretion (Sartorius 1926, Randhawa and Negi 1965, Winkler et al. 1974). The pollen grains contain a mass of protoplasm, stored metabolites and the two nuclei. The division of the nucleus of the generative cell occurs inside the pollen tube and produces two sperms (Pratt 1971), as in other angiosperms (Sedgley and Griffin 1989).

The epidermis of the anther wall is stretched and partly lost when the pollen grains are mature (Sartorius 1926, Esau 1965) and the single layer of cells immediately beneath

the epidermis (endothecium) develops fibrous thickening on the cell walls, except at its point of attachment near the centre of the anther. On the inside of the endothecium there are two layers of cells which have collapsed at the time of anther maturity. The most internal layer is the tapetum which consists of multinuclear cells; these are rich in food and nourish the pollen mother cells and later the microspores and contribute to the sporopollenin deposition in the exine. The tapetum also produces enzymes that release the microspore from the callose wall (Laser and Lersten 1972, Warmke and Overman 1972, Bino 1985). The tapetal cells have degenerated when the pollen tetrads have separated (Pratt 1971).

At the time of anther dehiscence the endothecium breaks away from its thin-walled points of attachment at the centre of the anther and turns inward thus allowing pollen to be released from the pollen sacs (Sartorius 1926, Pratt 1971).

Many abnormalities in pollen formation have been observed, (a) imperfect individual pollen grains, (b) one or more completely empty, white anthers among otherwise normal, yellow anthers within a flower (P. May, personal communication) and (c) genetically based sterility of all pollen grains. Due to the grapevine being predominantly autogamous, such irregularities, if frequent, may lead to low yield. In functional anthers, abortion of individual pollen can result from irregularities in chromosome distribution during meiosis (Pratt 1971) or from the absence of pores, reported for cvs Riesling and Sylvaner (Wagner 1960) and Picolit giallo (Lombardo 1976). Unfavorable environmental conditions, in the form of low temperature during the post-meiotic microsporogenesis, have also been reported to lead to a high level of pollen sterility (Staudt and Kassrawi 1972b).

More important is the genetic sterility of all pollen grains. This occurs in 'female' varieties in which the pollen is sterile, lacks furrows and germ pores and shows postmeiotic degeneration of the generative or vegetative nuclei, or both, and the absence of the second mitotic division (Pratt 1971). Genetic pollen sterility has also been reported in hermaphrodite varieties. In the variety group Picolit, the proportion of non-functional pollen was greater in the low-producing Picolit giallo than in more productive Picolit

biotypes (Cargnello et al. 1980). Lombardo et al. (1976, 1978) found that the sterile pollen is round, deprived of furrows and germinative pores, and surrounded by a continuous layer of bacula and tegmen. In Picolit giallo, the tapetal cells degenerate during the early part of microsporogenesis and do not provide the pollen grains with the "pollen-stigma recognition proteins" that are usually formed by the tapetum and transmitted to the pollen grains during the synthesis of their walls (Carraro et al. 1981).

Tetraploid mutants show lower pollen fertility than diploid biotypes. This was shown for Riesling (Staudt and Kassrawi 1972 a, b) where pollen fertility was 16% for the tetraploid and 34% for the diploid biotype, and for Barbera by Me et al. (1984).

Pollen development is liable to be damaged by unfavourable environmental conditions such as temperature, light, drought and carbohydrate or mineral deficiency (Laser and Lersten 1972, Saini et al. 1984, Hormaza and Herro 1992) through preventing the normal development of tapetal cells. Iwao (1983) showed that exposing rice plants to 12°C for four days particularly at the young microspore stage (an early stage of microsporogenesis), resulted in lower numbers of mature pollen grains at flowering time.

2.1.3.2 The gynoecium

The grapevine gynoecium conforms to the typical model of the angiosperm female sex organ, as has been described by many authors (eg. Sartorius 1926, Pratt 1971). The pistil is superior and formed at the apex of the receptacle. The ovary is initiated at the periphery of the meristematic apex of the floral receptacle. It gives rise to tissues which form usually two carpels, each enclosing a locule. The placenta, where the ovules originate, is formed at the edges of the carpels. The stigma and style are formed through the continuing growth at the upper part of the carpels. Within the style, and continuing into the ovary, the inner carpel walls form a canal which is later partly filled with the transmitting tissue. The rows of cells of the transmitting tissue continue into the open and

the cells at their tips become the papillae, their collective assembly representing the stigma (Sartorius 1926).

Normal development of the ovule

The ovules appear at the placenta, near the base of the carpellary initials, starting as undifferentiated cell masses from divisions in one or two subepidermal layers at the base of the locule, and enlarge quickly (Winkler and Schemsettin 1937, Fougère-Rifot et al. 1993a).

The mature ovule consists of a massive nucellus and two integuments. In his detailed description of the development of the grape flower, Sartorius (1926) stated that "the ovules do not display any abnormal characteristics" (translated quote), compared with the general ovule development in angiosperms. The development of the ovule given by Pratt (1971), based on work of Sartorius (1926), Stout (1936), Periasamy (1962) and Kim (1967), has been more clearly defined recently by Fougère-Riffot et al. (1993a). At development stage F (Baggiolini 1952, i.e. E-L stage 12, Coombe 1995), the ovules appear as undifferentiated cell masses at the placenta. They grow quickly within days after appearance to bodies of 90 x 65 μm and are surrounded by an epidermis. The archesporangium originates sub-epidermally near the tip of the meristematic cell mass and mitotic division leads to two cells, one becoming the nucellar primordium after several further mitotic divisions, while the other, the sporogenic cell, undergoes meiosis and becomes the female gametophyte. After archesporangium formation the ovules, first orthotropous, become anatropous because active growth of the ventral tissues of the ovule causes the funiculus to turn the ovule apex by about 180° toward the ovary wall.

The nucellus develops from periclinal divisions in the subepidermal layer of the ovule primordium in a position on the dorsal flank of the tip. The inner integument is formed by the extension of a ring-shaped initial surrounding the middle part of the ovule primordium. This first outgrowth is followed by a second, the initial of the external integument. However, according to the quote by Pratt (1971), Kim (1967) stated that the outer integument develops from periclinal divisions in sub-epidermal cells at the base of the inner integument when the ovule is partly anatropous. The inner integument consists

of two or three cell layers and extends beyond the outer integument to form the collar around the micropyle at anthesis. According to Periasamy (1962), and Fougère-Rifot et al. (1993a), the inner integument has a cuticle on the cells of its outermost layer, and cells filled with tannin in its innermost layer. The outer integument consists of two to five cell layers and of up to nine layers in the region of the raphe (Stout 1936 cited by Pratt 1971, Periasamy 1962, Kim 1967, Pratt 1971). Its outermost layer contains tannin-bearing cells (Periasamy 1962, Fougère-Rifot et al. 1993a, b).

The embryo sac is formed at the button stage just before anthesis (Fougère-Rifot et al. 1993a). As stated above, the archesporial cell, a sub-epidermal cell of the nucellus, divides periclinally. The outer daughter cell is the primary parietal cell, and the inner daughter cell is the primary sporogenous cell which enlarges to become the megaspore mother cell. The megaspore mother cell undergoes meiosis and cytokinesis to produce a usually linear tetrad of four megaspores. The chalazal megaspore enlarges to form the embryo sac and the other three megaspores degenerate. The embryo sac becomes eight-nucleate through three mitotic divisions. Three of these nuclei migrate to its micropylar end. They form the egg apparatus consisting of the egg cell and two synergid cells. The walls of the synergid cells are characteristically thickened at the micropylar end, forming a long finger-like structure called the filiform apparatus. Being rich in polysaccharides, it is the point where the pollen tube enters the embryo sac. At the chalazal end of the embryo sac there is a group of cells, the three antipodal cells. According to Fougère-Rifot et al. (1993a), the morphology of the antipodal cells in *V. vinifera* deviates from that of most other angiosperms, being small, with little cytoplasm and with a large vacuole which pushes the nucleus towards the centre of the embryo sac. The antipodal cells degenerate early before anthesis (Kim 1967, Pratt 1971, Sedgley and Griffin 1989, Fougère-Rifot et al. 1993a). The two remaining nuclei, the polar nuclei, occupy the shared cytoplasm of the membrane-enclosed central cell, the seventh cell of the embryo sac. The two polar nuclei fuse prior to fertilisation to form the secondary generative nucleus or polar fusion nucleus.

The cells of the nucellus just below the embryo sac form the hypostase, distinguished by having elongated, thick-walled, intensely staining cells (Kim 1967). The

hypostase increases in size during the course of integument development and grows into the nucellar tissue after fertilisation. In angiosperms in general, this tissue probably has an integral function in the translocation of metabolites into the megagametophyte and, after fertilisation, into the embryo sac (Tilton 1980).

Abnormal development of the ovule

Different types of abnormal development in the grapevine ovules have been reported. Development of some ovules may be arrested before meiosis, with accompanying deficiency or abnormal development of nucellus and integuments and sometimes incomplete anatropy (Pratt and Einset 1961, Kim 1967, Barrit 1970, Pratt 1971). In other nonfunctional ovules the embryo sac fails after meiosis, either by an arrest of the sac in its early stages or by degeneration of the egg apparatus of the mature sac (Barrit 1970, Pratt 1971, Carraro et al. 1979, Kassemeyer and Staudt 1982a, b, Vallania 1987). Other phenomena have been noticed in a tetraploid strain of cv. Barbera (by Vallania et al. 1987), namely difficulties in the fusion of the polar nuclei, non-migration of the secondary embryo sac nucleus just before anthesis, and/or irregular nuclear organisation in which the nuclei crowd together; these phenomena appeared to be related to tetraploidy. Fougère-Rifot et al. (1993a, b) found symptoms of degeneration in ovules of the cv. Chardonnay. The first sign of degeneration was noted at the tetrad stage of meiosis, when some cells of the outer epidermis ruptured and released their tannin contents into the cytoplasm, resulting in plasmolysis. Subsequent symptoms were separation of the inner and outer integuments, an abnormally large opening of the micropyle and the sub-micropylar chamber, culminating in slower growth and ultimately in ovule abortion. However, other ovules appeared to develop normally until macrospore maturity when they began to degenerate. Other degenerative conditions were evident either before or after megaspore formation. In a first type, the development of the structural parts of the ovule were affected either before or after megasporogenesis. In some cases, ovules were not anatropous and had only one integument. In other cases, the nucellus protruded from the micropyle and curved downwards, with its interior often broken down, the chalaza was partly necrosed and often the vascular bundle was branched. The cultivars White

Aspirant, Red Corinth and White Corinth belong to this first type (Müller-Thurgau 1898 cited by Pratt 1971, Pearson 1932). In the second type, the embryo sac showed irregular development after the megaspore mother cell was formed while the integuments and nucellus were normal (Calo 1965), or the embryo sac had degenerated (Pearson 1932, 1933, Gifford et al. 1960). When ovule abortion is an inherited trait, stenospermocarpy or even parthenocarpy may result, as discussed below.

2.1.4 Flowering

2.1.4.1 Anthesis

Opening of the calyptra

Normally, the calyptra are thrown off as a small cap after each of the five fused petals become detached from the receptacle while the distal tips remain fused. The opening of the calyptra is closely related to temperature and when the flowers reach the proper stage for opening, temperature alone is considered to control the time of anthesis. Below 15°C, few flowers open. As the temperature rises to 18°–20°C, calyptra fall (or anthesis) increases rapidly (Winkler et al. 1974). According to field observations of Sartorius (1926) in Germany, the diurnal increase in temperature during the early morning causes the concurrent opening of many flowers between 06.00 and 08.00. Under constant temperature conditions, endogenous periodicity comes into play, leading to the calyptra splitting open early in the morning and between 14.00 and 16.00. Irregular opening of the petals is not infrequent whereby the petals split at the tip instead of at their base or fail to detach themselves altogether, becoming brown and dry while still in position (Sartorius 1926). In some cases this is due to conditions inherent in individual vines or to cold or wet weather.

Opening of the anthers

According to the description by Winkler et al. (1974), the stamens move away from the pistil as soon as the calyptra is thrown off. It often occurs quite explosively with pollen dusting the entire flower. In opening, the two pollen sacs of one of the lobes of an anther break loose along their common, median attachment and on the side facing the stigma. In some varieties, dehiscence of the anthers takes place before anthesis but usually occurs after anthesis, leading to the transfer of pollen from the anthers to the stigma. However, it is not always the case and cleistogamy has been reported whereby some flowers are pollinated 4–24 h before anthesis. The extent of cleistogamy was about 60% for cv. Müller-Thurgau and 17% for cv. Pinot noir; therefore, for hybridisation, anthers need to be removed at least one day before anthesis (Staudt 1986). Randhawa and Negi (1965) showed that dehiscence started just before anthesis in Pusa Seedless; in cvs Bharat Early, Black Muscat and Alamwick, it started when 2-3 petals had detached themselves from the receptacle. Normally, the time taken for the completion of dehiscence was 5-12 minutes in these cultivars (Randhawa and Negi 1965).

The opening of the anthers, as described by Winkler et al. (1974), is influenced by temperature in the same way as the opening of the calyptra. It depends on stress arising when the outer surface of the anther dries, thereby pulling the walls of the pollen sacs from their median connection and forming the slit through which the pollen is liberated. High air humidity, be it through rain, cloudy-conditions or fog, and also through its effect on lowering temperature, will delay opening.

Stigma receptivity

Stigma receptivity may start from one day before anthesis, but this is not always the case in all grapevine cultivars, most having maximum stigma receptivity on the day of anthesis (Randhawa and Sharma 1960, Randhawa and Negi 1965, Carraro et al. 1979). The stigma can successfully retain its receptivity up to four days after anthesis and its receptivity is not necessarily related to the presence of a visible droplet of secretion produced by it (Staudt 1986). Stigma and style tolerate temperatures as low as 2°C for at

least two days without any injury (Staudt 1986). The period during which the stigma remains receptive is variable. If many viable pollen grains lodge on the stigma, its papillae degenerate within a few days, perhaps because fertilisation has occurred. For example stigmatic receptivity was longer in high-producing than in low-producing clones of cv. Picolit (Carraro et al. 1979).

2.1.4.2 The mode of pollination

Autogamy is thought to be the most common mechanism of pollination in the grapevine (Sartorius 1926, Pratt 1971, Winkler et al. 1974). Its frequency and that of other types of pollination are not clear, nor are the reasons why one or the other mode come into play. Entomophily has been reported in warm environments, while in cooler environments insect presence in the vineyard at bloom is very limited. It also has been reported that in cool climates the nectaries were non-functional (Pratt 1971, Lavee and Nir 1985). The pollen from the morphologically hermaphrodite flowers of wild grapevines is sterile and is the main food source for many insects including Halictidae and Syrphidae (Branties 1978). Sharples et al. (1964) showed that exposing Cardinal grapevines to insect pollination reduced the number of shot berries and increased the number of seeds per berry but did not affect the weight of bunches and thus yield, or the number of seeded berries per bunch. It was shown that insect pollination occurs in many *V. rotundifolia* cultivars (Lavee and Nir 1985).

Anemophily has also been shown to be involved in grapevine pollination. This finding was based mainly on fruit-set of female cultivars in relation to their distance from male or hermaphrodite vines. Improvement in fruit-set was achieved by blowing air through the vineyard (Olmo 1943, Lavee and Nir 1985) or by spraying a pollen suspension (Hale and Jones 1956). On the other hand, Sartorius (1926) showed that anemophily was effective for a distance of only a few centimetres.

There are a number of studies indicating a positive effect of open-pollination on fruit-set compared with self-pollination of bagged inflorescences (Rhandhawa and Negi 1965, Uppal et al. 1975, Shinde and Patil 1978). Supplementary pollination with a mixture of pollen from various cultivars was more efficient in setting fruit than was pollination with pollen from the same or another cultivar (Uppal and Mukherjee 1968, Samaan et al. 1981, Lavee and Nir 1985).

2.1.4.3 Pollen germination and pollen tube growth

As the pollen grains are released from the anthers and reach the stigma, they adhere to the papillae on its surface. These are covered by a sticky stigmatic fluid which covers them and fills all spaces within the style (Sartorius 1926). The course of the subsequent development of the pollen has been described by Staudt (1981) who distinguished four growth phases during pollen germination and pollen tube growth, their duration being temperature-dependent. During phase 1, a pollen grain, if viable, takes up water and immediately commences synthesis of protein and enzymes as well as enzyme activation. Phase 2 represents the formation of the pollen tube. A germination blister is extruded through one of the germ pores and becomes the pollen tube through its extension growth. The pollen tube grows strongly in phase 3. Once growth has reached its maximum, phase 4, ageing, commences and growth slows. Respiration is high in phase 1, low in phase 2, increasing again in phase 3 and decreasing in phase 4.

The pollen tubes grow intercellularly in the stigma and style and emerge onto the surface of the placenta and funiculus, enter the micropyle, and grow between the cells of the nucellus (Stout 1936, cited by Pratt 1971). As the pollen tube approaches the egg apparatus its tip swells to become a bubble, then bursts and releases the two sperm nuclei.

2.1.4.4 Temperature effects on pollen activity and pistil receptivity

All phases of pollen growth have been shown to be sensitive to temperature (Staudt 1981). Koblet (1966) working with cv. Sieger found that cold air (10° – 13° C) shortly before or during flowering almost completely destroyed pollen viability. Before or after this period, cold air retarded the opening of the flowers, but did not affect pollen viability. At 16° C, pollen viability was not affected but the development of the inflorescences was retarded. Kobayashi et al. (1964) working with cv. Delaware showed that pollen germination was best at 20° – 25° C. High temperature affected pollen germination *in vivo* because the associated low humidity reduced the effectiveness of the stigmatic fluid. On the other hand, pollen germinated well *in vitro* at 35° C in a humid environment. Staudt (1981, 1982) also found a positive relationship between temperature and pollen growth in *V. rupestris*. Both *in vitro* and *in vivo*, germination was completely prevented at 2° C. Holding pollen grains at 5° C for five days or more during phase 1 led to marked inhibition in germination and strong inhibition was still noted at 10° C. However, short-term exposure to low temperature, (5° C or even 2° C) had no effect on pollen tube growth if the inflorescence could subsequently continue to grow at conducive temperatures. Interestingly, Staudt (1982) stressed that exposure to 5° C was more damaging than exposure to 2° C. There was a positive relationship between temperature on the one hand and pollen germination and pollen tube growth on the other hand, between 10° C and 28° C. Pollen development was most rapid at 28° C. Therefore, it can be assumed that drops in temperature during the flowering season, even to as low as 2° C, will slow down but not permanently damage pollen activity, as long as such periods are of short duration only. Stigma, style and transmitting tissue can also remain functional when exposed for up to 15 days to temperatures as low as 5° C or 2° C (in contrast to the greater sensitivity of the ovules).

2.1.4.5 Pollen tube inhibition

Okamoto et al. (1989) found that the growth of the pollen tube was inhibited in the stigma, style or even further down in the ovary of the tetraploid cvs Centennial and Pione, resulting in lack of fertilisation and poor set of seeded berries. Self-incompatibility was excluded as ^{a possible} the reason. Pollen cultured *in vitro* in the presence of pistil extracts also failed to germinate normally, indicating that the extract may contain inhibitors against hydrolytic enzymes or suppressors that inhibit cell wall development. Okamoto et al. (1995) suggested that two quercetin glycosides, coded PGI-1 and PGI-3, present in the pistil of cv. Pione, may act as inhibitors of pollen germination and pollen tube growth, and furthermore, that the interplay of the flavonoid aglycons and glycosides may be involved in controlling pollen tube growth on the stigma and in the pistil.

Genetic and environmental conditions also can influence the progress of the pollen tube within the pistil. May (1992) noted irregular growth of pollen tubes within the pistils of field-grown Chardonnay vines when weather conditions were unfavourable to good fruit-set. In other plant species, this type of pollen tube growth has also been observed as an expression of breakdown between the male and female partners (Sedgley 1979).

2.1.4.6 The process of fertilisation

Fertilisation is defined as the joining of the nuclei of the egg cell and the pollen sperm cell, being the culmination of the growth of the pollen tube through the tissues of the pistil and the ovule. The process in angiosperms involves a double fertilisation whereby one pollen nucleus fuses with the nucleus of the egg cell and the other with the fused polar nuclei. This process initiates the change from ovule to seed. In the grapevine, this was found to occur 12 h after pollination at temperatures between 25° and 28°C under *in vitro* conditions but to take longer (24 h) under field conditions (Kassemeyer and Staudt 1981). However other workers stated that fertilisation occurs two to three days after anthesis,

judging from the presence of the pollen tube at the embryo sac or by changes in the ovule or ovary (Pearson 1933, Patel and Olmo 1955, Kim 1967, Pratt 1971).

2.1.5 Post-flowering development of the grapevine ovule

2.1.5.1 Functional seeds

Once the ovule is fertilised and starts to grow it becomes a seed. Once this process has occurred, the seed may become and remain functional or it may be functional initially but lose its viability. If it never grows it remains an ovule. The development of the fertilised ovule of grapevines was investigated mainly by Kassemeyer and Staudt (1983) in cvs Weisser Burgunder (syn. Pinot Blanc) and Gewürztraminer, as shown below.

Nucellus

Seed growth commences immediately after fertilisation due to the onset of cell division in the nucellus. The relative growth rate of non-fertilised and aborted ovaries may be due to stimulation coming from the pollen tubes which enter the ovary. The relative growth rate increases again between days 5 and 6 and reaches a maximum on day 7 in both varieties. At this time the nucellus passes from the cell division phase which lasts for ten days after anthesis, to a phase of intensive cell elongation. 18–20 days after anthesis, the nucellus cells continue to elongate, become strongly vacuolated and reach their maximal size. According to several authors (Nitsch et al. 1960, Alleweldt and Hifny 1972, Alleweldt 1977), the commencement of growth by cell elongation may be in causal relationship with the accumulation of phytohormones in the embryo and the endosperm, as an increase in the auxin content of the grape berry is noted on about day 10 after anthesis.

Integuments

After fertilisation, intensive meristematic growth in the funiculus, raphe, chalaza, and outer integument determines the change in form of the seed (Periasamy 1962). Cell

division in the outer integument reaches its maximum 20–25 days after anthesis but ceases 45 days after anthesis. The cells of the inner integument divide anticlinally, this tissue thereby keeping pace with the growth of the outer integument (Coombe 1960). The development of the testa and the differentiation of the integuments commences with strong growth by cell elongation between days 14 and 16 after anthesis. The formation of a secondary cell wall with many pores leads to the development of a sclerenchyma sheath around the nucellus and the embryo sac from days 18–20 onward (Kassemeyer and Staudt 1983). The nucellus grows within the integuments into the space available to them (Nitsch et al. 1960). As in other angiosperms, the hypostase increases during the course of integument development and grows into the nucellar tissue (Tilton 1980). In the third growth phase of the nucellus, the integuments develop into the testa by commencing the formation of sclerenchyma tissue (Kassemeyer and Staudt 1983). This definition of 'testa' differs from that by Esau (1977) which is used in this study, namely 'the seed coat that develops from integuments of the ovule after fertilisation and seed growth'.

Endosperm

One to two days after the fertilisation of the diploid polar nucleus by the pollen nucleus, the now triploid primary nucleus of the endosperm moves to the centre of the embryo sac and starts to divide by free nuclear divisions (Barritt 1970, Okamoto and Imai 1982, Kassemeyer and Staudt 1983). The first division of the endosperm nucleus produces two nuclei one of which moves toward the chalaza and the other to the micropyle; these then divide every 3–4 days to form the polynuclear endosperm (Kassemeyer and Staudt 1983). The previously non-cellular endosperm starts to become cellular 20–25 days after anthesis (Barritt 1970, Kassemeyer and Staudt 1983). Lipid droplets and starch inclusions appear within endosperm cells at the same time (Kassemeyer and Staudt 1983). As the endosperm expands it consumes nucell^{ar} ~~us~~ ^{tissue.}

In the mature seed the endosperm fills the whole space up to the testa and its cells are composed of uniform small polygonal cells with minute intercellular spaces (Pratt 1971). The cell walls, especially of the outermost layer, are more or less thickened. The

cells contain oil and aleurone (protein) grains with crystals of calcium oxalate (Harris et al. 1968).

Embryo

The formation of the globular proembryo and its suspensor coincides with the formation of cell walls in the endosperm, possibly because the endosperm can then provide the embryo with an optimal supply of necessary metabolites, e.g. organic nutrients and growth substances (Brink and Cooper 1947, Schulz and Jensen 1977).

The first division of the zygote occurs in most cases between 2–3 weeks after anthesis (Barritt 1970, Kassemeyer and Staudt 1983, Ledbetter and Ramming 1989). During this period, the volume of zygote nuclei increases (Kassemeyer and Staudt 1983) presumably due to the duplication of the DNA content caused by the fertilisation. The first division of the zygote is transverse, dividing it into a larger basal cell which faces the micropyle and a smaller apical cell which faces the chalaza. The apical cell becomes divided by a longitudinal wall. Subsequent divisions of the apical cells lead to the formation of the initials of the cotyledons and the epicotyl. The basal cell divides transversely and, after further divisions, the hypocotyl, root initial and suspensor are formed from it (Pratt 1971, Kassemeyer and Staudt 1983). The proembryo is hormonally supplied by the endosperm at this stage (Kassemeyer and Staudt 1983) instead of by the suspensor which is poorly developed in the grapevine. This tissue, which connects the embryo to the wall of the embryo sac, takes on this role in other plants, e.g. in *Phaseolus* spp. where it is strongly developed (Alpi et al. 1975, Cionini et al. 1976). The mature embryo occupies only a small part of the seed. It is straight, with two cotyledons, a short hypocotyl and an apical meristem representing the epicotyl (Harris et al. 1968; Kim 1967, Ramirez 1969, Kassemeyer and Staudt 1983).

The development of each individual seed will depend on its ability to assimilate resources provided through the distribution system of the parent vine. This ability is likely to vary between seeds in multi-seeded berries to the extent that the seeds essentially compete for these resources. Bouard et al. (1980) showed that the mineral, phenolic and

lipid composition of the seeds varied according to both the number of seeds per berry and their relative positions within the locules.

2.1.5.2 Non-functional seeds

The transition from ovule to seed is liable to be upset by a number of accidents leading to reduced viability or non-viability of the seed. Stout (1936, cited by Pratt 1971) differentiated the development of the following types of ovule development: (a) viable, hard seeds formed after fertilisation (fertile seeds); (b) viable seeds formed through the mechanism of apomixy; (c) seeds without endosperm and/or embryo (empty seeds or floater seeds); (d) seeds ceasing development soon after fertilisation without formation of the hard seed coat (aborted seeds, seed traces); (e) abnormal ovules which could not be fertilised and which therefore cannot become seeds (residual, sterile ovules). Berries of seeded grapevine cultivars may contain all of these types (Stout 1936 cited by Pratt 1971, Pratt and Einset 1961, Barritt 1970) but to develop and mature normally they need to contain at least one completely developed seed or an empty seed. The failure of ovules to develop into seeds leads to parthenocarpy which is of two types, depending on whether ovule development fails before or after megasporogenesis (Stout 1936 cited by Pratt 1971). Zante Currant is a typically parthenocarpic cultivar.

Stenospermy

Berries of stenospermocarpic, seedless cultivars contain seed traces which are aborted sterile or fertilised seeds (Stout 1936 cited by Pratt 1971, Randhawa et al. 1962, Barritt 1970). Seed traces may lose their embryo/endosperm at early stages of development before reaching half the normal seed size. They may have soft testas or hard and brittle testas, being at least partly lignified, and may float or sink. Barritt (1970) and Ledbetter and Ramming (1989) discussed the reasons for varying sizes of seed traces. They concluded that the size was related to the relative time of embryo/endosperm breakdown. Embryo/endosperm abortion in cultivars known for very small seed traces

occurs typically at an early developmental stage. In cultivars having larger seed traces, breakdown occurs at a later stage of development. The environmental conditions during the growing season are thought to play a role in seed trace development and thus size.

Sultana is the prime example of a stenospermocarpic cultivar where the trace is soft, relatively long and very stable. Wang (1990) found that seedlessness in cv. Himrod was the result of a high percentage of ovules having abnormal development of the embryo sac and egg apparatus and limited numbers of nuclear divisions in the endosperm. Staudt and Kassemeyer (1984) investigated the frequency and development of small juicy berries ('chickens') in nine cvs of *V. vinifera* and concluded that these berries were not parthenocarpic, as previously assumed, but stenospermocarpic because of the presence of endosperm in their largest seed; the increment in ovule size indicated that fertilisation had occurred.

Empty-seededness

Some seeds approach full size through the development of the integuments and their conversion to hard testa, and therefore appear normal externally. However they lack the internal components of embryo and/or endosperm, and their nucelli deteriorate before berry maturity. Being hollow they float on water, a characteristic which distinguishes them physically from viable seeds which sink (Ledbetter and Ramming 1989, Marasali 1993). There is little information in the literature at what stage of development the abortion of the essential seed components occurs.

2.1.6 Berry development

Fruit-set can be defined as the stage when the ovary starts its conversion into the berry by not abscising and commencing to enlarge. Berry enlargement which follows fertilisation and setting commences within the week after anthesis. The berry then increases rapidly in size and mass. Berry growth follows a double-sigmoid curve with two stages of rapid growth separated by a lag phase.

Growth stage 1: Stage I of berry development commences with growth of seed and pericarp tissue, but with limited growth of the embryo. Early pericarp growth is by cell division which ceases within three weeks of anthesis and passes into a prolonged phase of cell enlargement. The cessation of cell division proceeds from the placental tissue outwards to the epidermis. The green, hard berries accumulate organic (mainly tartaric and malic) acids. The duration of stage 1 is typically 40–60 days (Mullins et al. 1992).

Growth stage 2: Stage 2, the lag phase, is denoted by slow growth of the pericarp and of maturation of the seeds (Coombe 1960, 1976, Mullins et al. 1992). During this stage, general metabolism slows, but embryo development is rapid. The berries remain hard and green until the end of this stage. The lag phase lasts 7–40 days, its length being strongly variety-dependent.

Growth stage 3: At the beginning of stage 3, the berries start to soften and accumulate sugar, then to develop colour, a process called 'veraison'. The resumption of rapid growth during this period is due solely to cell expansion. Germination of the seed is possible at veraison if the seed is given a period of cold treatment (Rives 1965). Stage 3 lasts approximately 35–55 days (Mullins et al. 1992).

2.1.6.1 Berry development in relation to seed development

Nitsch (1953) defined fruit as a matured ovary and the pericarp as a tissue which supports the ovules and whose development is dependent on the events occurring in these ovules. While the first part of the statement is an over-simplification (Coombe 1976, Esau 1977) there is wide support for the generalisation in that seed development affects fruit development. For instance, it is well known that grape berry size increases with seed number (Müller-Thurgau 1898 cited by Hardie 1992, Winkler and Williams 1936, Schumann 1973), and also the degree of seed development (Olmo 1946). The berries of the wild grapevine *V. sylvestris* rarely, if ever, contain four fully mature seeds, the average number per berry being three, while this number in the berries of cultivated seeded varieties

of *V. vinifera* was given as two (Tutin et al. 1968) However, there are considerable variations in this number both between and within cultivars, caused respectively by genetic and environmental factors (climate and viticultural practices).

The development of the pericarp and the concentration of some of its major constituents is known to be conditioned by seed development (Olmo 1946). Sugar concentration of the juice became less as seed number increased in a number of German cultivars (Schumann 1973). Philosophically, the allocation of metabolic resources to the development of the pericarp could be regarded as being "related to the 'value' of the resources committed to the development of the seed, a likely consequence of the intrinsic potential for survival of the embryo in the next generation of the vine" (Hardie 1992).

Seedless berries remain small probably because the hormonal stimulus supplied by seeds is missing. In particular, parthenocarpic cultivars such as the Zante Currant have very small berries while those of stenospermic cultivars with seed traces such as the Sultana are somewhat larger (Winkler et al. 1974).

2.2 Fruit-set

Fruit-set is a particularly important part of the cycle of fruit development in the grapevine which starts with inflorescence initiation and ends with the ripe fruit. It is also one of the stages of this cycle susceptible to the influence of environmental factors such as temperature and light (Coombe 1989). Fruit-set in the vineyard usually ranges from 5% to 40% in most *V. vinifera* cultivars and in some, e.g. Chardonnay, Muscat Gordo Blanco and Grenache, seasonal variations occur frequently. Years of poor setting ('coulure') are usually attributable to adverse weather conditions during the flowering period. Of the weather factors, variation in temperature is considered the usual cause of variable setting (Koblet 1966, May 1992).

2.2.1 Effects of environmental factors

2.2.1.1 Temperature

Temperature may affect fruit-set in grapevine through its effect on pollination, pollen tube growth or the fertilisation of the ovules and subsequent seed growth (Roubelakis and Kliewer 1976). Many investigators have found that good fruit-set in grapevines occurs with day temperatures between 20° and 30°C (Tukey 1958, Kobayashi et al. 1960, Alexander 1965, Ewart and Kliewer 1977, Kliewer 1977, Kubota and Shimamura 1982). Alexander (1965), working with cv. Sultana, found no significant difference between plants growing at day temperatures between 21° and 30°C combined with night temperatures between 19° and 25°C. Temperatures outside the range considered to be favourable for growth and development have been shown to affect the development and function of both the female and male sex organs (Staudt 1981, 1982). May (1992) showed that, in cv. Chardonnay, ^{in which the} whose sexual apparatus is functional under favourable weather conditions, temperatures below 15°C for 2 weeks during the flowering period caused abnormality in those organs. Exposing vines to low temperature of 15°/10°C day/night temperature two or one week before anthesis resulted in fewer seeds per berry in comparison with vines exposed to higher temperatures (Roubelakis and Kliewer 1976, Ewart and Kliewer 1977).

High temperatures also have a detrimental effect on fruit-set (Kobayashi et al. 1965, Buttrose and Hale 1973, Kliewer 1977). Kliewer (1977) tested the effect of high temperatures of 35°–40°C from 8–12 d before flowering until 12–18 d after flowering in cvs Carignane and Pinot noir and found that ovule fertility and seed number were smaller at 35°C or 40°C than at 25°C. High percentage fruit-set was also obtained at 33°C but berry development was abnormal. Similar results were obtained in parallel investigations by Ewart and Kliewer (1977).

Night temperature was found to be important in fruit-set and a rise in night temperature from 19° to 35°C decreased fruit-set (Kobayashi et al. 1960). Root temperature was shown to be effective in increasing fruit-set in grapevines. Woodham and

Alexander (1966) showed that the percentage of fruit-set increased with rise in root temperature from 11° to 30°C.

2.2.1.2 Light

Light is another major component of the environment and its intensity has an important effect on the rate of photosynthesis in grapevines growing under otherwise optimal conditions. Trellising, training, vine density and row orientation play important roles in determining the effectiveness of the interception of photosynthetically active radiation in a vineyard (Mullins et al. 1992, Silvestroni et al. 1993).

Light intensity is an important factor for inflorescence initiation and shading is inhibitory only during the initiation period itself (May and Antcliff 1963). As this period commences at about flowering time in the season preceding harvest, low light at flowering (full bloom) or immediately after, imposed by shading, reduced flower initiation and thus next year's crop in cv. Sultana (May and Antcliff 1963).

Heavy shade has been known to be responsible for reducing fruitfulness and its effect is not dependent on the spectral composition of the residual light. Differently-coloured types of shade material, applied to individual buds, produced different light spectra but no differences in inflorescence initiation (May 1965). Similarly, heavy shade was detrimental even when changes in temperature were prevented (Buttrose 1970b). Heavy shade at the inflorescence zone may increase the physiological disorder called 'early bunch stem necrosis' which reduces yield (Jackson and Coombe 1988, Jackson 1991, 1994). Ollat (1993) also found that inflorescence initiation is a light-intensity dependent phenomenon and varies with different varieties. In combination with sufficiently high temperature, ample sunshine in late spring and early summer favours fruitfulness of the buds being formed at that time (May and Antcliff 1963, Buttrose 1970a, Srinivasan and Mullins 1981).

Some information has been gathered to show that full sunshine at flowering promotes fruit-set. This may be partly through the direct effect of high light intensity and partly through the indirect effect of raised temperature. It may create good conditions for pollination and improve current photosynthesis and thus assimilate availability. Artificial shading of grapevines caused a reduction of carbon assimilation per shoot of about 60% in a glasshouse and 80% in a vineyard (Ollat 1993). The effect of artificial shading is in some respects quite different from that of light reduction by clouds, as clouds absorb varying amounts of long-wave radiation; furthermore, climatic components apart from light intensity also change during cloudy weather (May and Antcliff 1963).

Artificial shading, thus reducing light intensity experienced by treated vines by 90% during five or more days immediately before flowering, led to a significant reduction in per cent fruit-set and this effect became more marked as the period of shading lengthened (Nuno 1993). The reduction of PAR to values below $200 \mu\text{E m}^{-2}\text{s}^{-1}$ during periods varying from 24 to 4 days before flowering to the beginning of flowering caused complete failure to set. Heavy shade of 85% in a glasshouse and 90% in a vineyard reduced fruit-set of cv. 'Merlot noir' by 12% and 7% respectively (Ollat 1993). Bagging the leaves of topped, girdled shoots for two weeks reduced set of cv. Grenache vines by 81% while bagging the bunches reduced set by 17% (Coombe 1962); similar effects were found with Muscat of Alexandria, except that leaf bagging doubled the percentage of seedless berries (ie 'chickens'). Per cent fruit-set and ovule fertility were about three and eight times greater at a light intensity of 2680 ft-c. than at 750 ft-c. (Roubelakis and Kliewer 1976).

2.2.1.3. Water stress

Water supply is another important environmental factor affecting fruit-set. Alexander (1965) applied treatments of varied temperature and water supply to small plants of cv. Sultana and showed that reduction in fruit-set was not due to high temperature *per se* but to inadequate water being available under such stress conditions.

Water stress was damaging for setting when occurring during and until one week after set was complete, but not later.

2.2.1.4 Mineral nutrition

Grapevines can adapt to a wide range of soil conditions and are less exacting than many other horticultural crops in the quantitative level of soil nutrients required (Winkler et al. 1974). The available evidence suggests that they draw upon reserves of carbohydrate and mineral nutrients during fruit-set and early fruit growth (Conradie 1991). However specific mineral nutrients have been associated with variations in grapevine fruit-set.

Nitrogen is a primary component of proteins and energy-transfer systems and of chlorophyll. It is one of the macroelements where deficiency may lead to decreased yield of grapevines. At the same time, ^{excessive}~~too heavy~~ fertilizing with nitrogen may result in poor fruit-set and the initiation of fewer inflorescences. Wolk and Pool (1988) found that fruit-set of non-fertilized vines was greater than fruit-set of vines that had received 84 kg nitrogen per hectare. High level of nitrogen may also lead to a disorder known as primary bud-axis necrosis which ultimately results in decreased yield potential (Perez-Harvey 1991).

Phosphorus is a macroelement ^{which}~~that~~ plays a critical role in the reproductive cycle of the grapevine. Skinner and Mathews (1989) showed that a deficiency of phosphorus caused significant decreases in berry number and bunch weight. They showed that inadequate phosphorus led to failure of inflorescence development even though initiation had occurred. A direct effect of phosphate application in improving the setting of Shiraz grapes was shown by Tulloch and Harris (1970) — a significant increase of 23% in berries per bunch; this is one of the few such findings that have been reported.

Boron plays an essential role in the biosynthesis of auxin in the meristems of the plant and plays an important part in cell division and cell enlargement in apical cells. Kobayashi and Okamoto (1967) found that boron deficiency prevented the normal

development and germination of pollen, thus reducing fruit-set or causing 'hen and chicken'. They also found that sprays of boron applied before flowering promoted pollen germination in Muscat of Alexandria and suggested that this might be associated with increased proline, alanine and total sugars in anther extracts. This was confirmed by Bamzai and Randhawa (1967). Gärtel (1974) showed that the boron deficiency of the stigma prevented pollen germination, and fertilisation did not take place. An increased fruit-set following boron spraying of vines in boron-deficient soils has been reported by Okamoto and Kobayashi (1970), Fregoni et al. (1979), Dabas and Jindal (1985) and Bavaresco (1989). Additionally, Fregoni et al. (1979) found that boron, applied as fertiliser to the soil or as spray to the flowers or leaves of grapevine cultivars with physiologically female or normal flowers, not only increased fruit-set, but also decreased the number of shot berries.

Zinc is important in fruit-setting. It has long been known that zinc is required for the synthesis of auxins, e.g. Skoog (1940). Zinc deficiency may cause poor fruit-set in grapevines e.g. in Sultana (Alexander 1964). In Muscat Gordo Blanco, Orton (1949, quoted by Alexander 1964) found that zinc treatment increased seed formation and fruit-set and reduced the proportion of seedless berries, thereby reducing the severity of 'hen and chicken'. Foliage application of zinc sulphate increased bunch weight and yield, presumably through improved setting (Alexander 1964, Myrianthousis 1983, Ezzili 1993).

Molybdenum is known to be involved in nitrogen metabolism. Kraus and Glos (1975) showed that spraying with ammonium molybdate at least 15–20 d before flowering improved fruit-set in grapevines.

2.2.2. Vine growth and fruit-set

2.2.2.1. Interactions with vegetative growth

It has been suggested that fruit-set in grapevine is influenced by the supply of organic nutrients to the inflorescences during and after anthesis (Coombe 1962, 1965, Koblet 1966). Organic nutrients are the end result of photosynthesis by leaves. Per cent fruit-set has been found to be directly proportional to the number of illuminated mature leaves adjacent to the inflorescence and, inversely, to be negatively affected by immature leaves, the shoot apex and by shading of leaves (Coombe 1962). Viticultural practices such as tipping (the removal of the terminal few centimetres of the shoot tip, Coombe 1962, Kobayashi and Okamoto 1967, Skene 1969, Naito and Kawashima 1980) and topping (the removal of 10–15 cm of the shoot which includes the shoot tip and some leaves, Coombe 1959, 1965, Cargnello et al. 1978) may increase fruit-set in grapevine. The mechanism of this effect may be a reduced competition between the inflorescence and those parts of the shoot which are net importers of organic nutrients, namely immature leaves and the shoot tip, both being very strong sinks. Girdling (removing a narrow complete ring of bark 2–5 mm wide around the vine trunk at some point below the foliage) is a practice which usually increases fruit-set (Coombe 1970, Chundawat et al. 1979, Jindal et al. 1982, Brown et al. 1988, Frisch 1991, Ibacache et al. 1992). A girdle interrupts the normal downward flow of phloem material to the roots and causes an accumulation of compounds such as carbohydrates and plant hormones in the parts above the wound. Other practices such as inflorescence thinning, flower thinning and canopy management have also been shown to improve fruit-set in grapevine (Moss 1964, Vergnes 1980, Myrianthousis 1983, Amarjeet and Daulta 1989).

2.2.2.2. Plant growth regulators

Stimulation of fruit-set is a way of increasing yield, and this has prompted much research on the use of plant growth regulators for this purpose in viticulture. Fruit-set is

influenced by several of the main groups of naturally occurring growth substances: auxins, cytokinins and gibberellins and, in addition, various growth retardants which do not occur naturally. Because of complex interactions between genotype, environment and growth regulators, it is difficult to ascribe specific roles to individual compounds (Mullins et al. 1992). One suggestion is that growth substances, endogenous or exogenous, influence fruit-set through effects on the partitioning of organic nutrients (Coombe 1973). This is consistent with promoting fruit-set by pinching, tipping or the removal of young leaves, all such treatments reducing the number of competing sinks (Coombe 1962). Promotion of fruit-set by metabolite diversion is also in accord with the phenomenon of hormone-directed transport in other growth processes. It is for instance hypothesised that hormones originating in the seeds or pericarp direct the import of nutrients from other parts of the plant (Mullins et al. 1992). The growth regulators that have been shown to affect fruit-set of grapevine fall into two groups: growth promoters (i.e. auxins, cytokinins, gibberellins) and growth retardants.

Growth promoters

Synthetic auxins were tested on grapevines in the late 1940s and early 1950s as a means of enhancing the development of berries and of stimulating the set of parthenocarpic fruit (Coombe 1950, 1962, Antcliff 1967a, b). There is considerable endogenous auxin activity in grape flowers before anthesis (Ito et al. 1969). Commercial use of auxins to promote fruit-set and development has been almost wholly confined to the principal seedless grape cultivars Zante Currant and Sultana. Only one report of a positive response to auxin on fruit-set in a seeded cultivar has been made but that has not been followed up (Weaver et al. 1961). Other seeded cultivars either showed no response and inhibition of seed development, or a promotion of set of 'shot' berries, parthenocarpic berries which do not soften and ripen (Considine 1983). 4-CPA (4-chlorophenoxy acetic acid) was one of the auxins which was widely used to increase fruit-set and berry weight in seedless cultivars (Coombe 1950, Gifford et al. 1960, Weaver and McCune 1960, Weaver et al. 1962, Bindra et al. 1983, Sharma et al. 1984). Use of 4-CPA in viticulture is now minimal because it has been largely replaced by other plant growth regulators, especially GA and

the growth retardant CCC (see below). In Zante Currant this is due to the greater susceptibility of 4-CPA-treated fruit to damage, e.g. to cracking and splitting by rain (Considine 1983).

Cytokinins have been shown to regulate inflorescence growth and substitute for the role of roots in maintaining the growth of young inflorescences (Mullins 1967, 1968). Precocious flowering has been induced by cytokinins. Srinivasan and Mullins (1981) showed that seedlings of the grapevine cvs Cabernet Sauvignon and Muscat Hamburg were induced to flower within four weeks of germination by repeated treatment of shoot apices with the synthetic kinin PBA (6-benzylamino purine). The same results were obtained by application of PBA to plantlets derived from somatic embryos of a *Vitis vinifera* x *Vitis rupestris* hybrid. In cytokinin-treated plants, tendrils were converted into inflorescences. Cytokinins were the first class of plant growth regulators shown to be capable of promoting substantial and, in some instances, almost complete set of all ovaries present at flowering in some varieties of grapevine (Weaver et al. 1966, Naito et al. 1974, Srinivasan and Mullins 1979); some types of growth retardants have the same potential. In Cardinal, a seeded cultivar, application of PBA at 50% anthesis doubled the number of seeded set berries (Leonard et al. 1961). In Sultana, PBA increased the proportion of berries set to just more than two-fold, but only the highest concentration tested (1000 ppm) caused a significant increase in berry weight (Weaver et al. 1966). There are some problems associated with the application of cytokinins which cause variation in results. These problems are probably associated with poor absorption and an unpredictability of the amount of cytokinin administered when it is added to the solvent in amounts exceeding its solubility (Considine 1983).

Gibberellin (GA) use on grapevine has been comprehensively reviewed by Considine (1983). He stated: "The most extensive work on plant growth regulators in viticulture concerns that of application of GA to grape flowers or young fruit in a wide range of seeded and seedless cultivars to encourage the growth in size of parthenocarpic fruits and to reduce fruit-set. Parthenocarpic grapes are the most responsive, followed by those fruit which are stenospermocarpic and which respond only after the embryo has aborted.

Those seeded cultivars which responded, did in a manner which was related to the degree and number of seeds per berry and the degree of their development. The presence and degree of seed development appears to regulate, not necessarily the responsiveness of fruit growth, but the timing of the response. The cultivars which respond do so most intensely in response to GA applied within a few days of full bloom; stenospemocarpic cvs and seedless fruit on clusters of seeded cvs appear generally to be most responsive to treatment at about the time of embryo abortion (about 10 days after bloom), while those seeded cultivars which respond do so at the end of the first phase of growth, at the end of growth of the testa and nucellus expansion. More recent research has demonstrated considerable variation in degree of response to applied GA in both seeded and seedless cultivars. Actual examples of GA-induced increase in numbers of fruit-set are rare; induced increase in fruit-set in the literature refers incorrectly to GA-enhanced fruit growth".

Grape seeds are a rich source of GA-like substances. A higher level of GA-like substances was found in pericarp tissue of GA-induced seedless berries than in seeded berries at the early stage of berry growth (Naito and Nakano 1971). After GA treatment, some flowers of cv. Muscat Bailey A appeared abnormal and the proportion of the flowers which set was low (Udea and Naito 1985). GA has been found by many authors to induce parthenocarpic berry formation (Kononov 1960, Muranishi 1968, Ito et al. 1969, Singh et al. 1971, Ueda and Naito 1981, Fukunaga and Kurooka 1988). "This effect was confined to cultivars whose ovules matured before anthesis" (Zuluaga et al. 1971). Pre-flowering spray of GA increased the proportion of seedless berries per bunch and this effect was stronger if it was followed by a post-flowering spray (Lee et al. 1986, Clark et al. 1993). Uarma (1991) suggested that the high number of seedless berries indicated that berries had set that would have abscised if not treated with GA. GA treatment increases inflorescence length (Branas and Vergnes 1960, Al-Dujaili et al. 1987) peduncle xylem development (Theiler and Coombe 1985) and causes berry lengthening (Muranishi 1968, Christodoul et al. 1968, Fukunaga and Kurooka 1988). In some cases GA also increases yield (Zuluaga et al. 1968, Singh et al. 1971, Funt and Tukey 1977, Al-Dujaili et al. 1987, Batukaev 1988). The germination capacity of the pollen from bunches receiving pre-

flowering GA treatment appeared to be reduced (Muranishi 1968), the degree of reduction varying with the variety and the timing of gibberellin application (Itakura et al. 1965).

Growth Retardants

The group of synthetic chemicals known as plant growth retardants only became known in the 1960s but since that time have attracted much interest. The effect first recognised was their ability to reduce the shoot length of plants without changing development patterns or being phytotoxic. This is achieved primarily by reducing cell elongation, but also by lowering the rate of cell division (Rademacher 1991). But, being a diverse group, growth retardants were found to have many other effects also.

The existing growth retardants form three main groups: (a) ethylene-releasing compounds e.g. ethephon, (b) inhibitors of GA translocation e.g. daminozide, and (c) inhibitors of GA biosynthesis e.g. chlormequat chloride (CCC) and mepiquat chloride (Rademacher 1991). In their effect on the morphological structure of plants, growth retardants are antagonists of gibberellins and auxins, the plant hormones that are primarily responsible for shoot elongation. Evidence is available that certain plant growth retardants affect the endogenous levels not only of GAs but also of cytokinins, ethylene and abscisic acid; the auxin status of plants appears to be less affected (Rademacher 1991).

Retardants have been commercially useful in modifying many aspects of plant growth and development e.g. reducing lodging in cereals, retarding senescence of harvested product and increasing fruit-set in grapevine (Coombe 1967). In fact, plant growth retardants are the only class of plant growth regulators that have found general acceptance as a means of increasing the setting of grapes (and also other fruits). Of these chemicals, those which have been most widely tested are CCC and daminozide.

CCC (2-chloroethyl trimethyl ammonium chloride, Cycocel) appears to be the most prominent representative of the group of growth retardants which inhibit GA biosynthesis by inhibition of ent-kaurene synthetase A, necessary for GA synthesis (Rademacher 1991). CCC increases the fruitfulness in grapevines e.g. in Muscat Gordo Blanco either

indirectly, by control of vegetative growth, or directly by inducing changes in the differentiation of anlagen through its effect on GA biosynthesis (Coombe 1967). CCC increases fruit-set of cultivars of *V. vinifera* or of inter-specific hybrids which include *V. vinifera* as a parent (Coombe 1965, 1967, Bairamowa et al. 1976, Peacock and Jensen 1977, Bajwa 1979, El-Hamady et al. 1979, Menary 1979, Nii 1979, Ahlawat and Dault 1981, Myrianthousis 1983, Kumar and Singh 1984, Kilany et al. 1986, Mohammad et al. 1987, Brown et al. 1988, Smirnov 1988). The effectiveness of CCC in increasing the number of set berries is independent of the presence or absence of seeds in fruit of particular cultivars and generally does not affect the ratio of seeded to seedless berries set (Coombe 1967). One exception is a report where CCC increased the number of seedless berries (Brown et al. 1988). Coombe (1967) found in addition that CCC on Muscat of Alexandria caused the development of inflorescences on almost every node of the lateral shoots.

Daminozide (succinic acid-2, 2-dimethyl hydrazide, Alar) is the second most important growth retardant which increases fruit-set. It appears most likely that daminozide reduces GA biosynthesis and the translocation of GAs or GA precursors to actively growing tissues but may also promote GA catabolism and conjugation (Komatsu and Nakagawa 1991, Rademacher 1991). Daminozide is used at concentrations an order of magnitude greater than CCC and has been shown by many workers to increase fruit-set in grapevine especially in *V. labrusca* hybrids (Hull 1966, Tukey 1970, Naito et al. 1974, Naito and Hayashi 1976, Morris et al. 1977, Morris et al. 1978, Nii 1979, Naito and Kawashima 1980, Okamoto et al. 1980, Naito et al. 1981, Naito et al. 1983, Isoda 1989). The application of daminozide to bunches of Kyoho grape depressed the growth of flowers, particularly the size of the ovary wall, when applied before and during bloom (Nii 1979, Naito et al. 1981, Naito et al. 1983). It increased fruit-set by improving the fertilisation of the ovules (Naito et al. 1983). Like CCC, daminozide applied during flowering and setting inhibited shoot elongation and stimulated the development of flowers (Naito et al. 1974, Kraus and Glos 1975, Smirnov 1988). The set of seeded berries was increased by applying daminozide before flowering (Naito et al. 1983, Isoda 1989).

Daminozide treatment increased the number of seeds per berry in addition to increasing the number of seeded berries per bunch (Naito and Kawashima 1980, Naito et al. 1983).

Mepiquat chloride (1, 1- dimethylammonium chloride, Mp-cl) is another plant growth retardant which inhibits GA biosynthesis as does CCC; it has been effective in increasing fruit-set. Mp-cl sprayed on one shoot of each vine close to anthesis increased the set and yield of *V. labrusca* cv Concord grapevines; it also restricted the length of not yet fully expanded internodes, but not of those formed after treatment. Unlike most growth retardants, MP-cl appears to exert its effect directly on the developing flowers rather than via vegetative growth control (Pool 1982) .

Paclobutrazol [1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl)- pentan-3-ol, PP 333] is one of the triazole-type compounds that inhibits GA biosynthesis as does CCC. It has attracted a great deal of interest because of its potential in shoot growth inhibition and in some cases in increasing fruit-set (Rademacher 1991). Paclobutrazol also increases the fruitfulness of seedless grapevines by controlling the vegetative growth, or by inducing changes in the differentiation of anlagen through its effect on GA biosynthesis. Nir (1991) working with the seedless cvs Thompson Seedless, Superior and Perlette found that paclobutrazol had the same effect as CCC in promoting the development of inflorescences on almost every node of the lateral shoots, suggesting that paclobutrazol induced changes in the differentiation of primordia from tendrils into inflorescences. These inflorescences produced a late maturing 'second crop' and it was also found to increase fruit-set in the seedless cvs Thompson Seedless and Superior and at the same time to reduce the size of the berries (Nir 1991). However, the relatively high persistency of paclobutrazol has restricted its use to ornamental plants (Rademacher 1991).

There are two different ideas about the mechanism of action of growth retardants in promoting fruit-set in grapes. The first proposal is that the effect of growth retardant is due to a correlated inhibition of shoot growth whereby organic nutrients are diverted to the developing ovaries. This was supported by inverse correlations found between rate of shoot growth and degree of fruit-set (Skene 1969, Coombe 1970). The second proposal is

that growth retardant treatment of leaves and shoots before flowering has a direct effect on berry set by increasing cytokinin level in flowers and thereby improving the fertilisation of ovules which is causally associated with improved berry set (Naito et al. 1974, Naito and Hayashi 1976, Naito et al. 1981). These authors found that ^{treatment of} inflorescences ^{dipping into} CCC or daminozide about three weeks before flowering consistently increased berry-set in seeded cultivars without any inhibitory effect on shoot elongation. Also, the endogenous cytokinin levels were higher in retardant-treated inflorescences. However, Naito et al. (1983) found subsequently that the difference in set found between bunches on strong and weak shoots could not be explained in this way. Thus it remains unresolved whether the ideas contained in one or the other proposal satisfactorily explain the mechanism by which growth retardants affect fruit-set in grapes.

For commercial applications, environmental considerations must come into play when the use of some growth substances and growth retardants is contemplated.

2.3. Model experimental plants

~~Horticultural research in general and including viticultural research faces a great number of problems relating to natural sciences and the application of their solution in the field. A major one is the testing of environment effects on crop plants, There are two possibilities for testing:-~~

For ^{woody perennial} crop plants, There are two possibilities for testing:-

1. Experiments with the plant under field conditions. These types of experiments, despite their advantages in dealing with natural environmental conditions, have important disadvantages in the difficulty of sufficient control of environmental variables to permit interpretation of results and in the large areas of vineyard required for detailed experimentation.

2. Experiments with the plant grown under controlled environmental conditions. This technique helps to clarify certain narrowly-defined questions in a short period of time. The main constraints which the method imposes are that of growing plants in

containers in a way that resembles their growth in the field. For herbaceous crop plants this aspect is not serious but, for woody perennials, special methods of plant culture need to be developed to produce a plant sufficiently small to fit into growth chambers but large enough to make its behaviour plausible, especially with respect to fruiting behaviour.

Model plants have been used successfully with a number of horticultural crops (Bünemann 1972), for example small fruiting orange 'trees' (Lenz 1967). The grapevine is another horticultural plant that has been successfully developed as a model plant. This followed from work by Alexander and Woodham (1963) and Alexander (1966) and the subsequent discovery by Mullins and Rajasekaran (1981) of the method of maintaining inflorescence retention, fruit-setting and berry growth on rooted grapevine cuttings by the removal of immature basal leaves as buds are bursting. Such plants have been used for many research topics including fruitfulness, flower development, fruit-set, competition between vegetative and productive growth, ontogeny of berries, nutrition, physiological disorders and hormonal studies (Alexander 1965, Buttrose 1966, Buttrose 1968, Buttrose 1970a, b, Mullins 1966, 1967, 1968 Buttrose and Hale 1973, Hale and Buttrose 1973, Roubelakis and Kliewer 1976, Ewart and Kliewer 1977, Kliewer 1977, Hawker and Walker 1978, Srinivasan and Mullins 1978, Srinivasan and Mullins 1979, Mullins and Rajasekaran 1981, Jackson 1991, Khurshid et al. 1992, Ollat 1993). The present research work has also been dependent on the application of this methodology (see section 3).

Chapter 3

General materials and methods

3.1 Controlled environment

Growth rooms

Two growth rooms were used to grow experimental plants. Temperature, light intensity and photoperiod were adjustable in these two growth rooms. The light source was two sets of 5 (32/80 watt) 'cool white' fluorescent tubes (Philips TLF 80/33) supplemented with two EYE Sunlux NHT 360-LX high pressure sodium lamps and two EYE Multi-Hi-Ace M400 LE/BUH high pressure Metal Halide lamps in each set. Light intensity was adjusted to $400 \mu\text{Em}^{-2}\text{s}^{-1}$ by pulling down the banks of lights and the light intensity was measured using a LI-COR model LI-185A Quantum/Radiometer/Photometer and a LI-COR Quantum sensor. In all experiments, plants were grown in a photoperiod of 14 h (06.00 to 20.00).

Growth cabinets

Controlled environmental growth cabinets (type Zankel, Adelaide) were used for exposing vines to the required temperature regime. Temperature was measured at the level of inflorescences with an alcohol-filled thermometer and a TDS digital-thermoelectric thermometer type 942. The photon flux density at the inflorescence level was maintained at approximately $350\text{-}400 \mu\text{Em}^{-2}\text{s}^{-1}$. The light sources and measurements of light intensity were the same as in the growth rooms. In all experiments, plants were grown in a photoperiod of 14 h (06.00 to 20.00). The relative humidity in the cabinet was not controlled.

3.2 Producing small fruiting plants

Dormant one-year-old canes of Chardonnay (clone I10V1) and Shiraz were collected at pruning time in the field and the canes were immersed for 5 h in a 5% solution of the fungicide Chinosol (8-hydroxy-chinoline sulfate). Cuttings of 25 cm length were prepared which had varying numbers of nodes. The two uppermost buds on each cutting were retained and all others were cut off. The basal end of each cutting was below node 3 of the cane from which it derived. The cuttings were planted in coarse sand in a box with a heating cable coiled over its base; the box was located in a cold room held at 4°C. The cuttings were placed so that their bases were 5 cm above the cable and thereby heated to 26°C while their uppermost 10 cm, including the two buds, were above the surface of the sand at 4°C. Tap-water was given every two days.

After four weeks, the rooted cuttings with the still-dormant buds were planted in pots of 10 cm diameter filled with a 6:3:1 mixture of perlite, vermiculite and peat moss and placed into one of two growth rooms. The temperature of this room (growth room 1) was initially 15°/10°C day/night, and was increased daily by 1°C until it reached 20°/15°C. Each vine was transferred to another growth room (growth room 2) when its first bud had burst. This room was held at 25°/20°C and a light intensity of 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ during the 14 hours per days.

Inflorescence retention and development was promoted by using the method of Mullins and Rajasekaran (1981). This involves retaining only the first-burst bud per rooted cutting, removing the newly appearing leaves up to the level of the inflorescence, tipping the developing shoot and permitting a single lateral shoot to grow at a node adjacent to and basal to the inflorescence; this shoot is tipped after it had lengthened to four leaves. Axillary buds are removed until two weeks after the first flower opens after that one axillary shoot is permitted to grow to produce about four additional leaves to support berry growth until harvest. Note that shoot development is much faster under these conditions compared with the same genetic material grown in the field, e.g. four

weeks from budburst to flowering in model vines compared with nine weeks in the vineyard.

Some vines were held throughout in growth room 2 at 25°/20°C until the berries were ripe; they represented the control treatment (C) of the experiments. Other vines remained in growth room 2 for most of the time, but were transferred for one week to one of two growth cabinets held at different and lower temperatures, and then returned to growth room 2. This was done with different plants during four specified stages of vine development.

3.2.1 Water and nutrient supply

Plants were irrigated with half-strength Hoagland solution prepared by using different nutrient compounds as follows:

Potassium nitrate	KNO_3	500 mg/L
Calcium nitrate	$\text{Ca}(\text{NO}_3)_2$	82 mg/L
Potassium dihydrogen orthophosphate	KH_2PO_4	136 mg/L
Magnesium sulfate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1010 mg/L
Iron citrate/ Fe-EDTA	$\text{FeC}_6\text{H}_5\text{O}_7 \cdot 5\text{H}_2\text{O}$	5.00 mg/L
Manganese sulfate	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1.52 mg/L
Zinc sulfate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.20 mg/L
Copper sulfate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08 mg/L
Boric acid	H_3BO_3	0.32 mg/L
Ammonium molybdate	$(\text{NH}_4)_6 \text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.11mg/L

Stock solutions were prepared for each of the compounds, sufficient to make up 1000 litres of half-strength Hoagland solution. Nutrients were dissolved and the solutions later diluted. ^{Water purified by (RO water)} Reverse-osmosis water was used to make up the solutions.

3.2.2 Timing of watering

Plants were irrigated with half-strength Hoagland solution when 60% of 'available water' had been used up. To determine available water, a sample of planting medium (mixture of 6:3:1 of perlite, vermiculite and peat moss) was taken. It was air-dried at room temperature for 24 h and then dried in an oven at 105°C for 24 h. The difference in weight of these two measurements showed the dry weight of the sample of planting medium. The sample was divided equally into two sub-samples by weight and both were saturated with water. One of the sub-samples was held at 33.8 kPa and the another at 1521 kPa pressure inside a pressure plate. The sub-samples were weighed and the difference between their weights was termed the "available water".

Ten plants each of Chardonnay and Shiraz cuttings, planted in an equal amount of compost (on a weight basis) were weighed at the beginning of each experiment and from then on daily to determine when 60% of the 'available water' was consumed. Then, irrigation was applied to all plants of the experiment. This exercise was repeated every third day when the plants were at an early stage of growth and every second day later on.

3.3 Microscopic examination

Light microscopy

Samples of flowers and berries were fixed in FPA50 (formalin: propionic acid: 50% ethanol, 5: 5: 90) for 3 to 7 days. For light-microscopic observations, ovules and seeds were dissected and then processed according to Feder and O'Brien (1968). They were dehydrated in a series of ethanol, propanol and butanol, two hours in each . Infiltration was done by transferring samples into a 50 : 50 mixture of butanol and GMA (93 ml of 2-hydroxyethyl methacrylate polyethylene glycol, 7 ml of polyethylene glycol 400 and 0.6 g benzoyl peroxide) for 2 h. Finally samples were placed twice into GMA for 24 h. Each sample was then placed in a gelatine capsule and kept at 60°C for two days. Serial sections were cut longitudinally at 4 µm thickness with Microtome 2050 Supercut

(Reichert-Jung). The sections were stained with periodic acid Schiff's reagent and toluidine blue O. For all solutions, reverse-osmosis water (RO water) was used. The procedure was as follows:

1. immersed in 2, 4-dinitrophenyl hydrazine solution for 30 min;
2. rinsed in running water for 1 h;
3. placed in 0.2% periodic acid for 30 min;
4. rinsed in running water for 5 min;
5. placed in Schiff's reagent (BDH) for 1 h;
6. immersed three times for 2 min into 5 : 5 : 90 solution of sodium metabisulfate (10 g sodium metabisulfate in 100 mL water, 5mL; HCl 1 N, 5mL; water, 90mL);
7. rinsed in water;
8. placed for 5 min in 0.05% toluidine blue in benzoate buffer pH 4.5 (0.29 g sodium benzoate, 0.25 g benzoic acid, 200ml water, mixed for 30 min);
9. rinsed in water until plastic around tissue is discoloured;
10. dried in fume hood for 20-30 min and mounted using Histoclad mounting medium (Clay Adams Parsippany, NJ, USA).

Whole pistils were used for the observation of pollen germination and pollen tube growth. They were fixed in FPA50 (see above) for one week, hydrated in ethanol (70% and 30%, 30 min each) and immersed twice for 30 min in RO water. Pistils were softened in 0.8 N NaOH at 60°C for 1 h (when harvested 2 d after opening) or for 1.5 h (when harvested 4 d after opening). Samples were then stained overnight in decolourised aniline blue (0.1% water-soluble aniline, 0.1N K_3PO_4 (7.67g K_3PO_4/L + 1.0 g/L aniline blue in 1 L water). They were mounted in 80% glycerol, squashed gently by pressing onto the cover slides, and studied, using a microscope with ultra-violet illumination.

Experiments

Three main experiments were done which are described in the various chapters as follows:

Table 3.1: List of experiments

Experiment	Cultivar	Environment facility	Temperatures	Light intensity ($\mu\text{Em}^{-2}\text{s}^{-1}$)	Described in chapter
1	Chardonnay and Shiraz	Growth room	25°/20°C,	400	4, 6, 7, 9
		Growth cabinets	17°/14°C, 12°/9°C	400	
2	Chardonnay and Shiraz	Growth room	25°/20°C	400	4, 9
		Growth cabinets	17°/14°C, 12°/9°C	370, 240, 112	
3	Chardonnay	Vineyard	—	—	5, 8

3.4 Statistical methods:

The plants in experiments 1 and 2 were allotted in factorial arrangement to a Randomised Block Design with five replications of 4-vine plots in the growth rooms, but to a Completely Randomised Design in the growth cabinets during their one-week exposure to different temperature regimes or light intensities.

There was just one set of control plants, therefore the data of all sets of observations were analysed in two ways: firstly, as a factorial analysis of variance (coded ANOVA type A) where the Control treatment was excluded; secondly, by ignoring the factorial arrangement, by including the Control and analysing the data in a one-way ANOVA (coded type B). The type A analyses for experiment 1 contained the factorial combinations of 2 treatments of the variable 'temperature' (17°/14°C, 12°/9°C), of the 4 treatments of the variable 'stages' (the period of one week when the temperature treatments were applied, at E-L stages 15, 16, 17, 20) and the 2 treatments of the variable 'pollination' (+, -). In experiment 2, the ANOVA type A had only 2 treatments of 'stages' (15, 17) and pollination treatments were not applied. However, there were 3 treatments of the variable 'shading' (8%, 40%, 72%). In ANOVA type B, Control was included, giving 17 treatments in experiment 1 and 13 treatments in experiment 2, both being non-factorial. Genstat 5 statistical program was used to analyse the data. Where appropriate, Tukey's test of means separation was applied.

Various stages of experimental procedures are illustrated in the following figures.

Fig. 3.1. Chardonnay shoot at E-L stage 12, deleafed up to the first inflorescence and tipped at that node. Insert: budburst, *E-L stage 4.*

Fig. 3.2. Chardonnay inflorescence at E-L stage 15, flowers in compact groups.



Fig. 3.3. Chardonnay inflorescence at E-L stage 16, pedicel and rachis lengthening; lateral shoot elongating and at 4-leaf stage.

Fig. 3.4. Chardonnay inflorescence at E-L stage 17, single flowers separating; shoot tipped to 4 leaves.



Fig. 3.5. Shiraz inflorescence at E-L stage 15, flowers in compact groups.

Fig. 3.6. Shiraz inflorescence at E-L stage 16, pedicel and rachis lengthening; lateral shoot elongating and at 4-leaf stage.

Fig. 3.7. Shiraz inflorescence at E-L stage 17, single flowers separating; shoot tipped to 4 leaves.



Fig. 3.8. Plants growing in the growth room, 25°/20°C, showing the light system; two sets of five cool white fluorescent tubes supplemented with high pressure sodium lamps and high pressure Metal Halide lamps in each set).

Fig. 3.9. Model plants at flowering time, showing paper cones to collect calyptrae.



Fig. 3.10. Pre-rooting of cuttings in a hot box, located in a 4°C room.

Fig. 3.11. Chardonnay bunch after fruit setting.



Chapter 4

Effect of short-term temperature and shading treatments on fruit-set and berry number in *Vitis vinifera*, cvs Chardonnay and Shiraz

4.1 Introduction

This chapter describes two experiments in which the consequences for fruit-set were tested when temperature and light intensity were varied during flower development and early flowering. The background for these experiments was described in chapter 1 and relevant literature references on the subject were presented in chapter 2.

The hypothesis underlying this study was that the conditions of temperature and light intensity during certain periods of the development of the male or female flower parts, or of both, affect their functional efficiency, and that this in turn is important for the subsequent events of flowering, fruit-set and yield. Furthermore, any such changes in 'floral efficiency' are morphogenetic in nature and due to changes in the environment over short periods, and as such not controlled by the correlative effects of whole-plant responses. Information in the literature supports the view that at least some of the physiological processes involved in the sequence of events of flower formation, flowering, fruit-set, and growth of seeds and berries are sensitive to fluctuation in the environment, and that some cultivars are more sensitive than others.

The hypothesis was tested by using two cultivars, Chardonnay and Shiraz (syn. Petite Syrah). These two cultivars are of major economic importance, the latter particularly in Australia, and were selected because of their suspected differing sensitivity to changing weather conditions which may lead to problems of fruit-set. The impossibility to study the related problems in short-term field experiments made the use of controlled environment facilities necessary.

4.2 Materials and methods

Experiment 1

Vines for this experiment were produced as described in chapter 3, section 3.2. Budburst occurred in Chardonnay between days 5 and 10, and in Shiraz between days 9 and 14, after placement into growth room 1. Each vine was watered throughout the experiment with 35 mL of half-strength Hoagland solution whenever the water content of ten sample pots had been lowered to 40% of 'available water'. More plants than necessary were produced to allow selection for uniformity.

The vines were held from budburst onward in growth room 2 at 25°/20°C. Some remained throughout in that growth room as the Control treatment. Others were taken from the growth room at four stages of inflorescence development, transferred for one week into one or the other of two growth cabinets held at lower temperatures, and then returned to growth room 2. They were the vines of eight treatments, namely the combinations of the variables 'temperature' and 'stage'. The two treatments of the variable 'temperature' (T) were day/night temperatures of 12°/9°C and 17°/14°C. The four treatments of the variable 'stage' (S) during which vines were exposed to the two treatments of T were chosen according to phenological stages defined according to the E-L scale of Eichhorn and Lorenz (Coombe 1995): stage 15 (flowers of inflorescence in compact groups), stage 16 (rachis and pedicels lengthening), stage 17 (single flowers separated) and stage 20 (flowering with 10-20% of flowers open).

In Chardonnay, a further variable, 'pollination' (P), was included which had two treatments, namely presence (+P) or absence (-P) of supplementary hand-pollination. To obtain pollen for hand-pollination, Chardonnay and Shiraz vines were grown in growth room 2. Their pollen was collected every morning throughout the flowering period, air-dried and mixed in equal amounts. It was applied at 9.00 next morning to the inflorescences of the (+P) vines to supplement their own pollen. This could not be done for the Shiraz vines because the fused petals ('caps') of the flowers did not detach themselves in time to allow hand-pollination.

There were five replicates of the nine treatments (C, 2T x 4S) using two-vine plots in Shiraz and four-vine plots in Chardonnay. In the latter, two of the vines were allotted to (+P) and two to (-P). For each cultivar, the vines were assigned at random to the treatments; these were arranged in a randomised block design in growth room 2, and completely at random in the growth cabinets.

On each inflorescence, the flowers (determined from the number of the shed caps collected in a paper cone surrounding the stem) and the ripe berries were counted.

The data for per cent fruit-set and number of ripe berries per inflorescence were statistically analysed as described in chapter 3, section 3.4.

Experiment 2

The plants for experiment 2 were raised in the same way as for experiment 1, with small changes as indicated below. The treatments applied differed from those of the first experiment as follows:

- 1) Three treatments of varying light intensity were applied during the week-long stay of the plants in the growth cabinets.
- 2) The temperature and shading treatments were applied at two phenological stages only, E-L stage 15 and E-L stage 17.

For the combined temperature/light intensity treatments, three growth cabinets were used, first at 12°/9°C and subsequently at 17°/14°C day/night temperature. The plants to be exposed to the higher temperature regime were planted later to start their development after an appropriate delay. Light intensity in the three cabinets, when fully lit, was 370, 350 and 470 $\mu\text{E m}^{-2}\text{s}^{-1}$. Variations in light intensity were provided by interposing three types of shade cloth which caused light reductions of 8%, 40% and 72%. To provide the required six environmental conditions, each of the three growth cabinets was divided into two equal sections by a white-painted separating wall placed in such a way as to allow free air flow and thus uniform temperature conditions. The lights in each section were shielded

by an appropriate type of shade cloth. The plants were placed in the middle part of each section to avoid edge effects.

The treatments applied to the plants were the factorial combinations of two levels of the variable 'temperature' (T) and three levels of the variable 'shading' (L), applied at two stages of the variable 'stage' (S). Plants held at 25°/20°C throughout were used in Control. Stage 15 was reached one week after shoot tip removal and stage 17 two days before flowering.

After initially growing as in experiment 1 in a 6:3:1 mixture of perlite, vermiculite and peat, and being watered with half-strength Hoagland solution, the plants were transplanted into soil in trays when the berries were about 5 mm in diameter, watered from then on with tap water and held in a glasshouse at 25°/ 20°C ± 2°C until the fruit was ripe.

In both cultivars, the number of flowers (from counts of shed flower caps) and of berries at maturity were counted and per cent fruit-set calculated.

All data of each measured or calculated set of observations were statistically analysed as described in chapter 3, section 3.4.

4.3 Results

Experiment 1

Flower numbers and time of flowering

Flower number per inflorescence did not vary significantly between the treatments in either Chardonnay or Shiraz in experiment 1 (Table 4.1). This is not surprising as all vines were growing under uniform environmental conditions just before and after budburst when the individual flowers were formed. Average flower number per inflorescence was 309 for Chardonnay and 323 for Shiraz. Although the vines had been selected for uniformity,

flower number varied considerably, from 192 to 474 in Chardonnay and from 218 to 577 in Shiraz.

The time elapsed from removing the shoot tip to the opening of the first flowers was about 18 days in the Control vines of Chardonnay and 21 days in those of Shiraz. Lowering the temperature temporarily slowed the rate of development of the inflorescences as shown by the increase in the number of days up to flowering (Table 4.1). With Chardonnay, the delay was from one to eight days: delays were longer in vines subjected to 12°/9°C, and especially so when the lowered temperatures were imposed close to flowering.

Table 4.1: Flower number of Chardonnay and Shiraz vines. For treatment description and ANOVA type A analyses see chapter 3, section 3.4.

Temperature	25°/20°		17°/14°				12°/9°				F
	Control	15	16	17	20	15	16	17	20		
Flower Number											
Chardonnay	290	314	282	332	328	269	335	306	323	ns	
Shiraz	321	333	290	310	305	349	372	372	296	ns	
Days to Flowering											
Chardonnay	18-9	19-20	20-21	21-22	18-19	21-22	23-24	26	18-19	n.a.	
Shiraz	20-21	22-23	22-23	24-25	20-2	23-24	23-24	26-27	20-21	n.a.	

Significance of F value: ns not significant; n.a. not applicable

None of the flowers opened when the vines were held at 12°/9°C during stage 17; they commenced flowering two days after being returned to 25°/20°C and the flowers shed their caps normally. The shedding of the flower caps of to 17°/14°C at stage 17 started during day 3 of the stay in the growth cabinet; four days later, at the time of their return to 25°/20°C, 20% of the caps had fallen and flowering ended two or three days later.

With Shiraz, the delay in flowering was 2–5 d at 17°/14°C and 3–7 d at 12°/9°C, and the delays were similar when the temperature treatments were applied during the pre-flowering stages 15 and 16. Many flower caps became brown and did not dehisce (they were 'stuck'). This occurred 3–4 d after the vines were returned from 12°/9°C to 25°/20°C

and affected all the caps of vines exposed to 12°/9°C and 40–50% of those exposed to 17°/14°C. Because of this supplementary pollination was not possible in Shiraz.

Fruit-set and berries per bunch

Table 4.2 shows per cent fruit-set and berry number per bunch as the means of the variables, namely of 'temperature' (T), 'stages' (S) and 'pollination' (P) for Chardonnay, and of T and S for Shiraz, together with the mean of the control treatment (C) in experiment one. The levels of significance of the analyses of variance type A are also given. This information is further illustrated in Fig. 4.1 and complemented in Appendix, Table 3 which give the treatment means of Control and of each of the two temperatures at each of the four stages (type B), together with the significance levels of the analyses of variance type B for the treatment differences in Chardonnay and Shiraz, in the former separately for the vines that did or did not receive supplementary pollination.

In Chardonnay, there were significant differences between the respective treatments of the variables T, S and P for both fruit-set and berry number. None of the treatment interactions reached significance, although the differences between 'stages' must have been due in part to differences in the timing of the temperature effects. Per cent fruit-set was smaller on vines exposed to 12°/9°C compared with that for vines exposed to 17°/14°C or held at 25°/20°C throughout; fruit-set was also lower when temperature treatments were applied late during flower development ^{and early flowering} (stages 17 and 20), and without supplementary pollination.

The reasons for these differences are shown in Fig. 4.1. With or without supplementary pollination, the vines exposed to the lower temperature regime shortly before or during the early part of flowering (stages 17 and 20) set fewer berries. When the vines were exposed to 17°/14°C, per cent fruit-set was not reduced significantly in comparison with that on the Control vines although it tended to be somewhat smaller at stages 17 and 20. The effects of the various treatments on berry number per bunch closely resembled those on per cent fruit-set.

Table 4.2: Per cent fruit-set and berry number per bunch of Chardonnay and Shiraz vines. For treatment description and ANOVA type A analyses see chapter 3, section 3.4.

Variable	Control	Temperature			'Stage'					Pollination		
	Treatments	25°/20°	17°/14°	12°/9°	F	15	16	17	20	F	—	+
Chardonnay												
% Fruit-set	9.9	8.0	5.9	***	7.6 ^{ab}	8.8 ^b	5.1 ^b	6.0 ^b	**	6.2	7.8	***
Berry Number	27.1	25.1	18.7	**	21.1 ^{ab}	24.5 ^a	17.3 ^b	18.1 ^{ab}	**	18.2	23.3	*
Shiraz												
% Fruit-set	14.2	12.3	12.2	ns	14.2	12.2	10.8	12.9	ns	n.a.	n.a.	n.a.
Berry Number	37.4	36.5	35.5	ns	38.8	37.5	33.7	34.2	ns	n.a.	n.a.	n.a.

Significance of F value: ns not significant; *, **, ***: significant at $p < 0.05$, $p < 0.01$, $p < 0.001$. Values marked by different superscript letters differ significantly according to Tukey's test

In Shiraz, per cent fruit-set overall was greater than in Chardonnay, and the bunches had about twice the number of berries. There were no significant differences due to the variables 'temperature' or 'stages' (Table 4.2) and the differences between the treatment means shown in Appendix, Table 1 were comparatively small, even though berry number per bunch of vines exposed to 12°/9°C at stage 20 tended to be smaller than that of vines of the best treatment.

Experiment 2

Flower numbers

Flower number per inflorescence was not affected by any of the treatments as was the case in experiment 1, and for the same reasons. Although plants had been selected for uniformity when allotting them to the various treatments, flower number per inflorescence still varied, in Chardonnay from 181 to 447 and in Shiraz from 345 to 726. Mean flower number per inflorescence per treatment varied in Chardonnay from 270 to 370, with a mean of 285, and in Shiraz from 378 to 585, with a mean of 497.

Fruit-set and berries per bunch

When tested by type A analyses of variance (data not shown), the Chardonnay means for per cent fruit-set and berry number per bunch proved to be significantly less in the plants exposed temporarily to 12°/9°C instead of to 17°/14°C or in plants given the differential treatments of temperature and shade at stage 17 instead of at stage 15. However the three levels of temporary shading did not cause significant differences in either of the two variables. In contrast to Chardonnay, the Shiraz plants did not differ significantly in either per cent fruit-set or in berry number per bunch as a consequence of their exposure to the treatments of the variables 'temperature', 'shading' or 'stage'.

These results are further illustrated in Fig. 4.1, and shown in Appendix, Table 2 where the results of the type B analyses of variance are shown. In Chardonnay, interrupting the 25°/20°C temperature regime for one week by exposure to either 17°/14°C or 12°/9°C at stage 17 reduced significantly per cent fruit-set and berry number per bunch compared with Control 25°/20°. Although not significant, there was a trend for both fruit-set and berry number to decrease with increasing intensity of shading imposed at stage 17 and at 17°/14°C. No values for stage 15 differed significantly from Control.

The number of berries classified as 'chickens', was small. During the examination of all the bunches only 22 such berries were found. Of these, 18 were on the bunches of treatment 12°/9°C, and 15 of the 18 on bunches exposed to this temperature regime at stage 17.

4.4 Discussion

Normally in the field, the interval from budburst to flowering takes about 9 weeks whereas in the present study, using model vines under controlled environment conditions with temperatures of 25°/20°C, this interval was only 4 weeks. Despite careful selection of the cuttings for uniformity of size before the commencement of the experiments and again when selecting the plants for uniformity in inflorescence size after bud burst, flower number per inflorescence varied considerably in both cultivars between the plants within each experiment and also on average between the plants of the two experiments. These variations were unrelated to any treatments applied during the course of the experiments. Such variability can only be reduced by having large numbers of plants available for pre-experiment selection. Limitations of space and other resources then come into play. The differences in mean flower number per inflorescence between the two experiments were caused by the different origin of the cuttings and were obviously due to local and seasonal effects on bud fruitfulness. The cuttings had been collected in different vineyards and in two different seasons,

The temperature treatments in both experiment show that, by transferring small vines in pots from 25°/20°C to 12°/9°C day/night temperature for one week shortly before and during the early period of flowering, fruit-set was reduced. A period of 'cold' earlier during inflorescence development did not have the same detrimental effect. The temperature effect was more pronounced in Chardonnay—a cultivar susceptible to irregular fruit-set in the vineyard—than in Shiraz in which fruit-set is more regular. The period of 'cold' imposed near flowering time delayed the onset of flowering.

Ewart and Kliewer (1977) found no differences in per cent fruit-set between Cabernet Sauvignon vines exposed continuously to 25°/10°C, 25°/20°C or 15°/10°C starting one week before anthesis. This is in contrast to the findings in both of the present experiments for Chardonnay where one week of exposure to 12°/9°C reduced fruit-set, but agrees with what was found for Shiraz. On the other hand, Buttrose and Hale (1973) showed that exposing Cabernet Sauvignon vines to 14°/9°C for the whole period from budburst to flowering completely prevented fruit-set. In this case, the effects were presumably due to a reduction in metabolic activity, mainly carbohydrate accumulation, combined perhaps with a diversion of the limited metabolites to more sinks each more active than the inflorescences. The effects after an exposure to 'cold' temperatures for one week, shown here, are more likely to be directly on the development of the pollen and the ovule.

Fruit-set in the vineyard usually ranges from 5% to 40% in most *V. vinifera* cultivars (Coombe 1973). According to May (1992), field-grown vines of Chardonnay suffered from reduced fruit-set and some abnormalities in the sex organs when daily temperature maxima were below 15°C for two weeks during the flowering period. Vines near those from which the Chardonnay cuttings for the present experiment were collected had three-year averages of 270 flowers per inflorescence and a fruit-set of 35% which resulted in about 100 berries per bunch (May, unpublished data). The corresponding values for the control vines of the present experiment were ca. 300 flowers, ca. 10% fruit-set, and 27 berries. Thus, the number of flowers per inflorescence of the rooted cuttings were within the range of that of the field-grown vines but the cuttings had smaller per cent fruit-set and therefore fewer berries per bunch.

Fruit-set under field conditions is considered to be strongly influenced by the supply of metabolites to the inflorescence during and after anthesis (Coombe 1973). On this basis alone, the small experimental vines used here could not be expected to support the level of fruit-set obtained in the field. The limited amount of reserves stored in the cuttings had already been reduced by the formation of the roots, and the cuttings lacked photosynthetic activity due to the early defoliation.

Temperatures outside the range considered to be favourable for growth and development have been shown to affect the development and function of both the male and female sex organs (Staudt 1981, 1982).

Applying foreign pollen can substitute for faulty autogenous (own) pollen. Randhawa and Negi (1965), Uppal and Mukherjee (1968) and Samaan et al. (1981) ascribed positive effects on fruit-set to supplementary pollination, at least in some instances. In the present experiment, fruit-set was also improved by cross-pollination as shown by supplying a mixture of Chardonnay and Shiraz pollen, produced under favourable conditions, to Chardonnay inflorescences that were held at 12°/9°C just before and during the early phase of flowering. Thus, foreign pollen may have substituted autogenous pollen of weakened viability due to its development under low temperature. In the treatments without supplementary pollination, the necessary arrangement of the experimental vines in the controlled environment facilities did not entirely exclude the possibility of cross-pollination whereby inflorescences with pollen formed under unfavourable conditions would have received pollen from plants held throughout under favourable conditions. However the possibility of the results being affected by cross-pollination can be discounted. The ~~gentle~~ air movement within the climate facilities ~~would~~ ^{was} ~~have been~~ ^{considered} unlikely to carry sufficient numbers of pollen grains to make them compete successfully with the large numbers supplied from within each inflorescence. Furthermore, the timing of the receptivity of the stigmata varied sufficiently between the treatments which set well or poorly to exclude cross-pollination.

Light, apart from temperature, is another important environmental factor which has been shown to have morphogenic effects during two periods of the seasonal cycle of grapevines, namely floral initiation and flowering. Field experiments on the effect on fruit-set of placing shades over whole vines have given variable results, from complete inhibition of fruit-set to almost no effect. For instance, Nuno (1993) found no effect of shading vines on fruit-set in one season but significant on effects in the other season. Coombe (1959) described his results of shading as erratic in contrast to other authors who showed significant effects (Roubelakis and Kliewer 1976, Ollat 1993). It can be concluded that

there is considerable variation in descriptions of the effects of reduced fruit-set after shading, some indicating damaging effects during pre-flowering, others during flowering, some leading to 'hen and chickens', while others do not, as in the present experiment. The heavy shading required to cause reduction in set is not likely to be reached in the field for any length of time, if ever. It is also clear that genetic differences between cultivars play an important role. Overall, temperature is an important – probably the single most important – environmental factor in affecting fruit-set.

4.5 Conclusions

- In Chardonnay, per cent fruit-set and berry number per bunch were greatly reduced by exposing plants for one week to 12°/9°C just before and at the early stage of flowering. There was a small but not significant tendency for fruit-set to decrease with increasing shade applied at this time.
- Supplementary pollination significantly increased per cent fruit-set and berry number per bunch in Chardonnay although the effect was smaller than that of temperature.
- In Shiraz, the differences between the various treatment means were small and mostly not significant confirming the important role that genotype plays in grape fruit-setting.

Chapter 5

Ultrastructure of the flowers of grapevine cultivars Chardonnay and Shiraz

5.1 Introduction

The ultrastructure of male and female sexual organs of grapevine flowers has been investigated by a number of workers (Scholefield and Ward 1975, Lombardo et al. 1976, Cargnello et al. 1980, Carraro et al. 1981, Ahmedullah 1983, Santa Maria et al. 1994, Roytchev 1995). Pollen morphology including pollen shape, exine sculpturing, and size and frequency of perforations of the exine accompanied with polar axis/equatorial diameter ratios have been used as means of indentifying grapevine cultivars (Ahmedullah 1983, Santa Maria et al. 1994, Roytchev 1995). Here structural differences of stigma and pollen between two cultivars were examined in an attempt to determine whether differences in structure or function of pollen and stigma may relate to fruit-set and seed development.

5.2 Materials and methods

Flowers of Chardonnay and Shiraz were collected on day 2 after anthesis from 4-year-old vines in the vineyard. Flowers were fixed in FPA 50 for 3 days and dehydrated in an ethanol series (50%, 75% and 100%), 15 minutes in two changes of each. Pollen grains were collected as described in chapter 6. Flowers were transferred to a mixture of ethanol and acetone (1:1) for 10 min, and then transferred to 100% acetone. They were dried in a Balzers critical-point-drier, and both flowers and pollen grains were mounted on stubs covered with double-sided tape. Samples were coated with 100 angstrom spectroscopically pure carbon and 200 angstrom gold palladium and observed by field emission scanning electron microscopy (FESEM) at 10 kV.

5.3 Results

The five petals of the grape flower are fused at the apex (Figures 5.1 and 5.12). However they detach themselves at their base and are thrown off as 'caps' (calyptra) when flower opening proceeds normally. This was the case with all flowers of both cultivars here examined.

Pistil

The pistil consists of a bilobed stigma, a short style and a well developed ovary. Nectaries are located on the receptacle at the base of the ovary (Figures 5.2, 5.4 and 5.13). The stigma carries papilla cells. These were turgid in Shiraz on the day of flower opening (Figures 5.15 and 5.16) but had collapsed by day 2 (Figure 5.14). In Chardonnay, the stigma was normal in external appearance on day 2 after flower opening (Figure 5.3). Deterioration of stigma cells started at the centre of the stigma and created a shallow hollow in both cultivars (Figures 5.5 and 5.17).

Pollen grain

Chardonnay pollen grains were found to be ellipsoid with flat ends (Figures 5.8, 5.9 and 5.19), tricolporate (Figure 5.10) with a germ pore in each furrow. The pattern of the exine showed many small perforations, at greater density in Shiraz (Figure 5.20) than in Chardonnay (Figure 5.11). Density of perforations was uniform in Shiraz but not in Chardonnay, in which some grains had as many perforations as those in Shiraz (Figure 5.8) while others had very few (Figure 5.9). Exine perforations were larger in Shiraz than in Chardonnay, such that the size of the large perforations of Shiraz was about twice that in Chardonnay (Figure 5.20). There were many pollen grains on the stigma surfaces of both cultivars. In Chardonnay, most of the pollen grains were dehydrated and shrivelled, and only a few were hydrated and spherical (Figure 5.6). A germinated pollen grain with a pollen tube growing into the stigma was observed in one of the Chardonnay flowers (Figure 5.7). In Shiraz most of the pollen grains had a spherical hydrated appearance (Figure 5.18).

5.4 Discussion

The stigma of Shiraz was completely collapsed on day 2 of flower opening, but this was not the situation in Chardonnay. This may indicate that Shiraz pollen was more viable and had produced many pollen tubes, resulting in stigma collapse (Sedgley 1982). In Chardonnay many pollen grains failed to hydrate and did not produce pollen tubes. The failure of pollen grains to germinate in Chardonnay could be due to faulty pollen grain development, or problems in pollen-stigma recognition. As per cent germination of Chardonnay pollen grains on artificial media was about half of that in Shiraz (see chapter 6), it appears likely that there was a problem in pollen grain development rather than in pollen-stigma interaction.

Pollen studies in *Vitis vinifera* L. for the identification of cultivars are of limited use, in themselves but helpful when complementary to other observations. However, pollen morphology with regard to size, shape and exine patterns may be more useful for species identification, stated by Ahmedullah et al. (1981). By classifying 42 cultivars of *Vitis vinifera* into five groups based in pollen shape, i.e. morphology, ultrastructure, and polar axis/equatorial diameter ratios (P/E), he concluded that these characteristics can be useful for taxonomic purposes. Polar axis/equatorial diameter ratios and exine patterns by themselves did not reveal great differences among cultivars. However, when all features were considered together, the pollen of each cultivar had a unique pattern, and this can be used as a means for cultivars identification when added to leaf, flower, and bunch characteristics. The present study was not concerned with variety classification, but with a possible operational difference between the two cultivars in pollen or stigma. The observations suggest the existence of functional differences between them; while no definite judgment can be made, more detailed observations may be useful.

Fig. 5.1: Calyptra of Chardonnay, showing the demarcation of five petals at the top of the flower. Bar represents 500 μm .

Fig. 5.2: Chardonnay flower on the day of anthesis, showing anthers (a) close to the pistil. Bar represents 1 mm.

Fig. 5.3: Stigma and style of Chardonnay one day after anthesis, showing no indication of stigma collapse. Bar represents 100 μm .

Fig. 5.4: Chardonnay flower one day after anthesis, showing functional stigma (s), nectaries (n) and receptacle (r). Bar represents 500 μm .

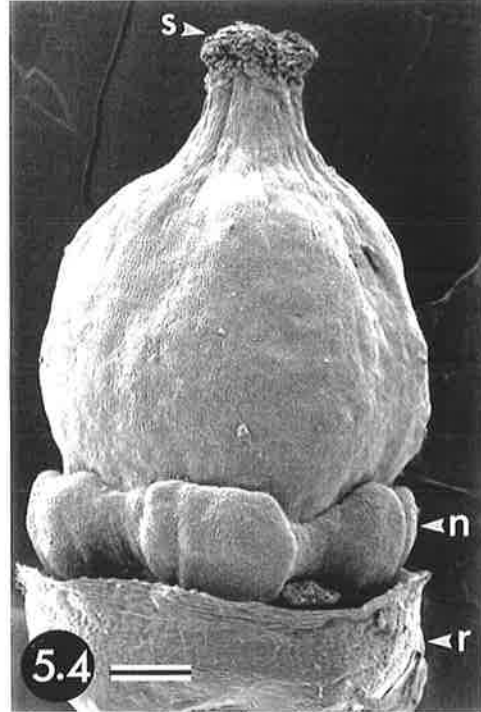
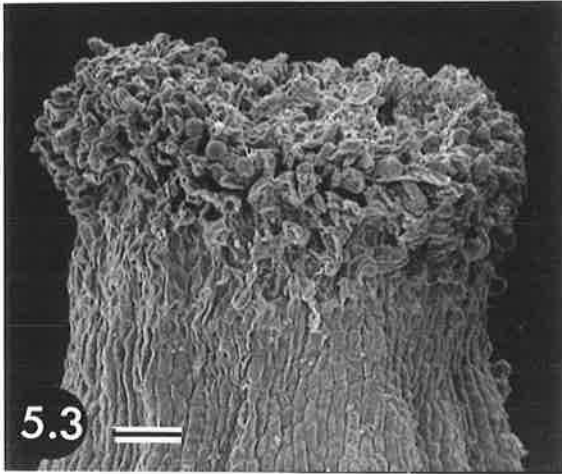
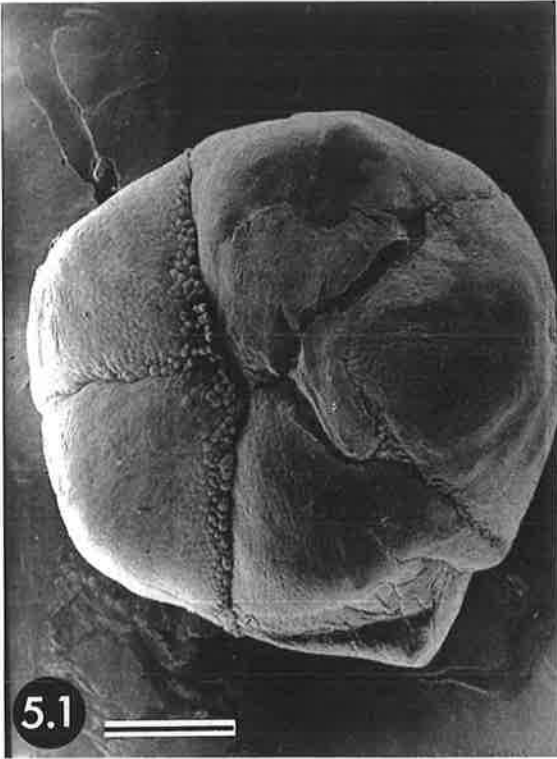


Fig. 5.5: Chardonnay stigma one day after anthesis, showing many dehydrated pollen grains. Bar represents 100 μm .

Fig. 5.6: Pollen grains on the Chardonnay stigma one day after anthesis, showing hydration of one pollen grain (arrow) compared with others which are dehydrated. Bar represents 20 μm .

Fig. 5.7: Germinated pollen grain on the stigma of Chardonnay one day after anthesis, showing the pollen tube. Bar represents 10 μm .

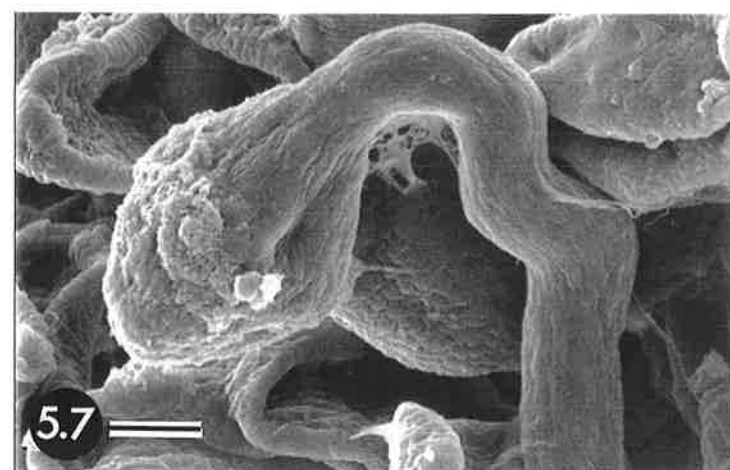
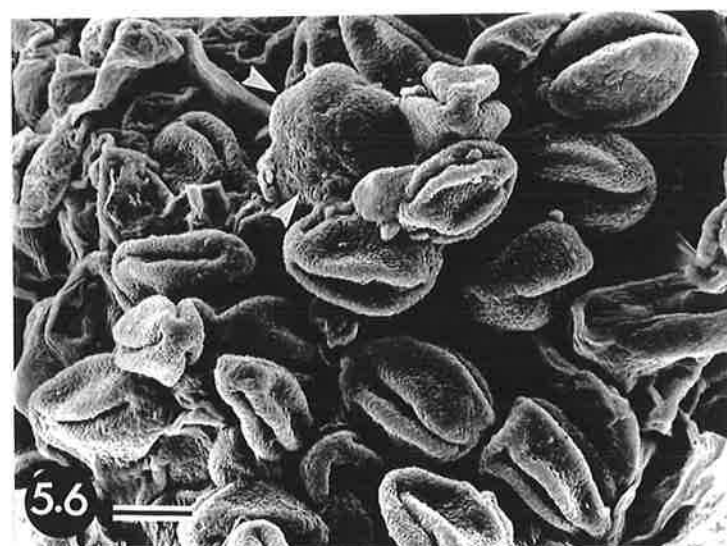
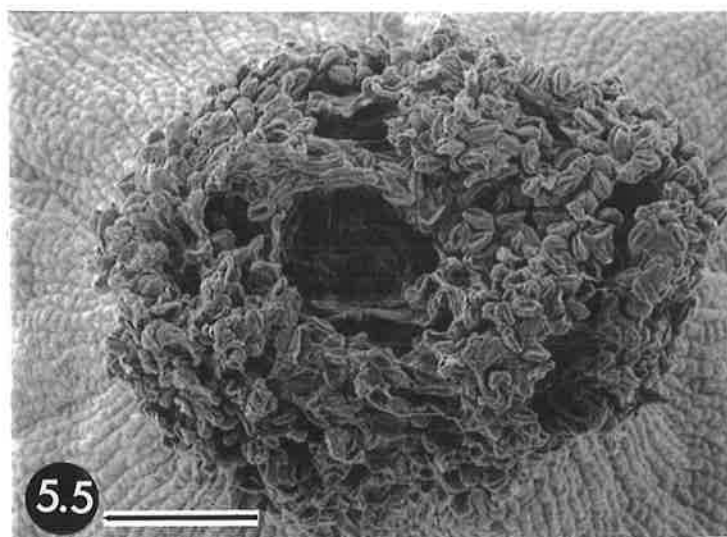


Fig. 5.8: Ellipsoidal pollen grain of Chardonnay, showing many perforations on the exine and one of three furrows. Bar represents 5 μm .

Fig. 5.9: Ellipsoidal pollen grain of Chardonnay, showing few perforations on the exine and one of three furrows. Bar represents 5 μm .

Fig. 5.10: Pollen grain of Chardonnay showing all three furrows from the top. The Bar represents 5 μm .

Fig. 5.11: Patterning of exine of Chardonnay pollen grain, showing small perforations. Bar represents 2 μm .

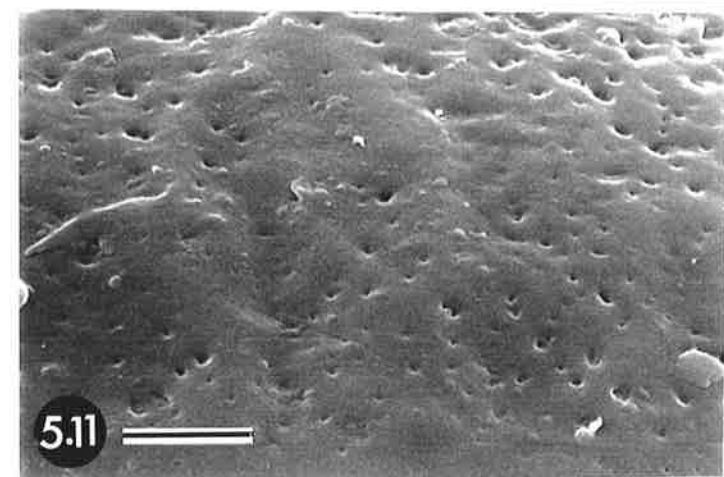
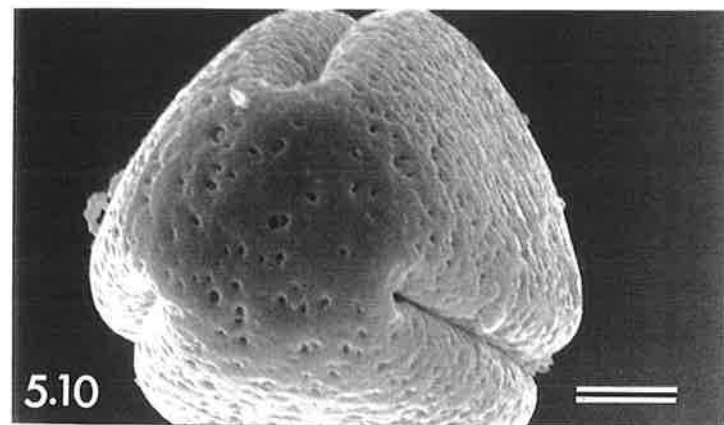
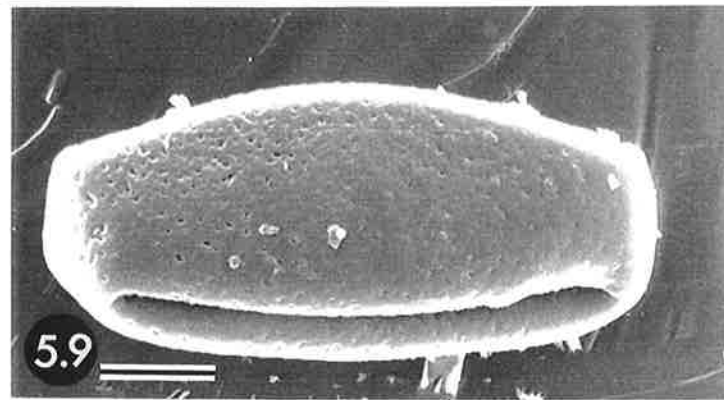
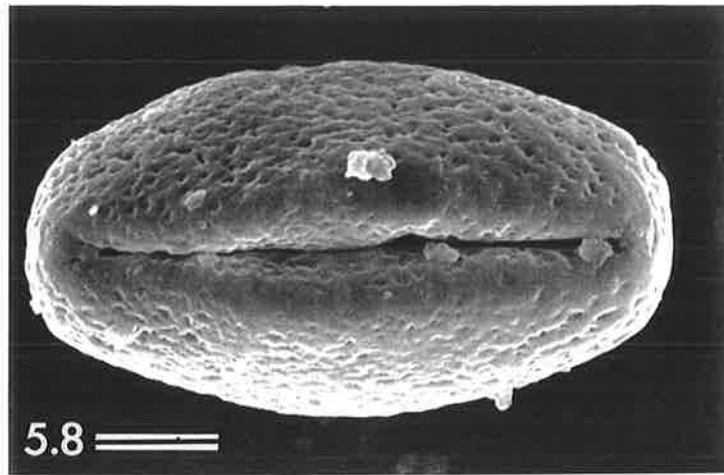


Fig. 5.12: Calyptra of Shiraz, showing demarcation of petals at the top of the flower. Bar represents 500 μm .

Fig. 5.13: Shiraz flower one day after anthesis, showing collapsed stigma (s), nectaries (n) and receptacle (r). Bar represents 500 μm .

Fig. 5.14: Collapsed stigma of Shiraz pistil one day after anthesis. Bar represents 100 μm .

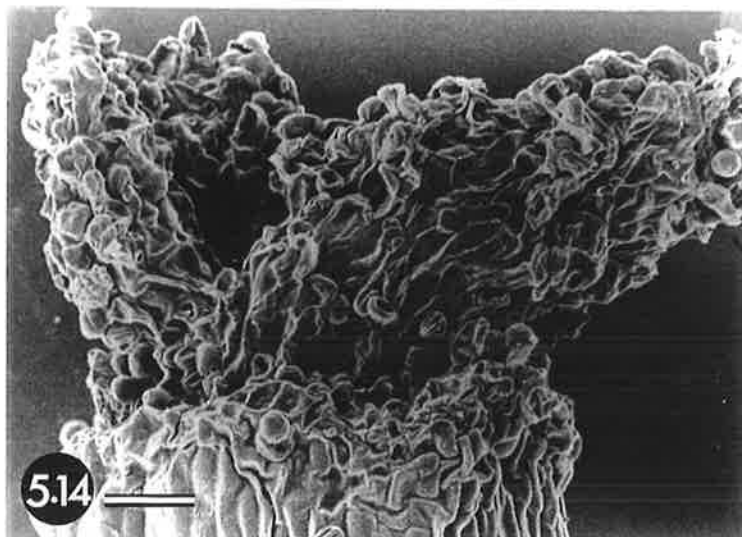
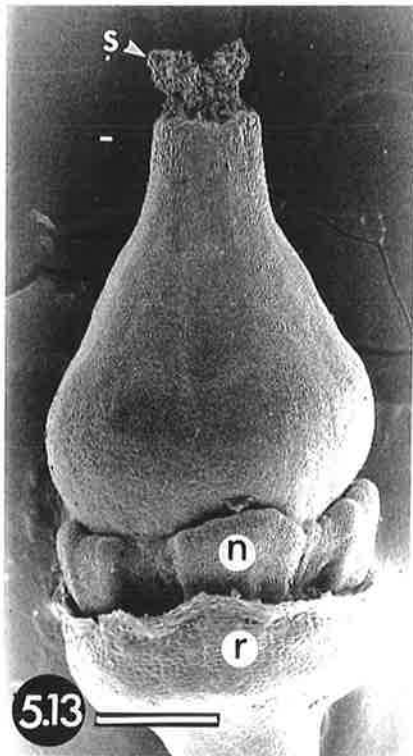
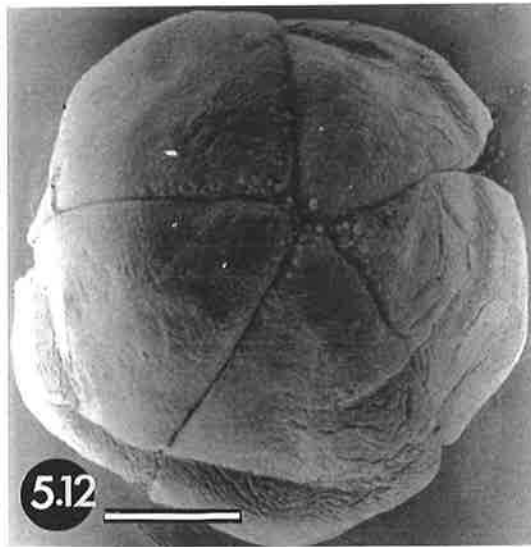


Fig. 5.15: Stigma and style of Shiraz flower on the day of anthesis. Bar represents 100 μm .

Fig. 5.16: Papillae tissue of the stigma of Shiraz with hydrated pollen grains on the day of anthesis. Bar represents 50 μm .

Fig. 5.17: Pollen grains on the stigma of Shiraz one day after anthesis, showing hydration of most pollen grains and a large collapsed area resulting from degeneration of stigma cells. Bar represents 100 μm .

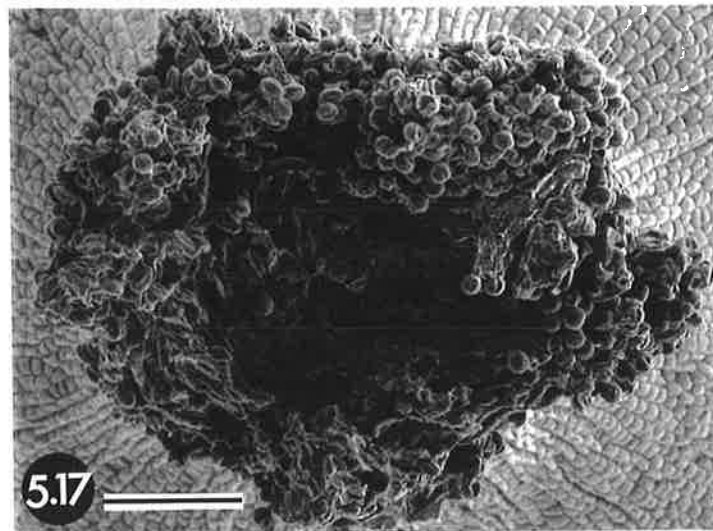
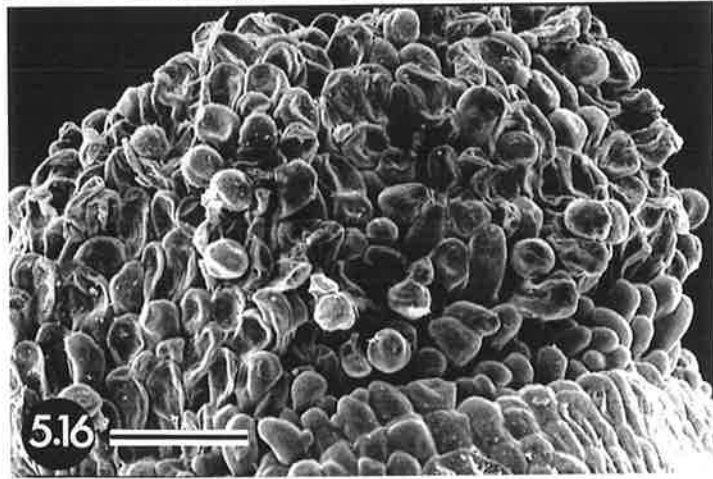
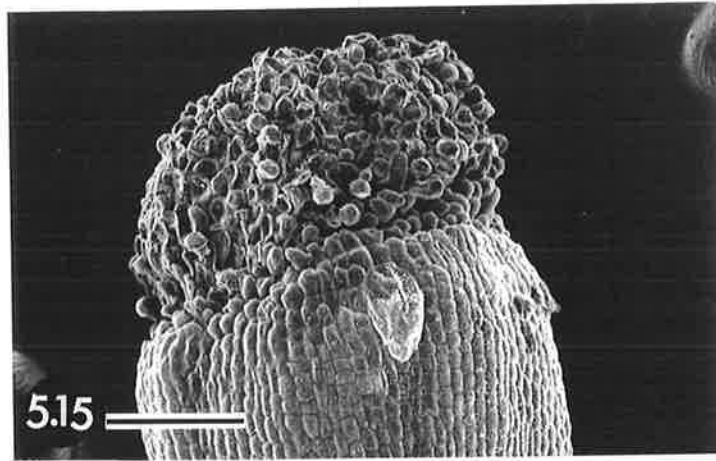
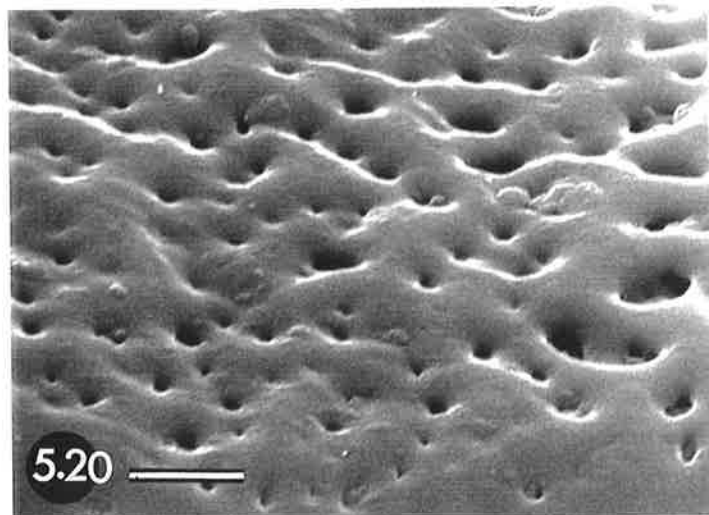
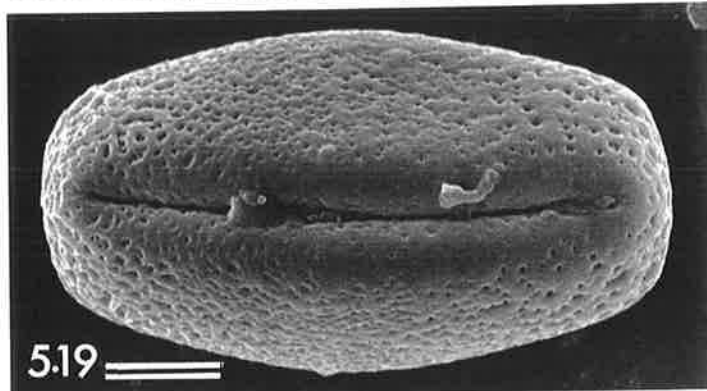


Fig. 5.18: Pollen grains on the stigma of the Shiraz pistil one day after anthesis, showing hydration of most pollen grains and degeneration of stigma papillae . Bar represents 20 μm .

Fig. 5.19: Ellipsoidal pollen grain of Shiraz, showing many large pores on the exine and one of three furrows. Bar represents 5 μm .

Fig. 5.20: Patterning of exine of Shiraz pollen grain, showing larger pores than in Chardonnay. Bar represents 1 μm .



5.5 Conclusions

- Scanning electron microscopy was used to display pollen and pistil morphology in grapevine cvs Chardonnay and Shiraz.
- Chardonnay pollen had a variable exine pattern, in many cases showing small, sparse perforations compared with Shiraz pollen.
- After lodging on the stigma, Chardonnay pollen tended to dehydrate and shrivel compared with Shiraz pollen which hydrated and swelled, a sign of better viability.
- On day 2 after pollination Chardonnay stigma remained unchanged in appearance while Shiraz stigma had collapsed evidencing successful pollination/fertilisation.

Chapter 6

Effect of short-term exposure to lowered temperature on pollen germination and pollen tube growth in grapevines of *Vitis vinifera*, cvs Chardonnay and Shiraz

6.1 Introduction

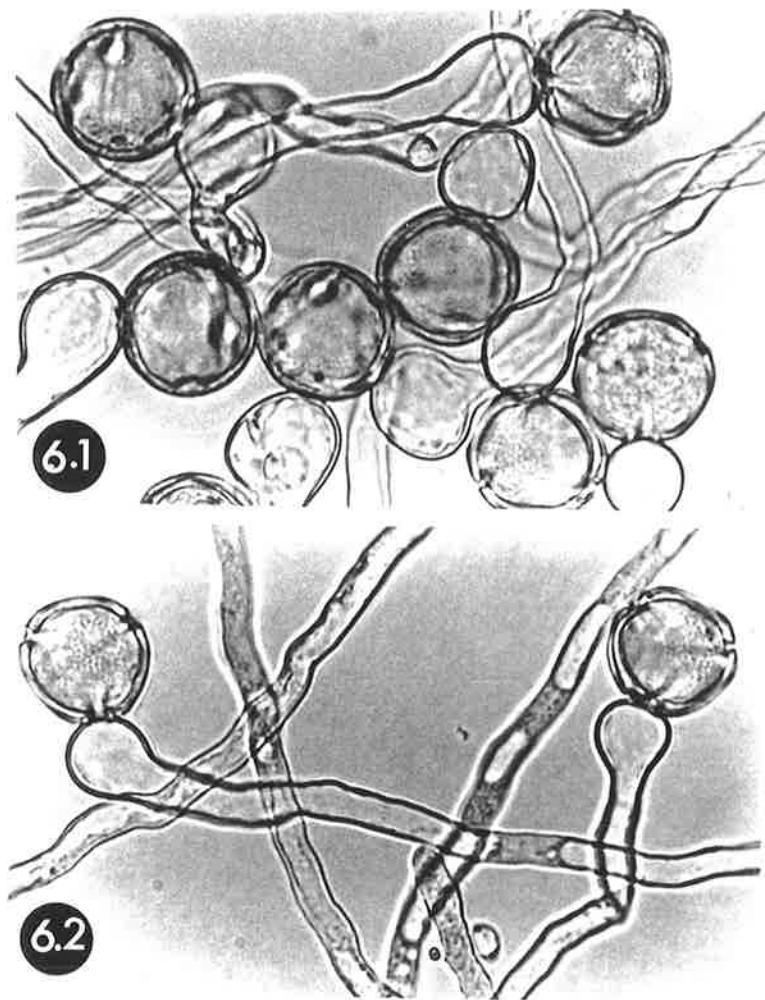
The results in chapter 4 showed that exposing plants to low temperature, and to a lesser extent to heavy shading, shortly before and up to the early part of flowering resulted in reduced fruit-set. This could have been due to deficiencies in the male or female part of the sexual apparatus or to lack of coordination between the two. Pollen development, germination and subsequent growth as pollen tubes within the pistil are susceptible to the effects of temperature variations. This has been reported among others by Koblet (1966) and Staudt (1982) for cultivars other than those tested here, and adapted to cool climate conditions. It appeared likely that genetic differences between cultivars would alter these responses. Tests *in vitro* and *in vivo* on the response of pollen to varying temperature conditions, with cvs Chardonnay and Shiraz, are described in this chapter.

6.2 Materials and methods

Evaluation of culture media for in vitro pollination tests

Three culture media were evaluated to select the appropriate and most efficient medium for *in vitro* tests of grapevine pollen germination. The media, spread in petri dishes, were:

- 1: 20% sucrose, 1% agar and 0.01% boric acid (J.F. Jackson, personal communication)
- 2: 20% sucrose, 0.03% calcium nitrate and 0.002% boric acid (Failla et al. 1992)
- 3: 15% sucrose, 1% agar, 0.01% boric acid, 0.03% calcium nitrate, 0.02% magnesium sulfate and 0.01% potassium nitrate (Brewbaker and Kwack 1963)



Figs. 1 and 2 Pollen grains germinated *in vitro* after 12 h incubation at 25°C. Pollen grains have three germination apertures and pollen tubes are swollen adjacent to the pore. Bar represents 50 μm .

To provide pollen for these tests, three ~~uniform~~ ^{of similar development,} small Chardonnay vines ~~were~~ ^{were used} raised as described in chapter 3. On day 2 of flowering, the anthers of newly opened flowers were cut off and dried on paper at 25°C for 24 h. Their pollen grains were then separated from the anther sacs. Pollen grains, taken in triplicate groups of 100 from the pollen of each vine, were placed in each of three petri dishes per culture medium. After 6 h (Figs. 6.1 and 6.2) and 24 h, the number of germinated pollen grains, ie. those producing pollen tubes at least as long as the diameter of the grain, was counted and the percentage of germinated grains calculated.

The results of this test of methods are shown in Table 6.1. Differences between the percentage of germinated pollen grains after 6 h and 24 h incubation were found on medium 1 but not on media 2 and 3. The pollen of all three vines germinated in largest numbers on culture medium 3. Therefore, this medium was chosen for the subsequent tests.

Table 6.1: Per cent germination of Chardonnay pollen on the three culture media after 6 h and 24 h.

Incubation	Medium 1		Medium 2		Medium 3	
	6h	24h	6h	24h	6h	24h
Vine 1	17.3	23.2	19.5	19.1	31.9	31.6
Vine 2	22.3	24.4	24.7	25.2	48.9	45.2
Vine 3	19.1	23.6	17.9	19.8	32.6	38.3
Mean±SE	19.6±1.5	23.7±0.3	20.7±2.1	21.4±1.9	37.8±5.5	38.4±3.9

Germination of pollen from vines grown at different temperatures

Uniform plants of Chardonnay and Shiraz, prepared as described in chapter 3, were selected, and were given the temperature treatments described in chapter 4, i.e. Control (C) —25°/20°C throughout, or 25°/20°C but transferred for one week at 17°/14°C or 12°/9°C (treatments of variable T), at E-L stages 15, 16 or 17 (treatments of variable S). Plots of single plants were used for each treatment and replicated five times. On the first day of

flower opening, all open flowers (less than 10 flowers per inflorescence) were removed. The anthers of flowers opening on day 2 were collected, dried and per cent germination of their pollen on culture medium 3 was determined after incubation at 25°C for 6 h, as described above. The data were submitted to two types of analysis of variance (chapter 3), the factorial type A with six treatment and excluding Control (3 S x 2 T) and the one-way analysis of variance type B with seven treatments including Control (3 S x 2 T, C). Where appropriate, means were separated according to Tukey's test.

Pollen germination on stigma and pollen tube growth in the pistil

Of 15 vines growing since bud burst at 25°/20°C, ten were transferred to and held for one week at 12°/9°C. Five of them were placed at the low temperature two days before flowering ('Cold'₂) and five on the day of flowering ('Cold'₀). The remaining five plants stayed at 25°/20°C as Control (C). On each inflorescence, the flowers which opened on day 1 of flowering were discarded; on day 2, the newly opened flowers of inflorescence branches 2, 3 and 4 were marked, to be harvested two or four days later. At each time eight flowers of each of branches 2, 3 and 4 per inflorescence were harvested.

The flowers were fixed in FPA50 and prepared for microscopic observations, as described in chapter 3. Counts were made of the number of deposited and germinated pollen grains on the stigma, of the pollen tubes in the style and in the various parts of the ovary, and of the number of ovules which were penetrated by at least one pollen tube. The experiment designed as a Completely Randomised Block Design with five replications of 1-vine plots and the data were analysed statistically by analysis of variance types A and B as in chapter 4 and with means separation according to Tukey's test where appropriate.

6.3 Results

Effect of temperature treatments of vines on pollen germination

Per cent pollen germination of Shiraz was about double that of Chardonnay (data not shown). In Chardonnay, the treatments of the variable 'temperature' were not significantly different while those of the variable 'stage' were (Table 6.2). The reason for this is shown in Table 6.3: exposing vines to 17°/14°C or 12°/9°C at stages 16 or 17 gave per cent pollen germination similar to each other and also to control. However germination at stage 15 was at a low level. In fact, the lowest germination found (from vines treated with 12°/9°C at stage 15) was half that of pollen from Control vines. Statistically, the T x S interaction failed to reach significance.

Shiraz pollen germinated better than Chardonnay pollen and was less affected by temporary exposure to lower temperatures. As shown in Table 6.2, differences due to the effects of the treatments of temperature or stage were not significant. However, Table 6.3 shows that a significant decrease in per cent pollen germination occurred following exposure of Shiraz vines to 12°/9°C at stage 15, and also at stage 17. Note that such a reduction at stage 17 did not occur in Chardonnay.

Table 6.2: Per cent germination of pollen from Chardonnay and Shiraz. Data from experiments 1-4 for Chardonnay and Shiraz (analysed by ANOVA type A)

	Control	Temperature			Stage			F
	25°/20°	17°/14°	12°/9°	15	16	17		
Chardonnay	31.4	25.9	23.3	ns	17.3 ^a	28.3 ^b	28.1 ^b	***
Shiraz	67.8	55.0	49.4	ns	49.7	56.8	46.7	ns

Significance of F value: *** significant at $p < 0.001$ Values marked by different superscript letters differ significantly according to Tukey's test.

Table 6.3: Per cent germination of pollen from Chardonnay and Shiraz. Data from experiments 1-4 for Chardonnay and Shiraz (analysed by ANOVA type B)

Temperature	25°/20°C	17°/14°C			12°/9°C			
'Stage'	Control	15	16	17	15	16	17	F
Chardonnay	31.4 ^c	19.4 ^{ab}	28.9 ^{bc}	29.5 ^{bc}	15.3 ^a	27.8 ^{bc}	26.8 ^{abc}	***
Shiraz	67.8 ^b	56.3 ^{ab}	55.2 ^{ab}	51.5 ^{ab}	43.1 ^a	58.5 ^{ab}	41.8 ^a	**

Significance of F value: **, ***: significant at $p < 0.01$, $p < 0.001$ Values marked by different superscript letters differ significantly according to Tukey's test.

Pollen germination on stigma and pollen tube growth in pistil

Data describing the course of pollination are presented in Table 6.4. In Chardonnay, the Control vines showed higher values than the 'cold'-treated vines for the course of pollination—more germinating pollen grains on the stigma and greater pollen tube growth in all parts of the pistil including penetration of the ovules. This applied irrespective of when the 'Cold' treatment was applied and when the flowers were harvested. In the Control vines, the pollen tubes reached the lower part of the ovary (Fig. 6.3) and ovules were penetrated (Fig. 6.4) in similar numbers on days 2 and 4 after flowering.

Shiraz flowers were less affected than Chardonnay flowers by low temperature applied either two days prior to or at flowering. More ovules were penetrated by pollen tubes in all cases and there were no significant differences between the harvests on day 2 or day 4 after flowering. An average of four pollen tubes reached the lower part of the ovary in Control pistils of both cultivars, but the mean number of ovules penetrated by a pollen tube was less than one per pistil in Chardonnay and about one in Shiraz. Double penetration of an ovule occurred in only two ovaries of Shiraz Control.

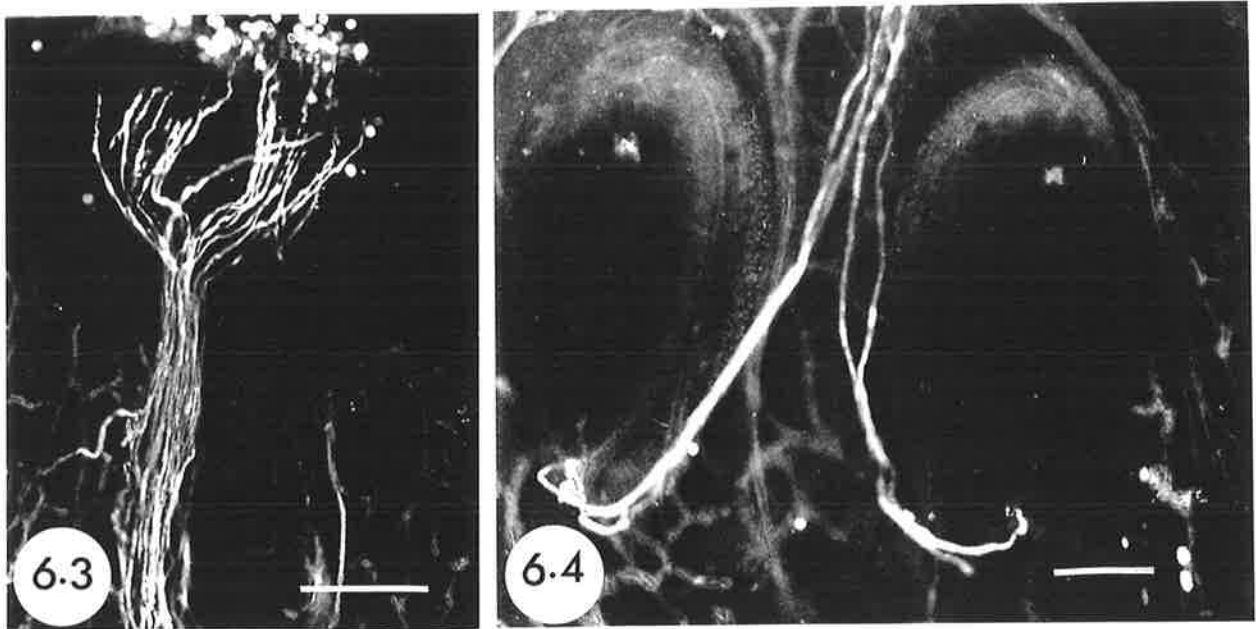


Fig. 6.3 Fluorescence micrograph of a squash preparation of a pistil of a Chardonnay Control vine 1 d after anthesis, showing pollen tubes growing from the stigma through the pistil to the ovules. The bar represents 1 mm.

Fig.6.4 Fluorescence micrograph of a squash preparation of a pistil of a Chardonnay Control vine 1 d after anthesis, showing one or two pollen tubes penetrating ovules. The bar represents 20 μm .

Note: In Fig. 6.4, the micropyle faces the bottom of the page.

Table 6.4: The course of pollination. Mean number per pistil of pollen grains deposited on the stigma, and of the pollen tubes, issued by them, in various positions within the pistil. Eight flowers each were sampled from inflorescence branches 2, 3 and 4 of five Chardonnay and Shiraz vines of treatments Control (at 25°/20°C throughout) and of 'Cold' [(at 25°/20°C until transferred to 12°/9°C either on day 2 before flowering ('Cold'₂) or on the day of flowering ('Cold'₀)], the flowers being harvested either on day 2 or day 4 after they had opened.

Treatment	Days†	Number per pistil of					ovules
		Pollen grains		Pollen tubes in:			
		on stigma	germinated	style	upper ovary	lower ovary	
Chardonnay							
Control	2	31.0	13.6 ^a	10.6 ^a	7.1 ^a	4.0 ^a	0.53
Cold₂	2	19.6	3.2 ^b	2.6 ^b	1.9 ^b	0.9 ^b	0.08
Cold₀	2	19.0	3.9 ^b	2.3 ^b	0.8 ^b	0.1 ^b	nil
F		ns	*	**	**	**	**
Control	4	23.3	9.3	7.6 ^a	6.1 ^a	4.8 ^a	0.54
Cold₂	4	21.6	6.8	5.7 ^b	3.2 ^b	2.4 ^b	0.12
Cold₀	4	26.6	6.3	2.3 ^b	1.0 ^b	0.4 ^b	nil
F		ns	ns	**	**	**	**
Shiraz							
Control	2	64.6 ^{ab}	19.1 ^a	10.8 ^a	7.8 ^a	4.7 ^a	0.91
Cold₂	2	27.5 ^b	11.0 ^b		7.1 ^{ab}	4.7 ^a	0.75
Cold₀	2	84.9 ^a	16.8 ^{ab}	8.3 ^b	4.5 ^b	2.2 ^b	0.31
F		**	**	*	*	*	*
Control	4	38.3 ^{ab}	15.8 ^a	10.7 ^a	7.9 ^a	5.2 ^a	1.14
Cold₂	4	26.3 ^b	9.8 ^b	7.6 ^{ab}	5.9 ^{ab}	3.2 ^{ab}	0.53
Cold₀	4	54.7 ^a	10.5 ^{ab}	6.1 ^b	3.9 ^b	2.3 ^b	0.23
F		*	**	*	*	*	**

† Number of days after anthesis when harvested.

Significance of F value: ns not significant; *, **: significant at $p < 0.05$, $p < 0.01$.

Different subscript letters within column groups represent a significant difference, according to Tukey's test.

6.4 Discussion

The treatments imposed on Chardonnay vines had some interesting effects on their pollen germination (Table 6.3). Those treated for one week at either 17°/14°C or 12°/9°C had similar germination percentages to pollen from Control vines (25°/20° throughout) if the treatments were given at E-L stages 16 or 17, but if the treatment was earlier—at E-L stage 15—germination was low, especially at the lowest temperature. At E-L stage 15 the inflorescence is only half of its final length, pedicels are very short and flowers are small and tightly grouped; by E-L 17, pedicels have lengthened and flowers are enlarging and

separating (Coombe 1995). During these events, the male gametophyte is undergoing some important development: pollen mother cells (which lie adjacent to cells of the tapetum layer) undergo meiosis, each producing four microspores as a tetrad; the microspores separate and form two walls, the outer being the distinctively structured exine layer that typifies a genotype.

The tapetum is considered to have a function in feeding the microspores and contributes to the sporopollenin deposition in the exine (Laser and Lersten 1972, Warmke and Overman 1972, Iwao 1983, Saini et al. 1984, Bino 1985, Hormaza and Herro 1992). It is likely that the imposition of low temperature for one week at E-L 15 interferes with the functioning of the tapetal cells or other processes in the formation of pollen, –thus causing pollen sterility.

In Shiraz the treatment effects on pollen viability were smaller but generally similar to those in Chardonnay. However, there were some subtle differences. None of the treatments of 17°/14°C applied at three stages showed differences in pollen viability from Control. Thus Shiraz pollen was not harmed by lowering temperature at stage 15 in the same way as was Chardonnay pollen from similarly treated vines. However, Shiraz vines exposed to 12°/9°C, showed lowered pollen viability compared with that temperature applied at stages 15 and 17, but not at stage 16. The explanation for the effects of treatment at E-L stage 15 in Shiraz is probably similar to that mentioned above for Chardonnay but the effects at stage 17 must differ; no explanation is available for the reasons why Shiraz showed different response to Chardonnay when treated at stage 17.

The results on the germination of pollen on stigmas and the growth of pollen tubes toward the ovules (Table 5.4) show the large and damaging effects of the exposure for one week to 12°/9°C on Chardonnay, evident at all stages examined. The effects on Shiraz were similar but smaller. Other authors have found that the rate of pollen germination and pollen tube growth is strongly dependent on temperature, for example Koblet (1966). Staudt (1982) found a positive correlation between temperature and the number of germinating pollen grains in *V. rupestris*. He also showed that there was a threshold

temperature between 10° and 15°C below which pollen grains could not germinate normally (Staudt 1981). The results here show that in Chardonnay plants held at 12°/9° very few pollen tubes reached the ovules even by four days after anthesis which would probably reduce, if not preclude, the chance of ovule fertilisation. For field-sampled flowers, it has been shown that fertilisation occurred 24 hours after flowering (Kassemeyer and Staudt 1981, Okamoto and Imai 1982).

It is possible that, in addition to direct effects of low temperature, the growth of pollen tubes suffer when attraction to the ovule is reduced due to the presence of a high proportion of defective ovules, such as those that develop with abnormal or missing embryo sacs (see chapter 6). Hence the number of pollen tubes passing through the ovary to reach the ovules may be reduced. In the present experiment, even abundant numbers of pollen tubes in the basal part of Chardonnay pistils did not lead to penetration of all ovules, with only about 13% penetrated by a pollen tube in Control plants which had occurred in 41% of ovaries. This percentage is subject to reduction by vagaries of fertilization and other factors so that per cent of berries is often much lower; in these particular vines, set was about 10% (see chapter 4).

In Control vines of both cultivars, the number of pollen tubes in the lower ovary was about four, which is the same as the number of ovules per pistil. This suggests that each individual ovule (or its embryo sac) exerts control over the growth of at least one pollen tube in the ovary. A similar phenomenon has been shown in flowers of other plants, for example in the base of the avocado style (Tomer and Gottreich 1975, Sedgley 1976). Few ovaries were observed in which ovules were penetrated by more than one pollen tube. This phenomenon has been reported in other plants also (Sedgley 1979).

The lack of penetration of more than one ovule per ovary conforms with the predominance of one-seeded berries in these two cultivars (see chapter 9). Single seededness of berries is common among winegrapes cultivars but should not be taken as the norm for *Vitis vinifera*. The selection of genetic material for wine making has probably led to an emphasis on smaller berries with the result that many have one seed.

6.5 Conclusions

- Viability of Chardonnay pollen was reduced by exposing plants for one week to either 17°/14°C or 12°/9°C at E-L stage 15 but not at later stages of E-L 16 or 17 which had similar germination percentages to pollen from vines held at 25°/20°C. This effect implicates an effect of cold temperature on the development of tapetal cells..
- Shiraz pollen had germination percentages double that of Chardonnay and was much less affected by the temperature treatments.
- At 25°/20°C, pollen tubes penetrated on average less than one ovule per ovary in Chardonnay and about one ovule per ovary in Shiraz.
- The number of pollen tubes in the lower ovary was about four in both cultivars held at 25°/20°C but it was reduced by cold to less than one or to almost nil when applied at stages E-L 17 and 20 in Chardonnay. A smaller reduction was found in Shiraz.

Chapter 7

Effect of low temperature near anthesis on ovule development in *Vitis vinifera* L., cvs Chardonnay and Shiraz

7.1 Introduction

Many authors have stressed that low temperature is one of the reasons for inadequate fruit-set. In chapter 4 it was shown that exposing vines to 12°/9°C shortly before or at early anthesis reduced Chardonnay fruit-set dramatically. The effect of low temperature on pollen development and its viability has been examined in chapter 5. However, the detrimental effect of low temperature is not limited to the development of pollen grains and could be attributed to its harmful effect on ovule development as well (Kassemeyer and Staudt 1981, May 1992). In the present chapter, the mechanism by which low temperature exerts a detrimental effect on ovule development is described.

7.2 Materials and Methods

Six vines each of Chardonnay and Shiraz, raised as described in chapter 3 and thus carrying one inflorescence, were selected for uniformity, and three each per cultivar were allotted to each of two treatments. Three vines were used as controls and held at 25°/20°C. The other three vines were selected for a 'Cold' treatment, whereby they were transferred from 25°/20°C to 12°/9°C for one week two days before anthesis. Based on previous observations (chapter 4), this time was judged to have arrived when the flower caps changed colour from bright green to slightly yellow-green. This is labelled as stage E-L 17 in this chapter although it is possibly more advanced and therefore intermediate between stages 17 and 18. After one week, these plants were returned to the control condition of 25°/20°C where their flowers started to open after two or three days. These flowers were also harvested one day after they had opened.

On each inflorescence, flowers were taken from branches 2, 3 and 4. The flowers sampled were the king flower and one central-lateral and one lateral-lateral flower (May 1987; see Figure 7.1). The flowers were harvested one day after they had opened. The four ovules of each pistil were excised and sections were prepared and stained as described in chapter 3.

To ensure correct interpretation of their morphological, anatomical and cytological details, all sections per ovule were retained. Treatment differences in the frequencies of occurrence in classes of normal ovules and of ovules with various abnormalities, including the absence of the embryo sac, were evaluated by χ^2 test. The four ovules of each ovary were measured for length and width, using an eyepiece micrometer, and their volume was calculated from the measurements of ovule length and width by approximating their shape to that of a rotational ellipsoid. The data were analysed statistically by analyses of variance, and the relationship between ovule size and frequency of occurrence of normal ovules was tested by weighted linear regression analysis.

7.3 Results

Measurements of ovule volume

Mean ovule volumes for the Control and 'Cold' treatments, averaged over the values for vines and branches, are presented separately for the king, central-lateral and lateral-lateral flowers (Table 7.1). For each type of flower, mean ovule volume was calculated for the average of the four ovules per ovary, for the largest and for the smallest ovule per ovary, as well as the ratio of the largest and smallest. There were clear tendencies of ovule size becoming smaller in the order, King > central-lateral flower > lateral-lateral flower, and Control flowers > 'Cold' flowers. However the differences in ovule size due to the temperature treatments failed to reach significance. The reduction in ovule size due to exposure to low temperature was large in two, but smaller in the third vine,

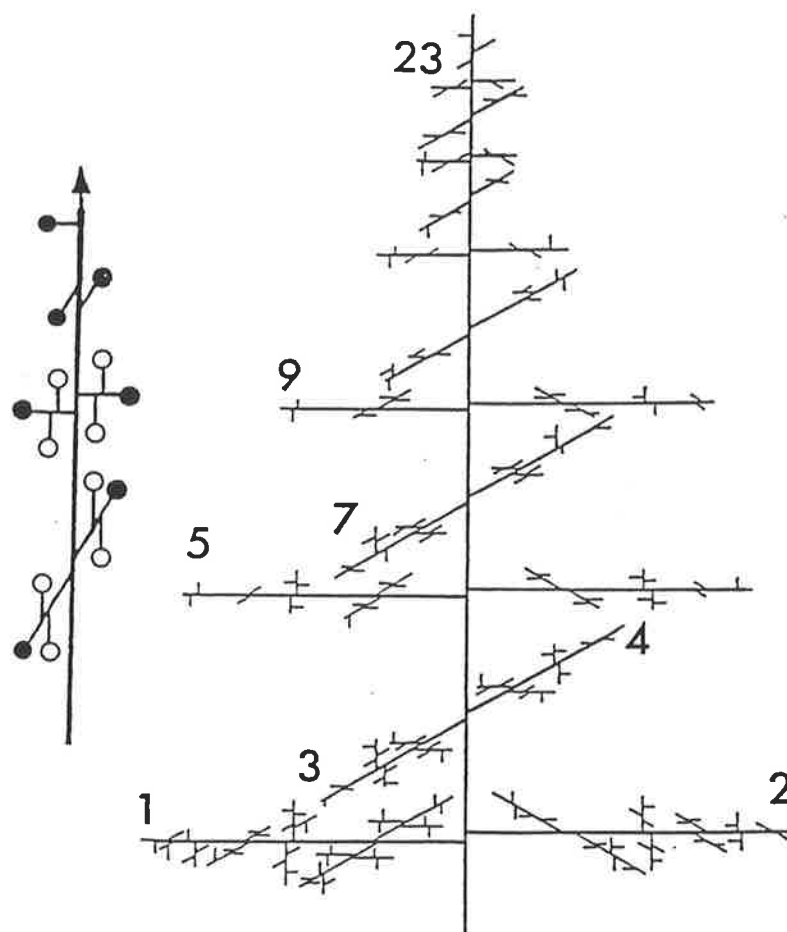


Fig. 7.1 Scheme of a grapevine inflorescence and of a branch showing the position of king (▲), central-lateral (●) and lateral-lateral (○) flowers (May 1987).

even though it was still evident, and the thus introduced variability was obviously responsible for the lack of statistical significance. Both cultivars showed large differences in the volume of the largest and smallest ovule per ovary in many, but not in all ovaries, the ratio Max/Min approaching or even exceeding a factor of two (Table 7.1). The coefficients of variation (CV) calculated for the volumes of the four ovules of each ovary ranged from 4.2 to 56.1 in Chardonnay and from 0.8 to 41.2 in Shiraz, the mean CV being 24.9 and 17.2 and the median CV being 24.0 and 16.5 respectively. However the ovaries of Chardonnay showed much greater variability in the size of their four ovules than those of Shiraz.

In agreement with information in the literature, the pistil was found to consist of a bilobed stigma, a short style and an ovary with two locules, each with two anatropous ovules. Each normal ovule consisted of the outer and inner integuments, the nucellus and the embryo sac (Figure 7.2), the latter with an egg cell, two synergids, two polar nuclei or a polar fusion nucleus (Figure 7.3) and three ephemeral antipodal cells (Figure 7.4). Fertilised ovules were identified by the presence of sperm nuclei or of free nuclear endosperm (Figure 7.5).

Table 7.1: Volume of the average, largest (Max) and smallest (Min) ovule (in $\text{mm}^3 \times 10^{-3}$) per ovary, and of the ratio Max/Min, of Chardonnay and Shiraz flowers. Each listed value (with its standard error) for flower types king (K), centalateral (CL) and lateral-lateral (LL) flowers in treatments Control and 'Cold' is the mean of nine flowers per type and its standard error, namely one on each of branch 2, 3 and 4 of the sole inflorescence of three vines. The Control treatment was at $25^\circ/20^\circ\text{C}$ throughout, the 'Cold' treatment at $25^\circ/20^\circ\text{C}$ except for one week at $12^\circ/9^\circ\text{C}$ starting on day 2 before anthesis.

Ovule type	K		CL		LL	
	Control	Cold	Control	Cold	Control	Cold
Chardonnay						
Average	752 ± 51	502 ± 65	688 ± 51	432 ± 77	636 ± 71	483 ± 67
Max	936 ± 80	622 ± 75	882 ± 69	519 ± 91	861 ± 111	625 ± 78
Min	616 ± 54	381 ± 53	541 ± 51	354 ± 68	471 ± 57	336 ± 65
Max/Min	1.57 ± 0.14	1.75 ± 0.15	1.71 ± 0.17	1.54 ± 0.08	1.87 ± 0.17	2.60 ± 0.63
Shiraz						
Average	674 ± 33	564 ± 17	606 ± 26	489 ± 32	513 ± 18	473 ± 43
Max	817 ± 53	659 ± 17	740 ± 32	561 ± 31	611 ± 25	575 ± 63
Min	564 ± 32	461 ± 29	451 ± 48	418 ± 34	427 ± 21	375 ± 43
Max/Min	1.48 ± 0.11	1.49 ± 0.11	1.78 ± 0.19	1.36 ± 0.04	1.46 ± 0.10	1.62 ± 0.20

The occurrence of abnormalities in ovules was assessed in samples one day after anthesis. All types of abnormality occurred in several ovules and with material prepared in different batches; therefore erroneous interpretation due to inadequate tissue fixation can be excluded. The most common abnormality was the absence of the embryo sac (Figure 7.6).

In other cases, the embryo sac had failed to develop to maturity —it was shorter than a normal embryo sac and without the complete number of nuclei (Figure 7.7) or had one or more cross walls (Figure 7.8). Deteriorating ovules showed shrinkage of the nucellus (Figure 7.9). With respect to the faulty development of the ovules we found that, after exposure to low temperatures, 54 of 108 ovules had no embryo sac; of the 54 ovules, 13 appeared normal in all other respects, 15 had an enlarged sub-micropylar chamber (7 with and 8 without an open micropyle) and 14 were small and with incomplete integuments.

Subjecting the vines to low temperature two days before anthesis decreased the size of the ovules (Table 7.1) and increased the frequency of abnormal ovules (Table 7.2). In the 'Cold'-treated Chardonnay vines, over half the ovules examined had either an abnormal embryo sac or no embryo sac; some had also a degenerated nucellus.

The ovules were not only smaller (Table 7.1) but their embryo sac, if present, was less developed than in the ovules of the Control vines which, at that stage, had mostly embryo sacs with degenerated antipodals, fused polar nuclei and a well developed filiform apparatus. The situation was similar in Shiraz, although low-temperature damage was less frequent than in Chardonnay.

Table 7.2: Number of occurrences of types of ovules. There were 108 ovules each of Chardonnay and Shiraz vines grown at two temperature regimes – Control (at 25°/20°C throughout) and 'Cold' (at 25°/20°C except for one week at 12°/9°C starting on day 2 before anthesis). Harvest was one day after anthesis. Treatment differences were tested by χ^2 test.

Ovule with	Chardonnay		Shiraz	
	Control	Cold	Control	Cold
<i>Embryo sac</i>				
Normal	84	44	86	70
Fertilised [†]	7	0	13	1
Abnormal	6	10	4	11
None	18	54	18	27
<i>Nucellus</i>				
Degenerated [‡]	3	5	2	7
χ^2	38.8**		19.4*	

* , ** significant at $p < 0.05$, $p < 0.01$

[†] These numbers included in those of the normal ovules

[‡] These numbers included in those without embryo sac

Fig. 7.2 Longitudinal section of an ovule of a Chardonnay Control vine 1 d after anthesis, showing normal development with outer integument (oi), inner integument (ii) which forms the micropyle (m), nucellus (n) and embryo sac (es). The bar represents 20 μm .

Fig. 7.3 Longitudinal section of an ovule of Shiraz Control vine one day after anthesis, showing micropylar half of embryo sac containing synergids (s) with well developed filiform apparatus (f), egg cell (e) and polar fusion nucleus (pn). The bar represents 3 μm .

Fig. 7.4 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12 $^{\circ}$ /9 $^{\circ}$ 2 d before anthesis and harvested 1 d after anthesis), showing chalazal end of embryo sac containing three antipodal cells (a). The bar represents 3 μm .

Fig. 7.5 Longitudinal section of a fertilised ovule of a Chardonnay Control vine 1 d after anthesis showing cytoplasm (c) and two nuclei (n) of free nuclear endosperm. The bar represents 3 μm .

Note: In Figs 7.2-to 7.5, the micropyle faces the bottom of the page.

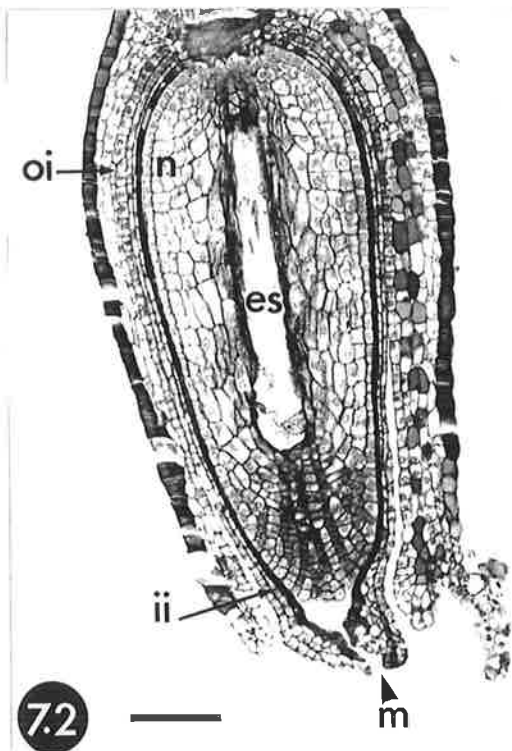


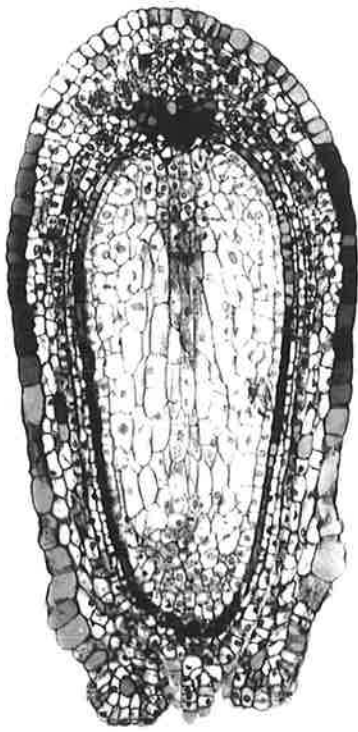
Fig. 7.6 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after the anthesis), showing absence of embryo sac and reduced overall size. The bar represents 20 µm.

Fig. 7.7 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing small, incomplete embryo sac. The bar represents 20 µm.

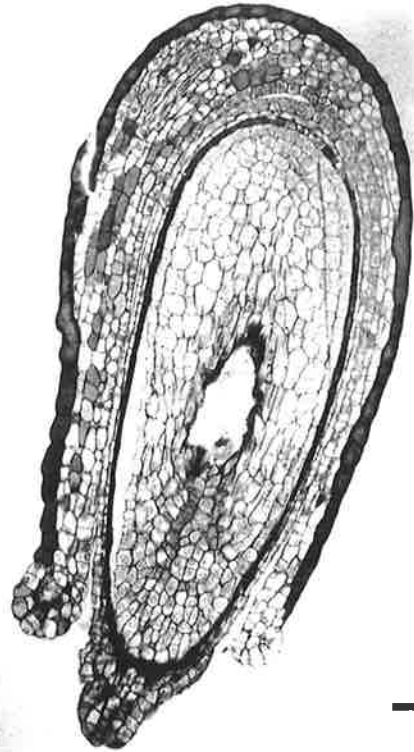
Fig. 7.8 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing cross wall (arrow) in the middle of the embryo sac. The bar represents 20 µm.

Fig. 7.9 Longitudinal section of an ovule of a Chardonnay 'Cold' vine (exposed to 12°/9° 2 d before anthesis and harvested 1 d after anthesis), showing deteriorating nucellus. The bar represents 20 µm.

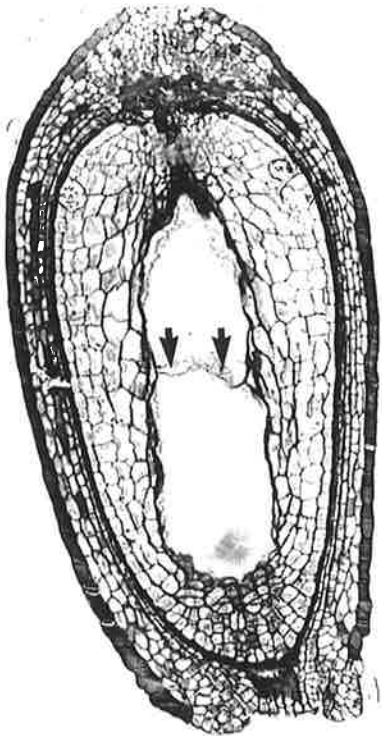
Note: In Figs 7.6-to 7.9, the micropyle faces the bottom of the page.



7.6



7.7



7.8



7.9

Table 7.3: Relationship between ovule size and ovule normality. The regression coefficients b of weighted linear regressions, relating numbers of normal ovules (as per cent of total ovules) per size-class of ovule length or width (y) with the mid values of ovule length or width per size-class (x), are shown, together with the number of ovules per size class (n) and the coefficients of determination R^2 . The data are for treatments of Control (25°/20°C throughout) and 'Cold' (25°/20°C except for one week at 12°/9°C, starting 2 d before anthesis) of Chardonnay and Shiraz vines. Harvest was 1 d after anthesis.

Treatments	Ovule length (μm)				Ovule width (μm)			
	n	Range	b	R^2	n	Range	b	R^2
Chardonnay								
Control	7	500-850	0.1278*	38.7	5	200-450	0.1475 ns	45.8
Cold	10	300-800	0.1464*	57.4	5	100-350	0.5229*	88.5
Shiraz								
Control	7	500-850	0.0983 ns	17.1	4	200-400	0.1236 ns	78.0
Cold	7	350-700	0.2938*	73.4	3	200-350	0.4490*	89.9

ns not significant; * significant at $p < 0.05$

The relationship between ovule size and normal or abnormal development of the ovules was tested by regression analysis. This was done by arranging the measurements of length and width of the 108 ovules per treatment into classes of 50 μm size-interval and relating the mid-value of each class (as independent variable) with the percentage of normal ovules per total ovules in each class (as dependent variable) (Table 7.3).

7.4 Discussion

Compared with ovules developing under warmer temperature conditions, flowers exposed to low temperature, for seven days starting two days before anthesis, had greater proportions of imperfect ovules with the embryo sac incompletely developed or even missing. There was also a tendency for the low-temperature ovules to be smaller, a trend supported by the fact that the proportion of normal ovules increased significantly as ovule size increased. Shiraz appeared more tolerant than Chardonnay and only 35% of observed ovules were damaged by the 'Cold' treatment compared to 54% in Chardonnay. Thus, low temperature not only affected pollination, by reducing and delaying pollen germination and

pollen tube growth (chapter 5), but also the development of the ovules and their state at the time when fertilisation is due to occur as shown here. The aspect of normal ovules just after anthesis resembled that described elsewhere, for instance by Pratt (1971).

It is possible that smaller ovule size and incomplete ovule development are associated with less frequent entrance of pollen tubes, and hence with less frequent transformation of the flower into a berry and also with smaller berries (because of the dependence of berry growth on the presence of seeds, see chapter 8). On average, cool conditions reduced the volume of all ovules per ovary, whether considering the largest, the smallest or their average size per ovary. Within each ovary, however, the ovules may or may not vary greatly in size, as shown by the range of the coefficients of variation for the ratio of largest over smallest ovule. The extent of this range does not seem to be influenced by either temperature or flower type (position of the flower on the inflorescence). The difference in size between the four ovules per ovary on day 2 of anthesis could be a size difference before fertilisation or the consequence of fertilisation.

Fougère-Rifot et al. (1993) described a number of symptoms of faulty ovule development in flowers of Chardonnay collected in vineyards near Bordeaux, France. The symptoms comprise the development and collapse of tannin-containing vacuoles, the lack of cell turgescence, detachment of the integuments, abnormal opening of the micropyle, an enlarged sub-micropylar chamber and a delay in growth. Interestingly, the absence of the embryo sac was not mentioned. In their study, the embryo sac was found to be formed at the 'button' stage, just before cap fall (corresponding to E-L stage 17 or possibly between stage 17 and 18; Coombe 1995). While the timing of embryo sac formation was not directly determined in the present study it can be deduced from the frequency of damage to embryo sacs which occurred when the treatment of low temperature was applied at stage 17.

The results here reported confirm that the stage E-L 17 is critical for the effect of low temperature on embryo sac development. Other comparisons between the French work and the present study can also be made. In both, ovules with enlarged sub-micropylar

chambers, some with open and some with closed micropyles, were found, as well as ovules with incomplete integuments. But there was also disagreement as far as the presence or absence of the embryo sac was concerned. The situation concerning the state of tannin vacuoles cannot be compared because tannin evolution seems to be determined at a stage preceding the observations in the present study. A further difference between the two studies is the frequency of healthy ovules per ovary. Fougère-Rifot et al. (1993) also stated that "in Chardonnay, at the time of pollination, we found only rarely an ovary with more than one healthy ovule" (translated here). In the present experiment there were about 50% of all Control ovaries with four normal ovules. However there was only one among the 27 'Cold'-treated ovaries examined in which all four ovules were normal. The question then arises whether the results of the study at Bordeaux were due to unfavourable environmental conditions or to the genetic make-up of the vines. This question cannot be answered with the information available.

While the results of the experiments described in chapter 4 pointed to the important effect of temperature on fruit-set, those reported in chapter 6 and in this chapter show that this effect is the result of low temperature being detrimental to the normal development and functioning of the ovules and the pollen.

Another, perhaps less telling but nevertheless viticulturally important effect of lowered temperature just before and during anthesis is the delay in anthesis. Anthesis did not start until after the plants had been returned from 12°/9°C to 25°/20°C. Temperatures as low as 12°/9°C are not uncommon during the anthesis period in regions where Chardonnay is grown. In all, the temperature response varies quantitatively between cultivars; in the present case it was much more pronounced in Chardonnay than in Shiraz.

7.5 Conclusions

- King flowers had larger ovules than central-lateral or lateral-lateral flowers. Larger ovules were more likely to be anatomically and cytologically normal than smaller ovules.
- Ovules exposed to 12°/9°C, tended to be smaller and less advanced in development, this effect being greater in Chardonnay compared with Shiraz.
- Over half of the cold-treated ovules in Chardonnay and about one third in Shiraz were abnormal, mostly due to an absence of the embryo sac.

Chapter 8

Seed development and abortion in cv. Chardonnay

8.1 Introduction

The process of seed development in the grapevine conforms to that described for angiosperm species in general. Before fertilisation, the ovule consists of the integuments and the nucellus which surrounds the embryo sac with its eight nuclei, the most important being the egg cell and the polar nucleus. Double fertilisation by the two sperm nuclei in the pollen tube results in the fertilisation of the egg cell to form the zygote and of the polar nucleus which initiates endosperm formation. The success of fertilisation signals the passage of the ovule to the developing seed (and of the ovary to the fruit). Mitotic divisions of the fertilised polar nucleus result in the formation of free nuclear endosperm (without cell walls). This is followed by the formation of cell walls in the endosperm coinciding with the development of the zygote into the proembryo through mitotic divisions. At seed maturity, the seed coat encloses the embryo and endosperm. Sedgley and Griffin (1989) stated that many plant species commonly produce mature fruits from only a small proportion of their fertile female flowers, and both flowers and immature fruits abort regularly. The reasons for abortion vary, but generally include problems in fertilisation and embryo development.

In the grapevine, studies of both normal and abnormal processes of fertilisation and post-fertilisation development have been described by a number of authors. In the cvs Gewürztraminer and Weisser Burgunder (syn. Pinot Blanc) fertilisation occurred in the field 24 h after flower opening (Kassemeyer and Staudt 1983), but *in vitro* from 12 h after pollination onward (Staudt 1986). The first division of the zygote occurred in most cases between two to three weeks after anthesis (Barritt 1970, Kassemeyer and Staudt 1983, Ledbetter and Ramming 1989) and this coincided with the formation of cell walls in the endosperm, possibly because the endosperm is unable to provide the embryo with an

optimal nutritional supply before this stage (Kassemeyer and Staudt 1983). This is also the case in other crops, e.g. cotton (Schulz and Jensen 1977).

Ovule abnormalities leading to abortion may occur before meiosis due to abnormal development of the nucellus and the integuments or due to incomplete anatrophy, or after meiosis due to arrested development of the embryo sac at an early stage, or degeneration of the egg in an apparently normal and mature embryo sac (Pratt and Einset 1961, Pratt 1971). The four ovules in an ovary of a normally-seeded grapevine cultivar are rarely developed to the same extent.

Fertilisation of at least one of its four ovules is necessary to ensure the development of the pistil into a berry; without this, pistil growth is limited and the pistils abort a week or so after flowering, the formation of an abscission layer starting between days 4 and 6. Other pistils, with ovules without embryo sacs but with normal nucelli, may remain on the bunch until harvest but fail to grow and soften (shot berries) (Kassemeyer and Staudt 1982). Pistil abortion may also occur after normal fertilisation evidenced by degeneration of the nucellus occurring from two to four days after flower opening (Okamoto and Imai 1982). On the other hand, imperfect development of the zygote and endosperm may result in seed abortion (stenospermy) and thus 'seedless' berries (stenospermocarpy) (Barritt 1970, Staudt and Kassemeyer 1984) or in berries with 'empty' seeds; the latter appear to be normal and well developed in external appearance but without contents, and thus devoid of embryo, endosperm and nucellus within the seed coat (Ledbetter and Ramming 1989).

Chardonnay is a cultivar which has normal, viable seeds when flowering occurs under favourable weather conditions, but which is sensitive to cool weather conditions (May 1992), leading to a poor set, or to a small number of seeds per berry, or to a high proportion of 'empty' seeds and consequently to yield reductions. The aim of this research was to investigate the mechanism and the timing of seed development in this cultivar in the field, with special reference to the abnormalities which may occur and to the differences between ovules within the same pistil. This included studies on the mechanism of empty-

seededness, a condition which has important implications for berry development and thus yield in this cultivar.

Terminology

The forms of seeds that are mentioned in this paper require definition. In grapevines, as in all angiosperms, a seed differentiates from an ovule after double fertilisation and further development of the fertilised structure (Esau 1977). Terms used in the literature include ovules, aborted ovules, small seeds, seed traces, stenospermic seeds, rudimentary seeds, normal seeds, floater seeds and sinker seeds. Some of the basis for these designations depends upon microscopic evidence. This is unsatisfactory for casual observations; terms are needed that avoid the need for prior microscopy. For these purposes we use here only three names for types of seeds (table 8.1). Ovules which remain unexpanded within the growing fruit continue to be termed 'ovules'.

Table 8.1: Seed terminology

Chosen name	Alternative name	Size	Bouyancy in water	Testa
Trace	Stenospermic	Less than half size	Float or Sink	Soft or hard & brittle
Floater	Empty, hollow	Full size	Float	Hard
Sinker	Normal	Full size	Sink	Hard

8.2 Material and Methods

Flowers and berries were collected from early- and late-flowering inflorescences (sample A, late October and sample B, mid November) of three-year-old Chardonnay vines growing in the Waite campus vineyard of the University of Adelaide (34° 56' south, 138° 35' east).

For sample A, used for microscopic examination of the ovules and seeds throughout the period of berry growth, two uniform inflorescences were marked on each of five vines. Only the flowers which opened on a single day, day 2 of flower opening, were retained on the inflorescences while all the other flowers were removed either on the first day or at the end of the second day of flowering. Samples of the retained flowers were taken on day 4 and of the developing berries on days 14, 28, 42 and at berry ripeness on day 98 after anthesis. Two post-anthesis flowers on each of the ten bunches were harvested at day 4 and two berries, one of large and one of medium size, on the other harvesting dates.

Sample B consisted of three uniform inflorescences growing on the same five vines but commencing flowering two weeks after those of sample A. All berries present at ripeness were harvested, their seeds were extracted and the proportion of floater seeds calculated. Ten such seeds were processed for microscopy. Temperature data were recorded during both the first and second sampling periods.

The sampled flowers and berries were fixed in FPA 50 (5% formalin, 5% propionic acid, 90% of 50% ethanol). Slices of the berries sampled on days 4, 14 and 28, and of the testa of the excised seeds of the samples of days 42 and at berry ripeness, were removed prior to vacuum infiltration for better penetration of the fixative. Ovules and seeds were selected for sectioning and microscopic examination as follows, their codes shown in brackets: On day 4, when berries (B) were small and had not yet developed clear size differences, the largest (B-1) and third largest (B-3) 'seeds' were taken. On days 14, 28 and 42, the largest seed of each large berry (LB-1) and the third-largest seed of each medium-sized berry (MB-3) were sampled. At berry ripeness, on day 98, only the largest ovule of large berries. i.e. LB-1, was taken.

Ovules or seeds were dehydrated through an ethanol series, embedded in glycol methacrylate, serially sectioned longitudinally at 5 μm thickness, stained with periodic acid-Schiff's reagent and toluidine blue O and observed using bright field optics (Feder and O'Brien 1968). Sections stained with aniline blue to detect pollen tubes were

observed using ultra violet light (Martin 1959). Structural details of the ovules were recorded, and the length and width of the ovule, embryo sac, endosperm and embryo was measured using an eyepiece micrometer.

Floater seeds were identified in the samples from days 42 and 98. All seeds of each bunch of sample A (early), except those taken for microscopic examination, and all seeds of the three bunches of sample B (late) were floated on water, and the numbers of sinkers and floaters counted. Ten floater seeds of each of the 42 and 98 d samples were processed for microscopy as described above.

8.3 Results

The temperatures in the vineyard (fig. 8.1) showed mean maxima and minima during the sampling period A of $19.2 \pm 1.1^\circ\text{C}$ and $11.4 \pm 0.8^\circ\text{C}$, and during sampling period B of $23.3 \pm 3.1^\circ\text{C}$ and $12.9 \pm 3.0^\circ\text{C}$.

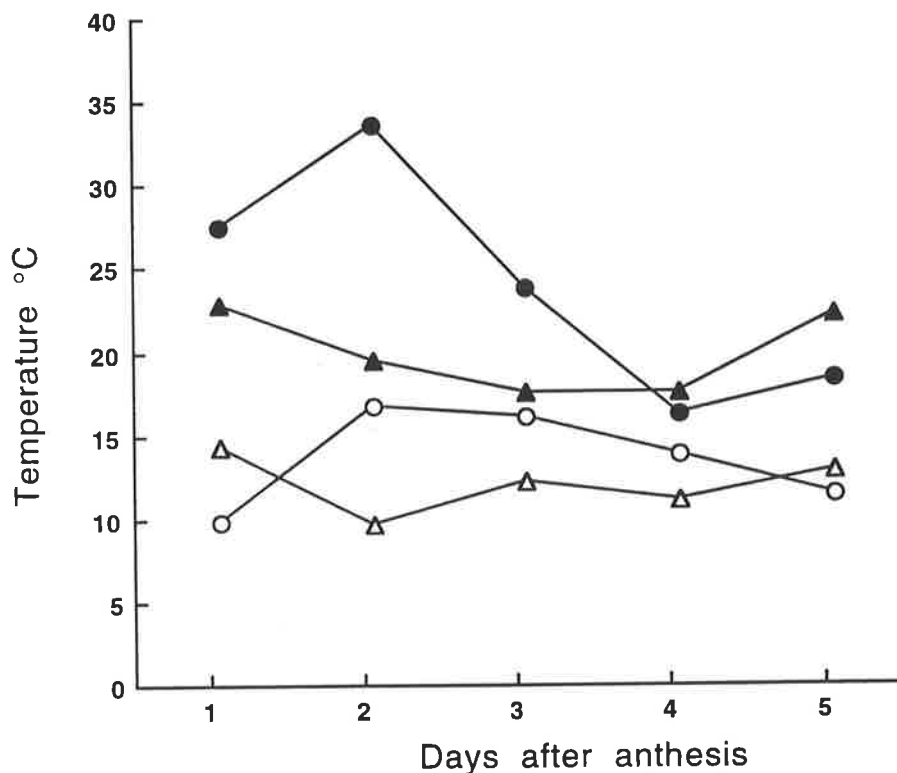


Fig. 8.1: Daily maximum (\blacktriangle , \bullet) and minimum (\triangle , \circ) temperatures during 5 days after the first flower opened. Early flowering \blacktriangle , \triangle (sample A), late flowering \bullet , \circ (sample B). Flowers used for microscopy opened on day 2 of flowering.

The course of normal seed development is shown by the data in columns 6 and 10 of Table 2, most of which relate to LB-1 types. On day 4, four of ten ovules had been penetrated by a pollen tube (Fig. 8.2) and five of 20 showed evidence of fertilisation by the presence of free nuclear endosperm and a zygote. On day 14, all normal seeds had free nuclear endosperm and a still undivided zygote (Fig. 8.3); by day 28, this state persisted in some seeds, but in others cellularisation of the endosperm and division of the zygote had commenced, leading to the start of the proembryo (Fig. 8.4). On day 42, endosperm was cellular and the proembryos were multicellular, the cell number ranging from 16 to about 40. The most advanced proembryo was globular and had a short suspensor (Fig. 8.5). At berry ripeness, endosperm filled most of the seed (Fig. 8.14), and embryos had well developed cotyledons and vascular tissues (Fig. 8.6). The development of the zygote and proembryo, and free nuclear and cellular endosperm proceeded as the seed grew (Table 8.3). The largest increase in size of the testa occurred between days 14 and 28, while that of endosperm, zygote and proembryo occurred later. Endosperm growth continued steadily throughout while the main growth of embryos took place after day 42.

Aberrations from normal seed development were observed as early as day 4 after flower opening and were particularly prevalent in B-3 and MB-3 ovules/seeds (Table 8.2). Fertilisation could not be recognised in the ten ovules examined for pollen tube penetration. In the other 20 examined ovules, only 3 had a zygote and free nuclear endosperm on day 4; on day 14 this was the case with 2 of 10. Thereafter, all failed to develop further.

Overall, two categories of aberrant development of ovules were identified, namely those without an embryo sac and those with a normal embryo sac but with no evidence of fertilisation. In the ovules of the first category, the nucellus was in the process of degeneration on days 4, 14 and 28 after flower opening (Fig. 8.7). The ovules of the second category were structurally normal, with a well developed egg apparatus (Fig. 8.8), and fused

Fig. 8.2 Pollen tube (arrow head) penetrating embryo sac (es) within ovule on day 4. Nucellus (n), integument (i). Bar represents 20 μm .

Fig. 8.3 Normal fertilised seed on day 14, showing embryo sac with zygote (z), one nucleus (arrow head) and cytoplasm (ct) of the free nuclear endosperm (fe). Bar represents 10 μm .

Fig. 8.4 Fertilised seed on day 28, showing proembryo (pe), the endosperm becoming cellular, showing formation of cell walls (cw). Bar represents 10 μm .

Fig. 8.5 Fertilised seed on day 42, showing globular multicellular proembryo (pe) with a short suspensor (s) and cellular endosperm (ce). Bar represents 20 μm .

Note: Micropyle faces the bottom of the page in all figures.

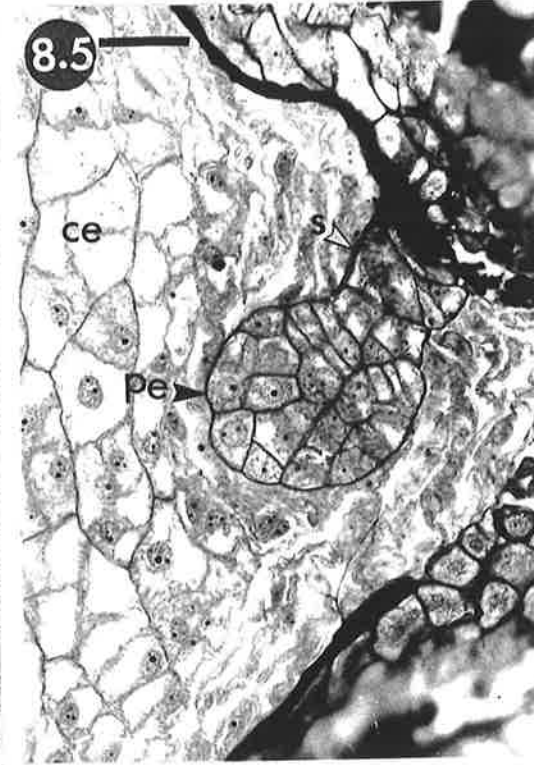
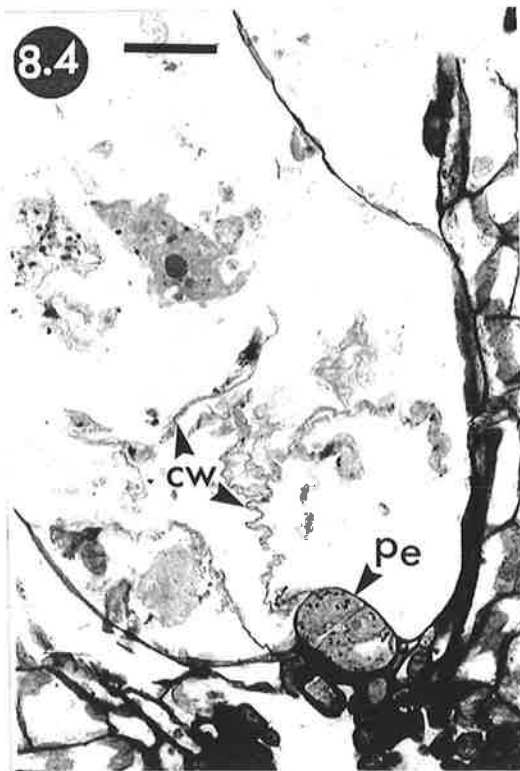
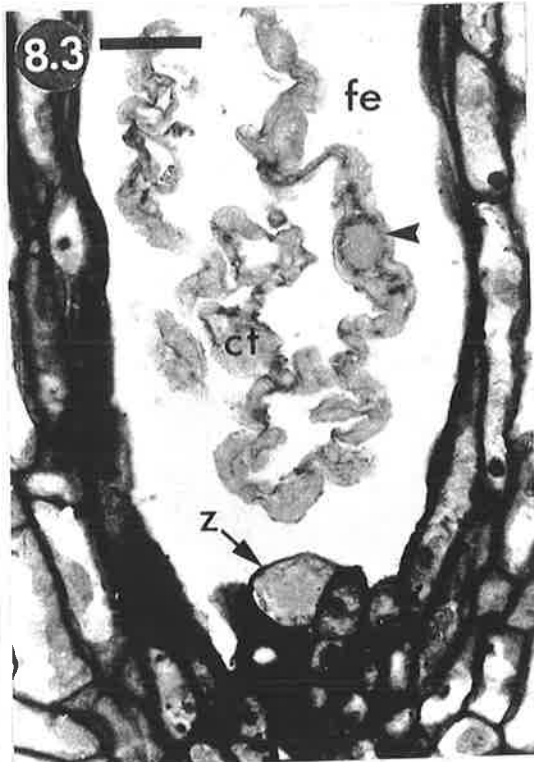
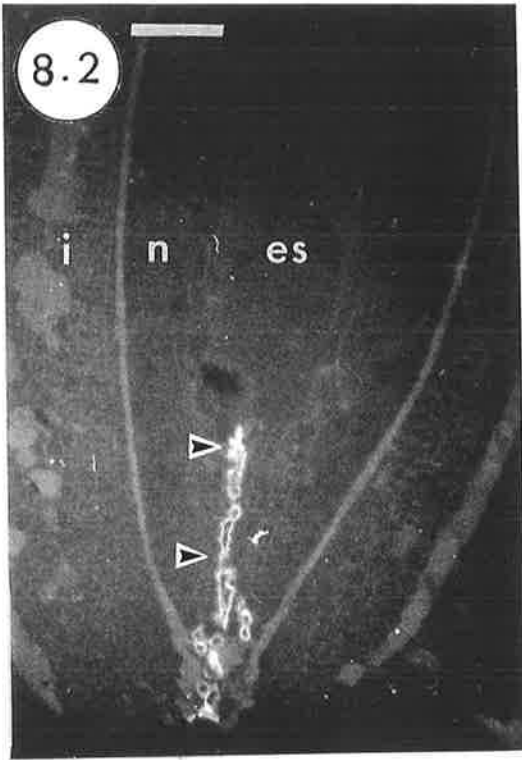


Fig. 8.6 Normal seed on day 98. Embryo (e) with vascular tissue (vt) surrounded by cellular endosperm (ce). Bar represents 100 μm .

Fig. 8.7 Ovule without embryo sac on day 28, showing degenerated nucellus (n) within large integument (i). Bar represents 50 μm .

Fig. 8.8 Ovule on day 14 with a normal but unfertilised egg apparatus (ea). Bar represents 10 μm .

Fig. 8.9 Seed trace on day 28 with a normal but unfertilised embryo sac (es) and proliferated nucellus (n), integument (i). Bar represents 50 μm .

Note: Micropyle faces the bottom of the page in all figures. Days are counted from the day when the first flower opened on the inflorescence.

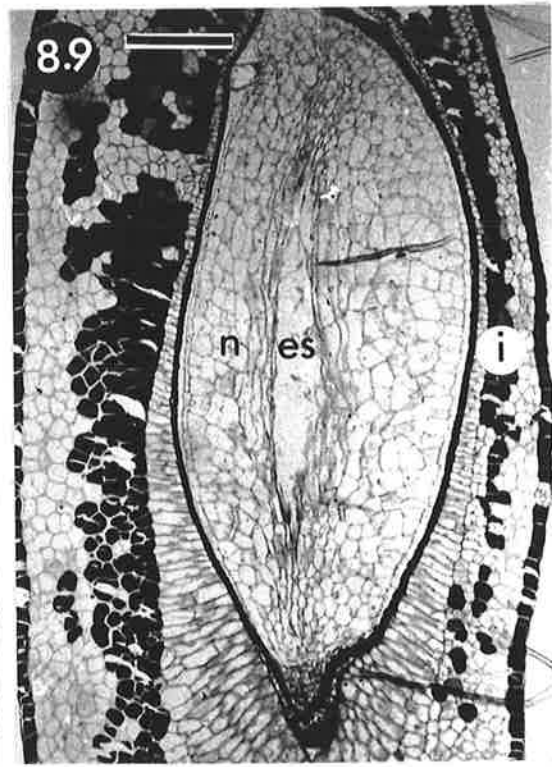
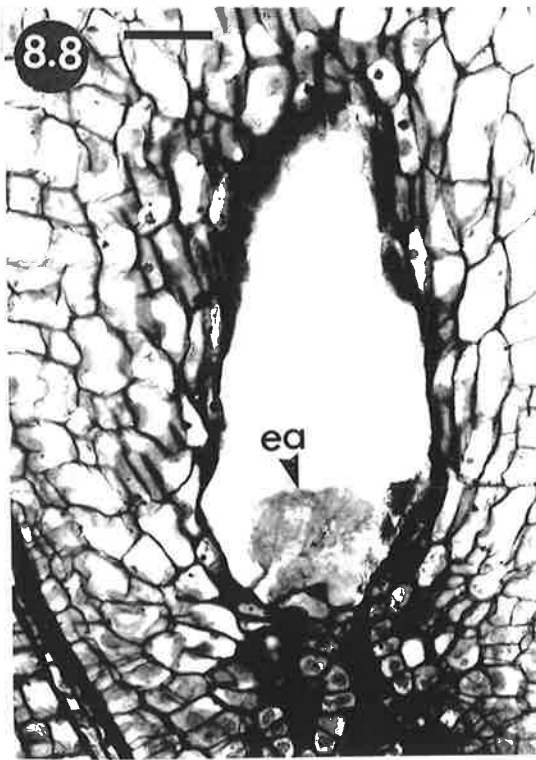
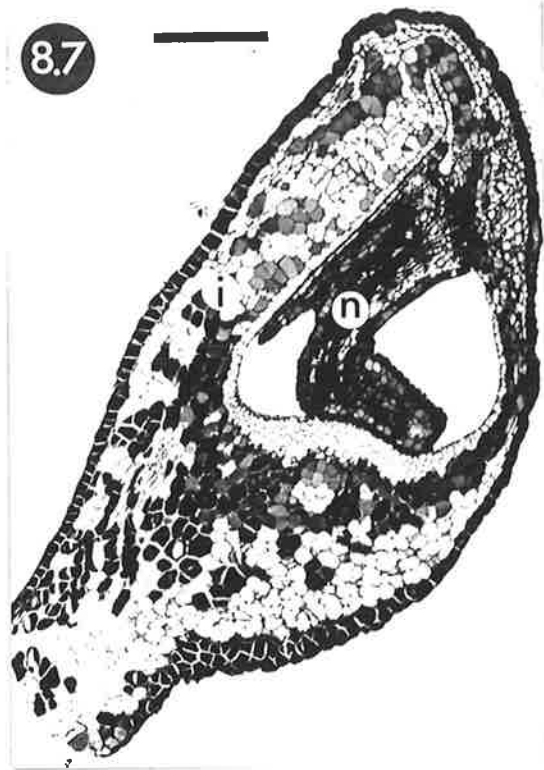
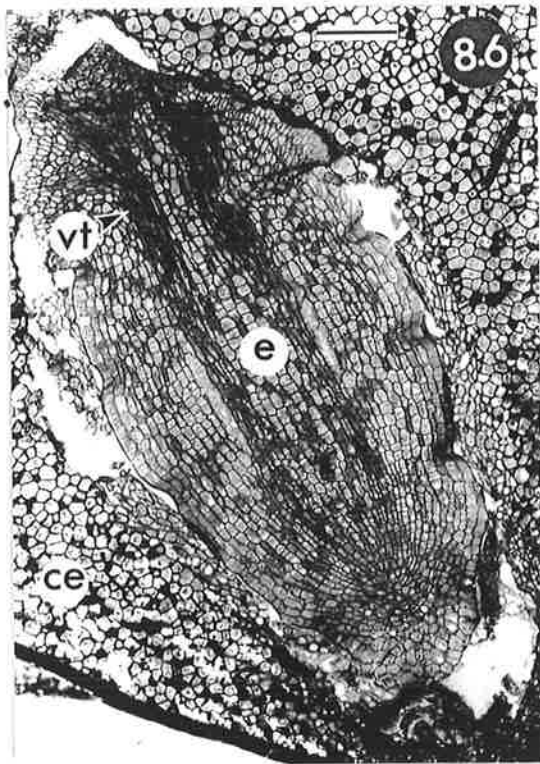


Fig. 8.10 Fertilised seed on day 28, showing undivided zygote (z), free nuclear endosperm (fe) having degenerated. Bar represents 5 μm .

Fig. 8.11 Seed on day 42 with degenerating free nuclear endosperm (fe) and without zygote or proembryo. Bar represents 10 μm .

Fig. 8.12 Seed on day 28, with no embryo, very small cellular endosperm (ce) and proliferated nucellus (n) beginning to degenerate. Bar represents 50 μm .

Fig. 8.13 Floater seed on day 98 showing well developed testa (t), with invaginations into the seed lumen, but no embryo or endosperm and with fragments of degenerating nucellus tissue (n). Bar represents 480 μm .

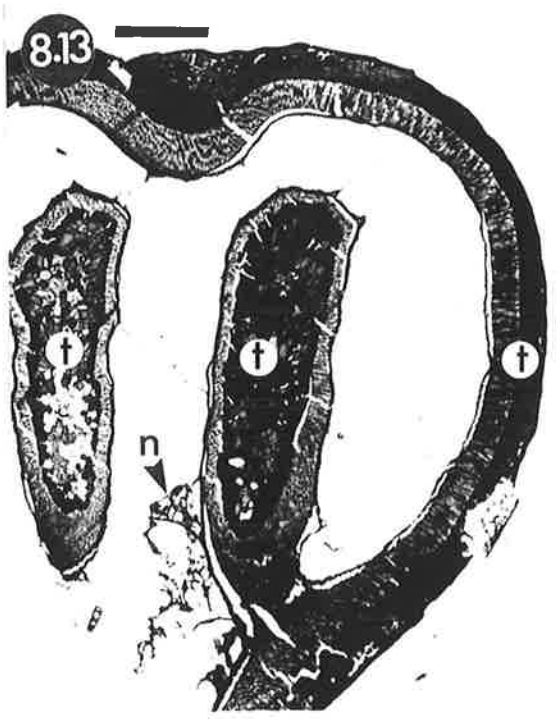
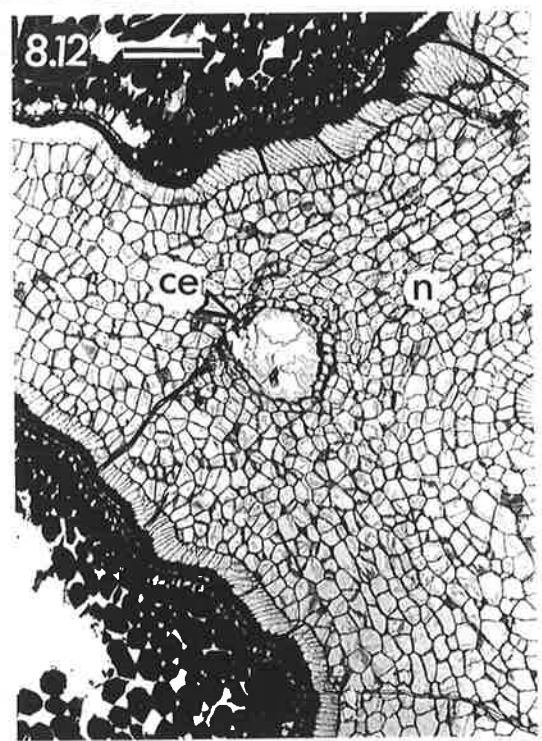
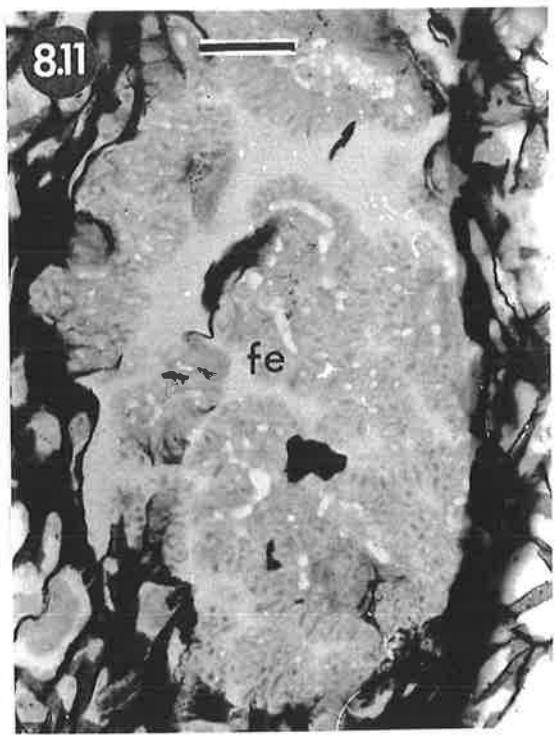
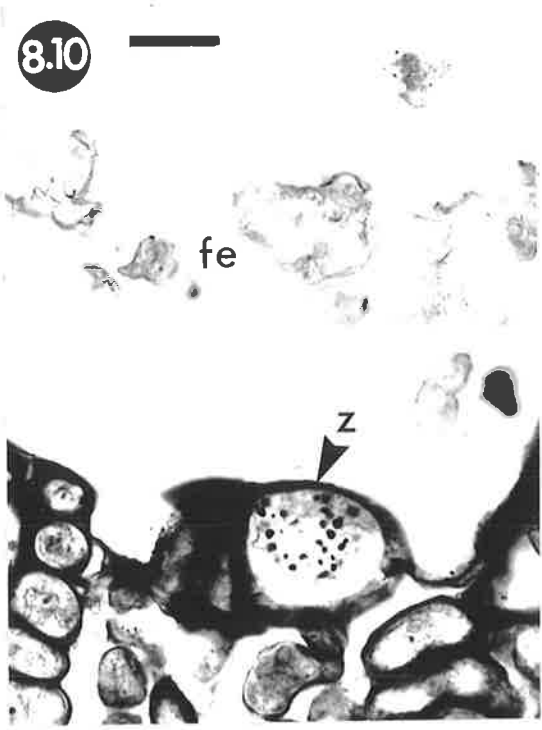
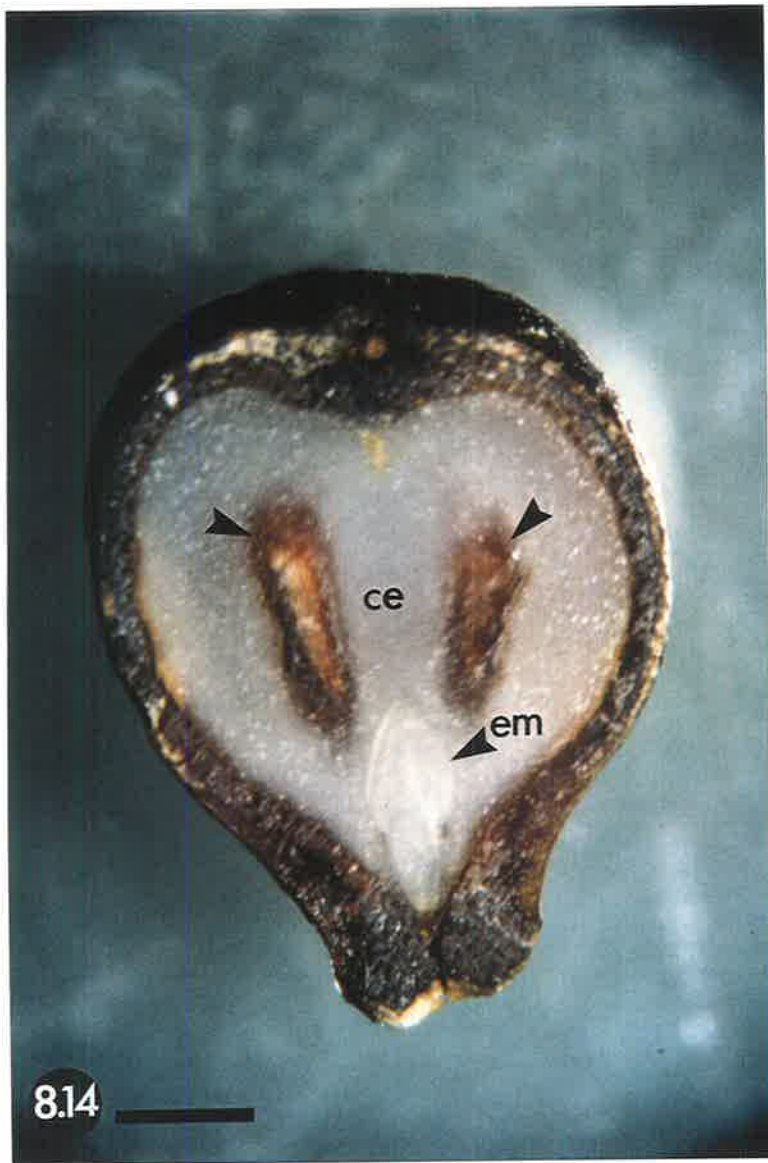


Fig. 8.14 Mature sinker seed, halved longitudinally, with testa (t), cellular endosperm (ce) and embryo (em), and invaginations of the testa (arrow head). Bar represents 1 mm.

Fig. 8.15 Mature floater seed, halved longitudinally, without embryo and showing empty space with some remnants of cellular endosperm (ce). Bar represents 1 mm.



polar nuclei. In the absence of fertilisation, they either aborted at an early stage or continued to grow by division and enlargement of the nucellus and integuments (Fig. 8.9).

Several types of abnormal development were found in ovules after fertilisation, preventing normal seed development. Degeneration of the zygote and free nuclear endosperm was observed on day 14 and thereafter (Table 8.2). In most cases, the zygote did not divide or proembryo development ceased after a few divisions while the free nuclear endosperm degenerated before cell walls were formed (Fig. 8.10). In other cases, there was no sign of zygote or proembryo development and the endosperm either degenerated (Fig. 8.11) or was cellular but very small (Fig. 8.12).

The dimensions of an array of ovules/seeds are shown in Table 8.3 and depicted as line diagrams in Fig. 8.16. On day 4, size differences between the large- and medium-sized berries were small. Therefore no distinction is made between the ovules/seeds extracted from them. The size differences between the B-1 and B-3 seeds (listed in Table 8.2) were also small at this early stage of development and this made it somewhat difficult to distinguish between them. Relatively similar numbers of B-1 and B-3 seeds were apparently normal although fertilisation, division of the primary endosperm nucleus to produce free nuclear endosperm, and zygote formation occurred in 5 of the 20 B-1 and in 3 of the 20 B-3 seeds. In contrast, there was no indication that B-3 ovules had been penetrated by pollen tubes as had been the case in 4 of 10 B-1 ovules.

On the subsequent sampling days, differences in the development of the largest seed of the big berries (LB-1) and the third-largest seed of the medium-sized berry (MB-3) (table 2) were clearly evident. On day 14, 8 of 10 of the LB-1 seeds had a zygote and free nuclear endosperm compared with only 2 of 10 for the MB-3 seeds, the other eight lacking an embryo sac or remaining unfertilised. On day 28, all MB-3 seeds showed

8.2

Table 1: The structure of Chardonnay ovules and seeds from four days after anthesis to maturity. Seeds showing normal development are represented in columns 5 and 10, while the other columns show various abnormalities. The value show the number of ovules/seeds of the specified type out of the total number examined. The types, B-3 and MB-3, are the third-largest ovules/seeds in medium-sized berries, and B-1 and LB-1 are the largest ovules/seeds in large berries.

Type	Days after anthesis	1 Embryo sac penetrated by pollen tube	2 3 Embryo sac (ES)		4	5 6 Zygote		7	8 9 No embryo Cellular endosperm (CE)		10 Embryo
			ES absent	ES present	No zygote Free nuclear endosperm (FE)	FE degen.	FE degen.		FE healthy	CE degen.	CE healthy
			Non fertil.		FE degen.	FE degen.	FE healthy	Nucellus degen.			
Third largest											
B-3	4	0/10	5/20	12/20	—	—	3/20	—	—	—	—
MB-3	14		5/10	3/10	—	—	3/20 2/10	—	—	—	—
MB-3	28		5/10	5/10	—	—	—	—	—	—	—
MB-3	42		—	—	—	—	—	10/10	—	—	—
Largest											
B-1	4	4/10	5/20	10/20	—	5/20	5/20	—	—	—	—
LB-1	14		—	—	2/10	—	8/10	—	—	—	—
LB-1	28		—	—	1/10	1/10	1/10	—	—	5/10	2/10
LB-1	42		—	—	1/10	—	—	—	—	3/10	6/10
LB-1	98		—	—	—	—	—	—	2/10	—	8/10
Fig. numbers		2	7	8,9	10		3, 4	11, 13		12	5,6

Abbreviations: degen.=degenerating; fertil. = fertilised;

Table 8.3: Dimensions ($\mu\text{m} \times 10$) of Chardonnay ovule and seed structures from 4 days after capfall to maturity (mean \pm SE).

Days after flowering	Structure	ovule		embryo sac/endosperm		embryo	
		length	width	length	width	length	width
4 d	no embryo sac/nucellus degenerating	86 \pm 2	44 \pm 1	–	–	–	–
4 d	unfertilised embryo sac	90 \pm 1	43 \pm 1	37 \pm 2	6 \pm 0	–	–
4 d	zygote/free nuclear endosperm	97 \pm 3	47 \pm 2	46 \pm 4	6 \pm 1	–	–
14 d	no embryo sac/ nucellus degen.	97 \pm 2	39 \pm 1	–	–	–	–
14 d	unfertilised embryo sac	92 \pm 3	48 \pm 5	25 \pm 3	7 \pm 0	–	–
14 d	no zygote/free nuclear endosperm degen.	124 \pm 6	75 \pm 6	50 \pm 7	10 \pm 1	–	–
14 d	zygote/ free nuclear endosperm	140 \pm 10	101 \pm 0 00	79 \pm 11	10 \pm 1	3 \pm 0	2 \pm 0
28 d	no embryo sac/nucellus degenerating	94 \pm 9	49 \pm 6	–	–	–	–
28 d	unfertilised embryo sac	197 \pm 52	122 \pm 42	57 \pm 12	10 \pm 4	–	–
28 d	no zygote/free nuclear endosperm degen.	420	323	46	35	–	–
28 d	zygote/free nuclear endosperm degen.	420	270	230	170	3	2
28 d	no embryo/endosperm cellular	430 \pm 15	311 \pm 13	60 \pm 20	23 \pm 5	–	–
28 d	zygote/free nuclear endosperm	4.0 \pm 15	285 \pm 5	108 \pm 8	59 \pm 24	4 \pm 1	2 \pm 0
28 d	embryo/endosperm cellular	440 \pm 10	285 \pm 25	102 \pm 10	57 \pm 42	4 \pm 1	2 \pm 0
42 d	nucellus degenerating	143 \pm 21	85 \pm 19	–	–	–	–
42 d	no zygote/free nuclear endosperm degen.	319	200	58	15	–	–
42 d	no embryo/endosperm cellular	469 \pm 21	399 399 \pm 27	271 \pm 19	180 180 \pm 35	–	–
42 d	embryo/endosperm cellular	538 \pm 10	416 \pm 08	289 \pm 43	101 \pm 18	9 \pm 1	6 \pm 0
98 d	no embryo/cellular endosperm degen.	426 \pm 6	406 \pm 6	–	–	–	–
98 d	embryo/endosperm cellular	495 \pm 9	401 \pm 12	368 \pm 12	347 \pm 12	78 \pm 10	40 \pm 7

degen. = degenerating

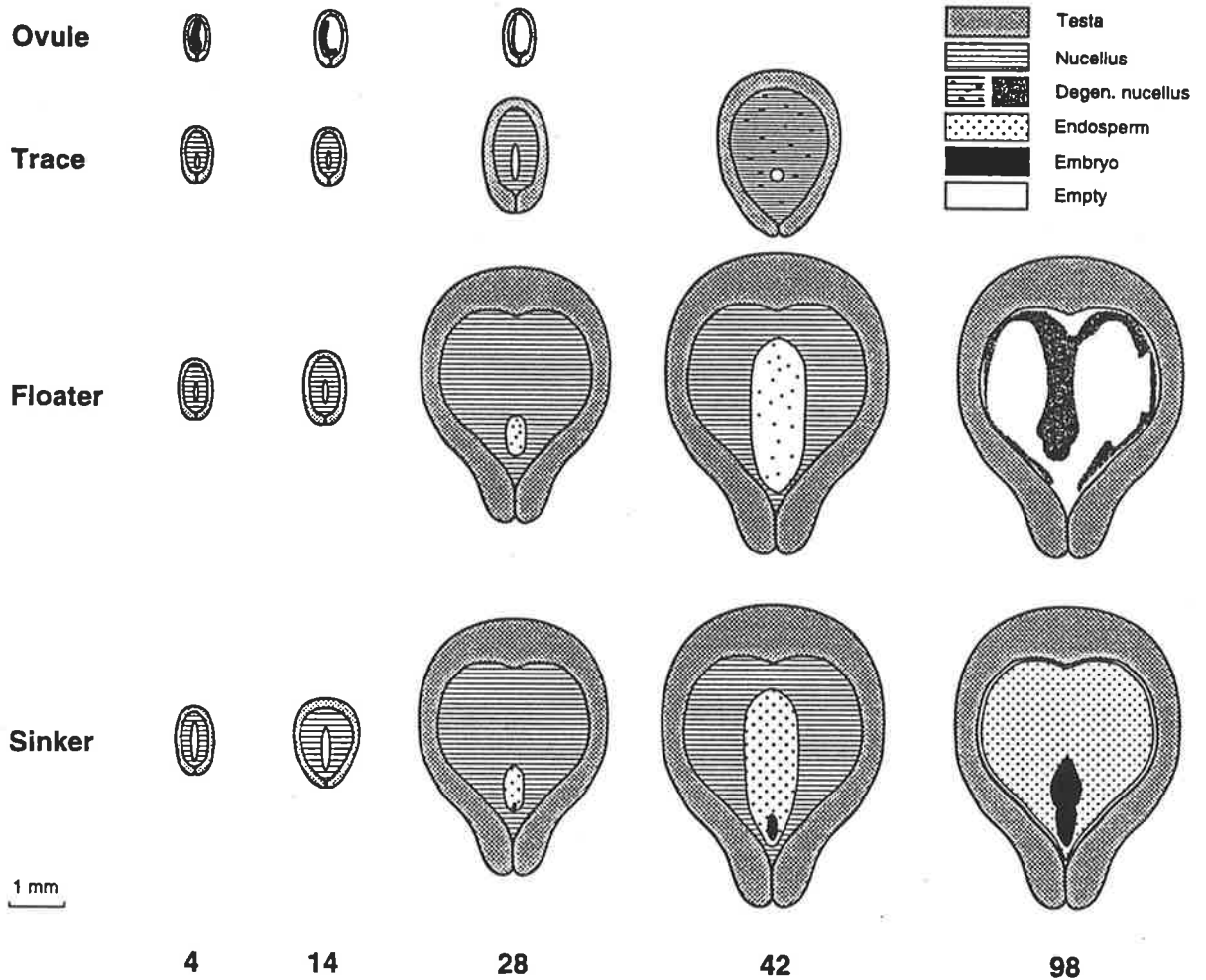


Fig. 8.16 Line diagrams drawn to scale to interpret the seed development shown by the data in table 8.3 and in Figs 8.2–8.13. For clarity, the invaginations of the testas as seen in fig. 8.14 have been omitted. The five columns show schematically development examined at the five sampling days. The four rows describe the development patterns as follows:

- ovule—no embryo sac, no integument growth and nucellus generated at all stages;
- trace—embryo sac present but unfertilised or, in some cases, fertilised, as evidenced by endosperm formation, with degeneration setting in at the free nuclear stage; no zygote; testa development incomplete; degree of sizing and hardening of the testa, and timing of the degeneration of the nucellus varied;
- floater—at early stage, embryo sac present, testa fully developed but nucellus, endosperm and proembryo degenerate later;
- sinker—testa, nucellus, endosperm and embryo developed normally.

abnormal development whereas three LB-1 seeds were normal and seven showed post fertilisation degeneration. Differences in the dimensions of seeds with an embryo and endosperm and those with a degenerated zygote were evident on days 42 and 98, at berry ripeness (Table 8.3). On day 42, all MB-3 seeds had degenerated nucelli and were small seed traces. All 10 LB-1 seeds had at least free nuclear endosperm and all but one had cellular endosperm; six seeds had also well-developed proembryos of more than 16 cells whereas the other four seeds had degenerated zygotes. At berry ripeness, only the largest seed of ten LB berries were examined; eight had an embryo with cotyledons, vascular tissue and extensive endosperm and were sinker seeds, while embryo and endosperm had degenerated in the remaining two, these being floater seeds.

Floater seeds were examined on days 42 and 98. On day 42, 3 of 10 seeds showed no evidence of embryo or endosperm and the embryo sacs were small and empty within the nucellus and testa. Small cellular endosperm was found in 4 of 10 seeds. Three others had a proembryo with up to four cells but no sign of endosperm or cytoplasm within the embryo sac, indicating that the endosperm nucleus had not divided or perhaps that normal double fertilisation had not occurred. At day 98, in ripe berries from late flowers (sample B), ten floater seeds examined had no embryo; they were either empty or had fragments of degenerated nucellus tissue or of cellular endosperm (Figs. 8.13, 8.15). The proportions of floater seeds was 31% for sample A bunches and 9% for sample B bunches.

8.4 Discussion

The sequence of events from fertilisation to the first division of the zygote and formation of cellular endosperm for Chardonnay was substantially similar to that described for other seeded grape cultivars (Figs 8.2–8.6). As found by Barritt (1970), Kassemeyer and Staudt (1983) and Ledbetter and Ramming (1989), the first division of the zygote occurred between 14 and 28 days after flower opening which coincided with cell wall formation of the endosperm. Such a relationship between endosperm and zygote

development is not unusual, e.g. it is found in cotton (Schulz and Jensen 1977) and is assumed to be due to the capability of the endosperm at this stage to provide the embryo with nutrients required for division. The results showed that embryo growth was slow from 14 to 42 days after flowering (Table 8.3) and that most of it occurred thereafter. Since the endosperm continues growth throughout and its embryo remains small, the grapevine has an endospermous seed in contrast to those angiosperms in which the endosperm has been consumed by the embryo at seed maturity (Sedgley and Griffin 1989).

Most investigations of seed development in grapevines have been restricted to the first few weeks after anthesis. Kassemeyer and Staudt (1983) showed for cvs Weisser Burgunder (syn. Pinot Blanc) and Gewürztraminer that the zygote started to divide 21 d after anthesis. Documentation exists on the abortion of seeds without embryo sacs, of unfertilised ovules (Pratt and Einset 1961, Carraro et al. 1979, Kassemeyer and Staudt 1982) or of fertilised seeds for the first few weeks after anthesis (Pratt and Einset 1961, Barritt 1970, Okamoto and Imai 1982, Staudt and Kassemeyer 1984). The present results, from field-grown Chardonnay vines, show that seeds aborted, not only when they were without an embryo sac or were not fertilised (Figs 8.7–8.9), but also after they had been fertilised (Fig. 8.10), such abortion occurring within a few weeks after the start of flowering. Furthermore, it was found that fertilised seeds which had developed with fully formed testas but lacked an embryo (Figs 8.11 and 8.12). In such cases, growth of the proembryo ceased after the first two divisions. Marasali (1993) also investigated seed development in the cultivar Cavus and found that empty-seededness in this cultivar resulted from zygote or embryo degeneration; however, the stage of embryo development at degeneration was not determined. In Chardonnay a divided zygote was rarely observed even on day 28 while, in many seeds, the embryos continued cell division past the 16-cell stage at day 42.

In earlier chapters, results obtained under controlled environment conditions were presented to show that Chardonnay is a cultivar susceptible to damage by low temperature before anthesis. The nature of this damage shown in ovules one day after flower opening is described in chapter 7. In addition, the results of this present experiment show that seed

development after fertilisation is also a problem. This is different from the finding of Kassemeyer and Staudt (1983) who found normal seed development in fertilised ovules of two-seeded cultivars. Faulty development of seeds in Chardonnay resembled to some extent that reported in seedless cultivars (Ledbetter and Ramming 1989).

The effect of temperature during flowering on the subsequent development of the berries and their seeds is illustrated by the difference in the number of floater seeds between the berries of samples A and B. The daily maximum temperatures during the first four days of flower opening (Fig. 8.1) were about 4°C lower for the earlier-flowering inflorescences (sample A) than for the later-flowering ones (sample B), both temperature levels being well above those found to be damaging for fruit-set by May (1992). The higher proportion of floater seeds (in which the embryo and endosperm aborted at an early stage) in the berries of sample A than in those of sample B was probably a consequence of this temperature difference. Ovaries containing floater seeds remain on the bunch, but become berries of reduced size in comparison with berries with viable seeds, and this will cause some reduction in yield.

It can be concluded that, in Chardonnay, arrested ovule development at an early stage may lead to flower abscission or the growth of very small berries which ripen (chickens). Later abortion may lead to empty seeds (floaters) which provide sufficient stimulation for the berries to mature but which are smaller than berries with normal seeds.

8.5 Conclusions

- Only about 20% of examined Chardonnay ovules were penetrated by pollen tubes and were fertilised as evidenced by a zygote and free nuclear endosperm.
- The first division of the zygote occurred between 14 and 28 d after anthesis and coincided with cell wall formation in the endosperm.

- Ovule abortion occurred shortly after flowering in ovules without an embryo sac or ovules with a normal embryo sac but without fertilisation. Abortion occurred also in fertilised seeds.
- Ovule abortion in fertilised ovules was observed from 14 days after anthesis leading to degeneration of zygote and endosperm and incomplete seed formation i.e. seed traces (stenospermy).
- Later degeneration of endosperm and nucellus after testa development was complete (by day 42) resulted in empty (floater) seeds; this is considered to be the consequence of zygote /embryo degeneration.
- Thus, aberrant embryo sac development before anthesis, lack of fertilisation at anthesis, and defective development of zygote/embryo after anthesis are seen as the causes of nil or defective seed growth in cv. Chardonnay.

Chapter 9

Effect of variations in temperature regimes and shading on seed and berry development of *Vitis vinifera*

9.1 Introduction

It is well known that berry size is dependent on seed number (Müller-Thurgau 1898, Winkler and Williams 1936, Schumann 1973) and the degree of seed development within any one cultivar (Olmo 1946) when environmental and cultural conditions are similar. The composition of the berries and the seeds is also correlated with seed number (Schumann 1973, Boselli et al. 1995). Seed development is influenced by climatic conditions of which temperature is probably the most important factor. Incidence of low temperature two or one week before flowering resulted in fewer seeds per berry (Roubelakis and Kliewer 1976, Ewart and Kliewer 1977). However, detailed information is not available on the interplay between climatic conditions before and at flowering which affect the development and functioning of the sexual parts of the flower on the one hand, and the post-flowering development of seeds and berries on the other. The study described in this chapter dealt with this subject, using bunches from the experiments described in chapter 4.

9.2 Materials and methods

Plants used in the experiments of chapter 4 were used for the observations described here. Experiment 1 (here coded exp. 1-4) dealt with variations of temperature, and experiment 2 (coded exp. 2-4) with combined variations of temperature and light.

For exp. 1-4, the number of seeds per berry was determined for all berries of a random selection of half of the bunches of each treatment of experiment 1 of chapter 4, i.e. two bunches on each of the five replicates of the 18 treatments of the variables 'temperature' (T), 'stage' (S) and 'pollination' (P) and of Control (C), namely [(2T x 4S x

2P), (C x 2P)] in Chardonnay, and in each of four replicates of the nine treatments [(2T x 4S), C] in Shiraz. The seeds were divided into sinkers and floaters according to their buoyancy in water. Per cent berries with one seed, total number of seeds per berry and number of sinker seeds and floater seeds per berry were determined.

For exp. 2-4, seed development was observed only in Chardonnay because the results of experiment 1 (chapter 4) had shown that seed number in Shiraz was less susceptible to varying temperature conditions prior to fruit-set. Random selection was made of two of the four bunches of each plot of the five times replicated 13 treatments, i.e. 2 'stages' (S; stages 15, 17) x 2 'temperatures' (T; 17°/14°C or 12°/9°C for one week, otherwise 25°/20°C) x 3 levels of 'shading' (L; 8%, 40%, 72%) and Control (25°/20°C; full light). Every berry of each bunch was weighed and the number, the individual weight(s) and the buoyancy of its seed(s) were determined. Pericarp weight was calculated for each berry by deducting seed weight from berry weight

All data of each measured or calculated set of observations of both experiments were statistically analysed (see chapter 3, section 3.4).

9.3 Results

Seed complement per berry

Per cent one-seeded berries

While the results of the two experiments 1-4 and 2-4 concerning the percentage of berries with varying seed complements (Tables 9.1 and Fig. 9.1, see also Appendix, Tables 3 and 4) are not clear-cut, certain trends are emerging even if not confirmed statistically in all cases. This may be due in part to the type of plants used in the experiments, i.e. their small size, but in part also because of the severity of Tukey's test.

Table 9.1: Per cent berries with one seed. Data from experiment 1–4 for Chardonnay and Shiraz and experiment 2–4 for Chardonnay (analysed by ANOVA type A)

Per cent berries with one seed																
	Control	Temperature			'Stage'				Pollination			Shading				
	25°/20°	17°/14°	12°/9°	F	15	16	17	20	F	—	+	F	8%	40%	72%	F
Chardonnay																
Exp. 1	67.7	77.8	73.1	ns	79.5 ^{ab}	67.1 ^a	74.0 ^{ab}	81.4 ^b	*	80.2	70.9	*	n.d.	n.d.	n.d.	n.d.
Exp.2	73.4	80.2	68.6	**	72.2	n.d.	76.6	n.d.	ns	n.d.	n.d.	n.d.	70.3	76.6	76.4	ns
Shiraz																
Exp.1	70.9	69.2	69.1	ns	67.7	68.5	70.1	70.4	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Per cent berries with one floater seed																
Chardonnay																
Exp. 1	22.2	24.4	32.9	ns	18.9 ^a	23.0 ^{ab}	28.7 ^{ab}	43.9 ^b	**	33.8	23.4	**	n.d.	n.d.	n.d.	n.d.
Exp.2	9.2	14.6	23.2	***	15.9	n.d.	21.9	n.d.	**	n.d.	n.d.	n.d.	16.6	19.7	20.3	ns
Shiraz																
Exp.1	7.1	7.3	9.5	ns	5.6	7.3	9.7	10.6	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Significance of F value: ns not significant; *, **, ***: significant at $p < 0.05$, $p < 0.01$, $p < 0.001$

n.d not determined

The large majority of berries was one-seeded in both experiments. Overall, and in both experiments, a larger proportion of one-seeded berries was present after exposure to 17°/14°C than after 12°/9°C, although this difference was significant only in experiment 2-4. This result is somewhat surprising, considering the generally detrimental effect of the lower-temperature treatment. A possible explanation for this may be found in the better fruit-set after exposure to 17°/14°C, compared with 12°/9°C, leading to more berries per bunch. The proportion of one-seeded berries among the additional berries may have been somewhat greater, increasing their overall proportion. The tendency toward more one-seeded berries being present at stages 15 and 20 than at 16 and 17 (exp. 1-4), causing a significant interaction, cannot be explained also. The response to supplementary pollination in experiment 1-4 was a significant decrease in the proportion of single-seeded berries, indicating that there were more berries with multiple seeds.

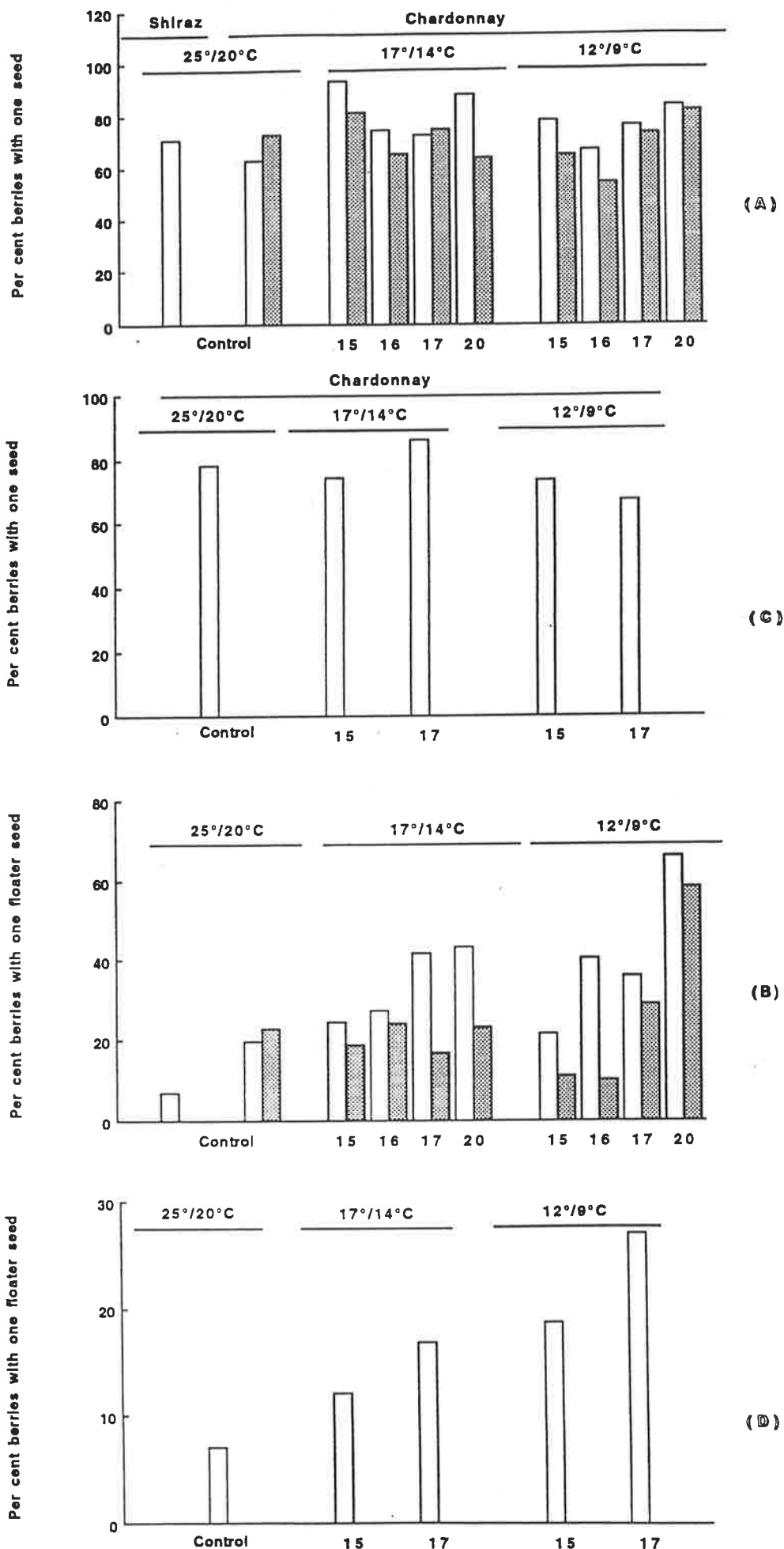


Figure 9.1: A. Per cent berries with one seed Exp. 1-4; B. Per cent berries with one floater seed Exp. 1-4; C. Per cent berries with one seed Exp. 2-4; D. Per cent berries with one floater seed Exp. 2-4. □ -Pollination, ▨ +Pollination

Thus, while the proportion of berries with a single seed varied somewhat erratically between treatments, that of berries with one floater but without sinker seed was quite clear-cut (Table 9.1): firstly, without supplementary pollination, the proportion of such berries was greater when 17°/14°C was applied close to flowering, stages 17 and 20, and much greater when 12°/9°C was applied at stage 20 (the result for stage 17 appears to be aberrant). Secondly, supplementary pollination reduced the proportion of berries with only one floater in all cases (leading to a highly significant difference, Table 9.1).

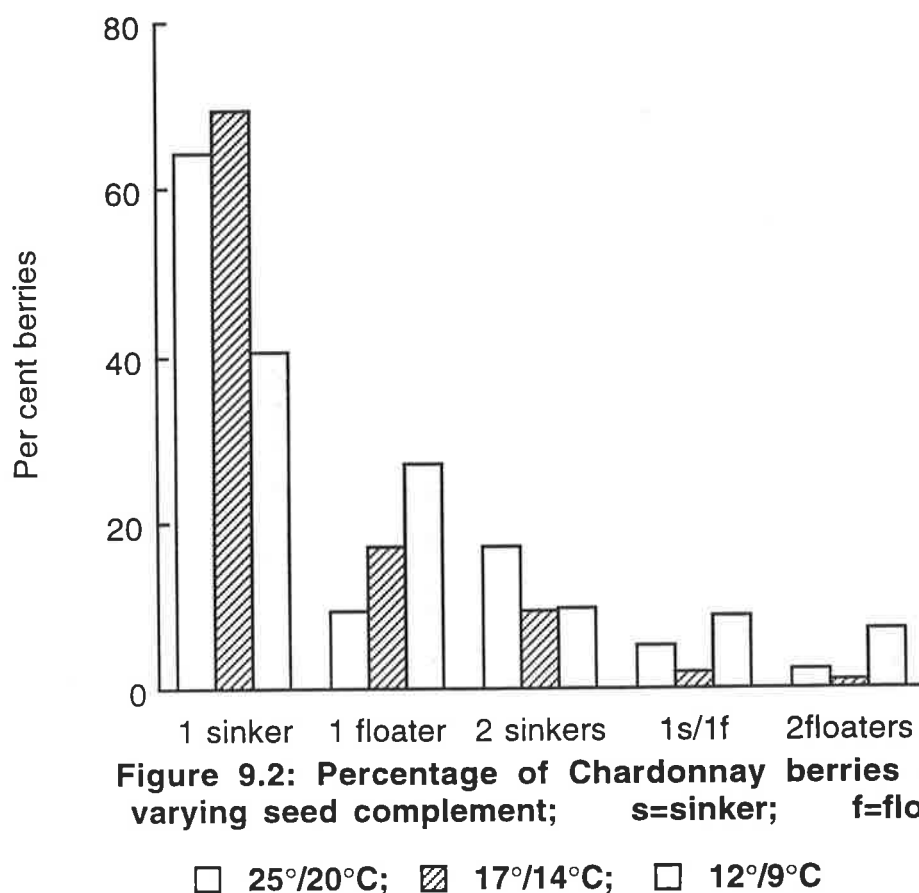


Figure 9.2: Percentage of Chardonnay berries with varying seed complement; s=sinker; f=floater

□ 25°/20°C; ▨ 17°/14°C; □ 12°/9°C

The shading treatments in experiment 2-4 had little effect on the proportion of one-seeded berries or on the proportion of berries with one floater. Therefore, the values in Fig. 9.2 for the four temperature treatments are presented as averages over the shading treatments. The increasing proportion of berries with one floater seed was evident as temperature became less. In particular, 12°/9°C applied at stage 17 increased the proportion of such berries. In Shiraz, none of the treatments caused significant differences in the proportion of one-seeded berries.

There were no berries without seed at maturity in experiment 1-4 and a number of small berries fell off before maturity. A few such berries continued to maturity in experiment 2-4.

Seed number per berry

The mean number of total seeds per berry was not affected significantly by any of the treatments in either varieties and in both exps 1-4 and 2-4, with the exception of supplementary pollination in Chardonnay in exp. 1-4 (Table 9.2). This treatment had a beneficial effect on seed number in the berries of all other treatments (as shown in detail in Appendix, Table 5). In ANOVA type B for total seed number per berry, there were no significant differences between any of the treatments in exp.1-4, Chardonnay (with the values of with or without pollination being analysed separately) or Shiraz, nor in exp. 2-4

Table 9.2. Number of total seeds per berry. Data from experiments 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay (analysed by ANOVA type A)

	Control	Temperature		'Stage'				Pollination			Shading					
	25°/20°	17°/14°	12°/9°	F	15	16	17	20	F	—	+	F	8%	40%	72%	F
Chardonnay																
Exp. 1	1.39	1.29	1.31	ns	1.31	1.39	1.30	1.19		1.21	1.39	***	n.d.	n.d.	n.d.	n.d.
Exp.2	1.32	1.23	1.29	ns	1.29	n.d.	1.23	n.d.	ns	n.d.	n.d.	n.d.	1.27	1.24	1.27	ns
Shiraz																
Exp.1	1.48	1.37	1.34	ns	1.44	1.34	1.41	1.22	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Significance of F value: ns not significant;

n.d not determined

(see Appendix, Table 5). The average seed number per berry in Chardonnay Control was 1.39 and 1.32 in exp. 1-4 and 2-4 respectively, the corresponding value for Shiraz, experiment 1-4, being 1.48.

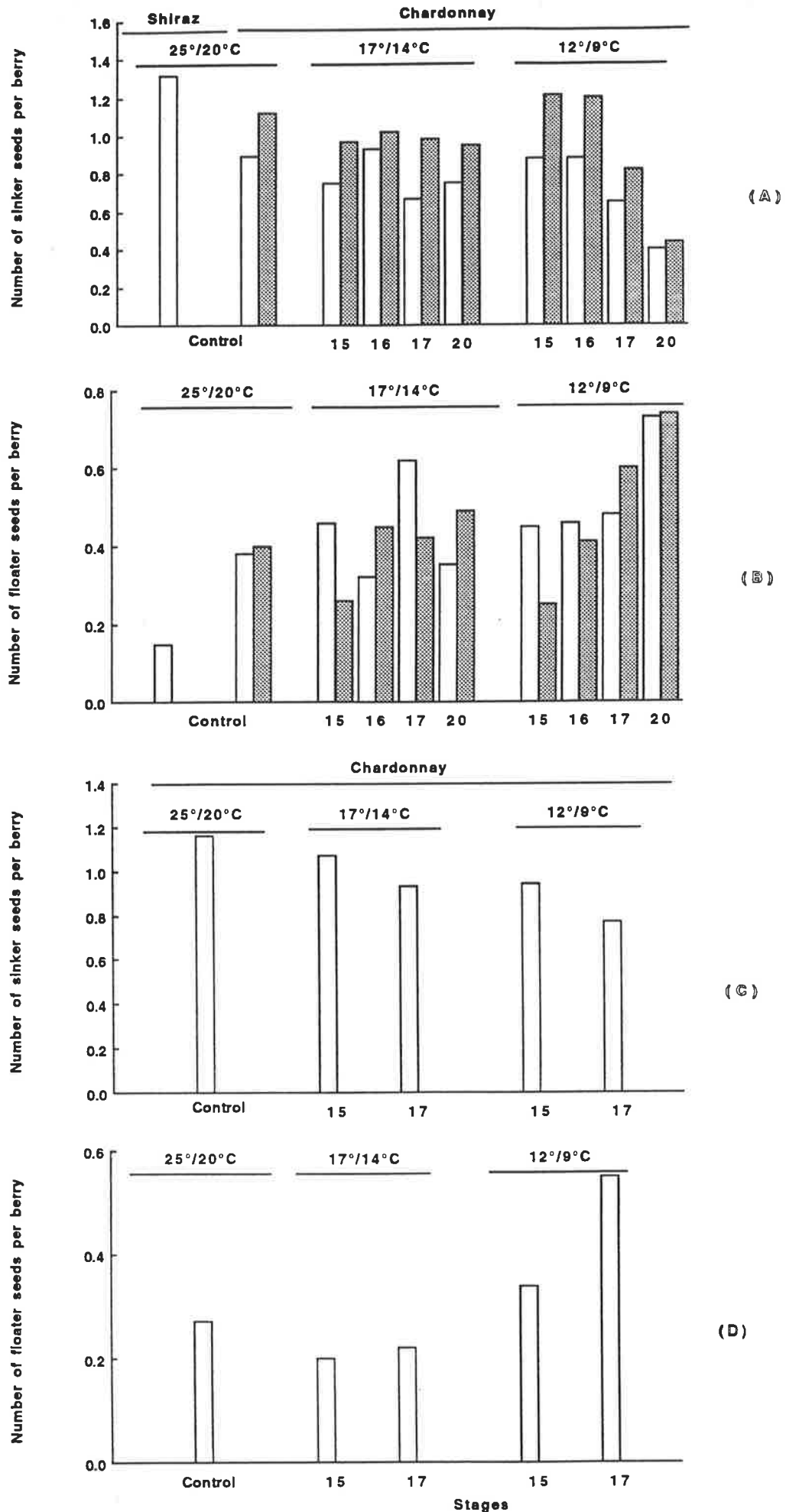


Figure 9.3: A. Number of sinker seeds per berry Exp. 1-4; B. Number of floater seeds per berry Exp. 1-4; C. Number of sinker seeds per berry Exp. 2-4; D. Number of floater seeds per berry Exp. 2-4 □ -Pollination; ▨ +Pollination

Table 9.3: Number of sinker and of floater seeds per berry. Data from experiments 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay (analysed by ANOVA type A)

Number of sinker seeds per berry																
	<u>Control</u>	<u>Temperature</u>			<u>'Stage'</u>				<u>Pollination</u>			<u>Shading</u>				
	25°/20°	17°/14°	12°/9°	F	15	16	17	20	F	—	+	F	8%	40%	72%	F
Chardonnay																
Exp.1	1.02	0.88	0.81	*	0.95 ^a	1.00 ^a	0.78 ^b	0.64 ^c	**	0.74	0.95	***	n.d.	n.d.	n.d.	n.d.
Exp.2	1.16	1.00	0.86	***	1.00	n.d.	0.85	n.d.	***	n.d.	n.d.	n.d.	0.98	0.93	0.87	*
Shiraz																
Exp.1	1.32	1.19	1.11	ns	1.28 ^a	1.18 ^{ab}	1.13 ^{ab}	1.01 ^b	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Number of floater seeds per berry																
Chardonnay																
Exp.1	0.40	0.41	0.50	ns	0.36 ^a	0.39 ^{ab}	0.52 ^{ab}	0.55 ^b	*	0.44	0.48	ns	n.d.	n.d.	n.d.	n.d.
Exp.2	0.27	0.23	0.44	***	0.29	n.d.	0.38	n.d.	ns	n.d.	n.d.	n.d.	0.30	0.31	0.39	ns
Shiraz																
Exp.1	0.15	0.19	0.23	ns	0.17	0.16	0.29	0.21	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Significance of F value: ns not significant; *, **, ***: significant at $p < 0.05$, $p < 0.01$, $p < 0.001$

n.d. not determined

In contrast to the total number of seeds per berry, the mean number of sinker seeds per berry differed significantly between the treatments of all three variables in experiment 1-4, (Table 9.3, Figure 9.3; see also Appendix, Table 6). Fewer sinker seeds were present in berries after exposure to the lower temperature regime, and when temperature treatments were applied late (in exp. 1-4, stages 17 and 20, and in experiment 2-4, stage 17). There was a statistically significant T x S interaction in experiment 1-4 due to the values of the two temperature treatments being similar when applied early, but quite different when applied later (exp. 1-4). As expected, the number of floater seeds per berry showed the inverse trend. Supplementary pollination was effective in increasing the number of 'sinker' seeds (Table 9.3, Figure 9.3). The increase was 37% and 36% at stages 15 and 16, but only 26% at stage 17 and 10% at stage 20. Shading had smaller effects in exp. 2-4; there was an indication that the most severe shading treatment (72%), when applied at stage 17, reduced the number of sinker seeds per berry (data in Appendix, Table 6). None of the other shading treatments seemed to affect the number of sinker seeds per berry to any extent. The severe reduction of the combined exposure to unfavourable temperature and heavy

shade applied at a critical time can be gleaned from the difference between the treatments of control and of 12°/9°C plus 72%, 1.16 sinker seeds per berry reduced to 0.67.

As would be expected the number of floater seeds varied in reverse order to those of sinkers in both experiments although their relatively small numbers reduced the possibility of treatment differences becoming statistically significant (see Figure 9.3 for data from experiment 1-4). Nevertheless, there was clear indication that their number increased with treatment 12°/9°C being applied closer to flowering, and that supplementary pollination tended to reduce the number of floater seeds to some extent. In experiment 2-4, there was a trend for heavy shading combined with low temperature and applied late (12°/9°C plus 72% at stage 17) to cause an increase in the number of floater seeds per berry (data shown in Appendix, Table 6), the mean value of control (0.27) increasing to 0.66 (difference not significant). This trend of heavy shading having a detrimental effect on seed development was somewhat reinforced by a (statistically non-significant) increase in floater number per berry found at 17°/14°C plus 72% shade applied at stage 17 (see Appendix, Table 6).

In Shiraz, the effect on seed number of applying different temperatures at various stages of development was similar but much less pronounced than in Chardonnay. Shiraz also showed an overall trend of more sinker seeds developing after exposure to 17°/14°C than to 12°/9°C (Table 9.3), but the difference between the means failed to reach significance. As shown in more detail by the ANOVA type B, exposure to 12°/9°C at the later stages tended to reduce the number of sinkers per berry compared with Control, the difference being significant at stage 20 (see Appendix, Table 6). As in Chardonnay, there was also a trend towards a reversal in the number of floaters per berry, compared with that of sinkers, but the differences if any were small (Table 9.3, Appendix, Table 6).

Seed weight

The mean weight per seed, determined in experiment 2-4, is shown in Figure 9.4 where the mean values for control and the temperature treatments at stage 17 are shown.

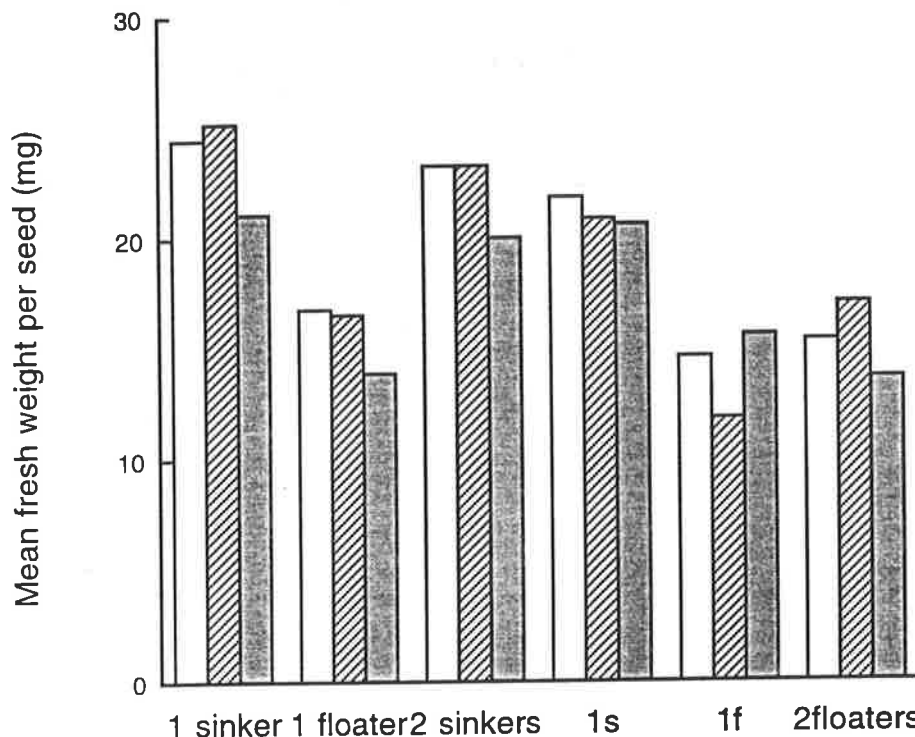


Figure 9.4: Weight per seed of Chardonnay berries with varying seed complement; s=sinker; f=floater

□ 25°/20°C; ▨ 17°/14°C; ■ 12°/9°C

Shading results are excluded because of the absence of significant differences; similarly stages 15 results are excluded because they were similar to stage 17 (the complete set of data is shown in Appendix, Table 7). Sinker seeds were heavier than comparable 'floater' seeds in all cases. The short-term exposure to the 12°/9°C regime caused a significant reduction in seed weight in berries with a single sinker seed, and there was a similar, non-significant trend in berries with a single 'floater' or two sinker seeds. On average, the weight of Chardonnay floater seeds was somewhat less than 2/3 of that of sinker seeds (the reduction being 32, 32, 34, 37 and 35%, calculated on the treatment means shown in Appendix, Table 8). In berries with two sinker seeds, the average weight per seed was about 6 % less than in berries with one sinker.

In Figure 9.5 the total weight of seed per berry is shown to demonstrate its relationship with the weight of pericarp per berry (see section seed weight and pericarp weight).

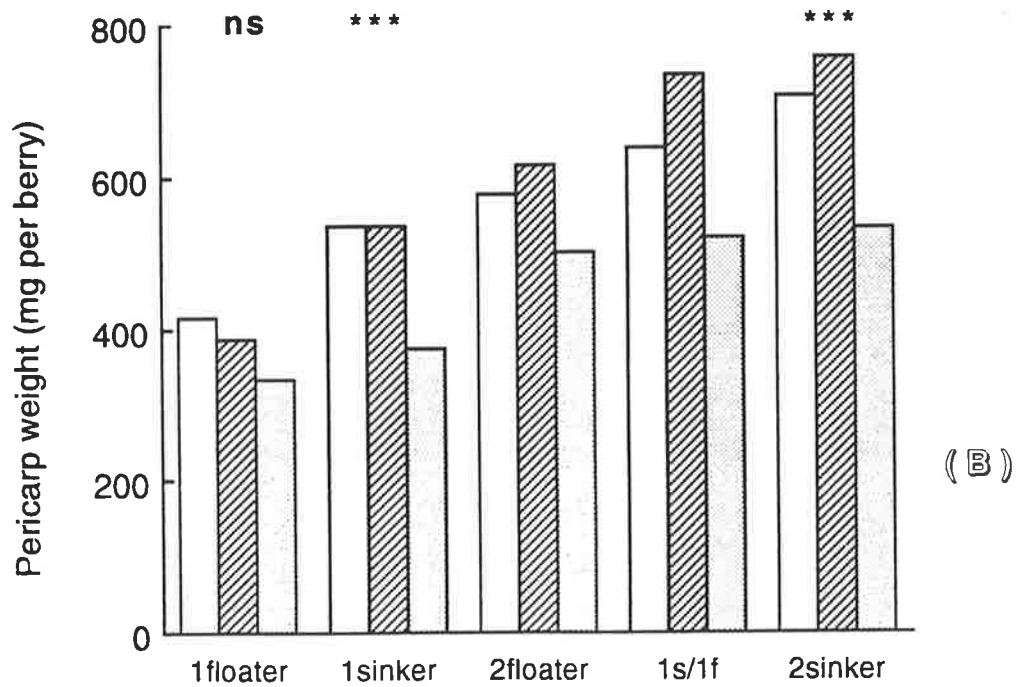
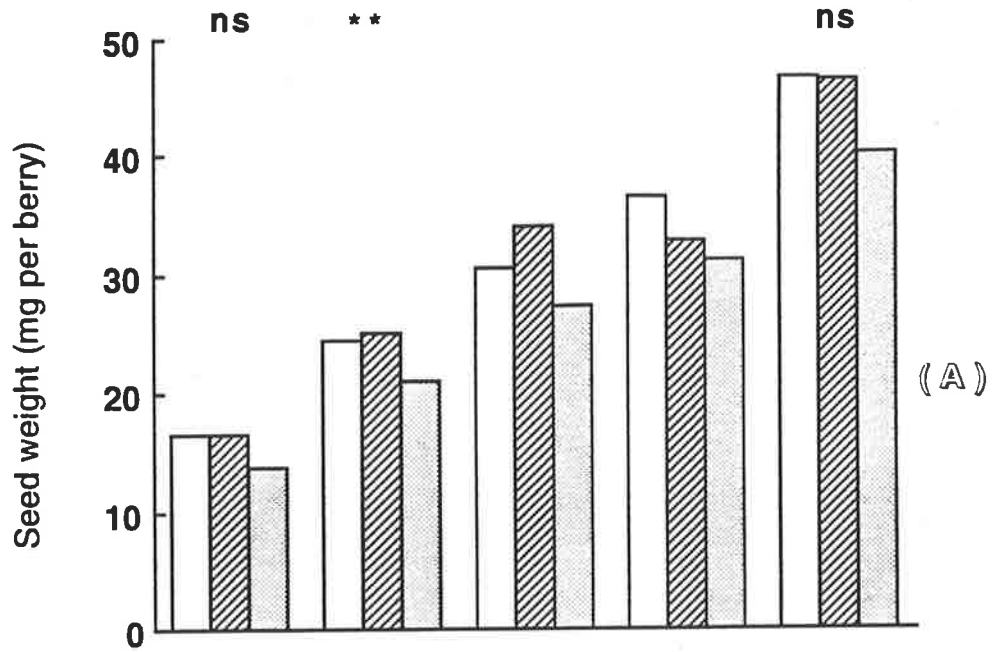


Figure 9.5: Seed (A) and pericarp (B) weight per Chardonnay berry with varying seed complement

□ 25°/20°C; ▨ 17°/14°C; □ 12°/9°C; s=sinker; f=floater

Pericarp weight per berry

The weight of pericarp per berry in exp. 2-4 (calculated from berry weight less seed weight) is shown in Figure 9.5B (and in Appendix, Table 8). The effects of exposure to the various environmental conditions at stages 15 or 17 were similar. Temporary exposure to 12°/9°C caused a large decrease in pericarp weight of berries with 'sinker' seeds and to a lesser (non-significant) extent in those with 'floater' seeds. Shading had no significant effect on pericarp weight. Overall, berries with 'sinker' seeds had considerably larger pericarps than berries with 'floater' seeds in the Control and 17°/14°C treatments, but not in the 12°/9°C treatments. Comparison of the pericarp weight of berries with two 'sinker' seeds and one 'sinker' seed supported the well-known effect of multiple seeds per berry causing increased pericarp size. The average pericarp weight of the few berries with no hard seeds ('chickens') was 172 mg, about one third of the pericarp weight of berries with one 'sinker' seed and about one half of berries with one 'floater' seed.

Relationship between seed content and berry development

As shown in Figure 9.6, both berries per bunch and the percentage of sinker seeds per berry in Chardonnay (data in Appendix, Table 9) decreased with exposure to lower temperatures and without supplementary pollination. The effect of this exposures was the more severe the closer they occurred to flowering. Supplementary pollination seemed to overcome the detrimental effect of treatment 12°/9°C on fruit-set, without improving the proportion of sinker seeds. In Shiraz, the proportion of sinker seeds (80-90%) was similar for all treatments, as was the number of berries per bunch (~40 berries).

Seed weight per berry had an important effect on pericarp weight, becoming obvious by comparing parts A and B of Figure 9.5 and inspecting Figure 9.7. In Figure 9.5, both seed weight (A) and pericarp weight (B) per berry increased in the order of: 1 floater < 1 sinker < 2 floaters < 1 sinker + 1 floater < 2 sinkers.

There were insufficient occurrences to extend this sequence to berries with three seeds per berry but the pericarp weights available for such berries indicate a continuation

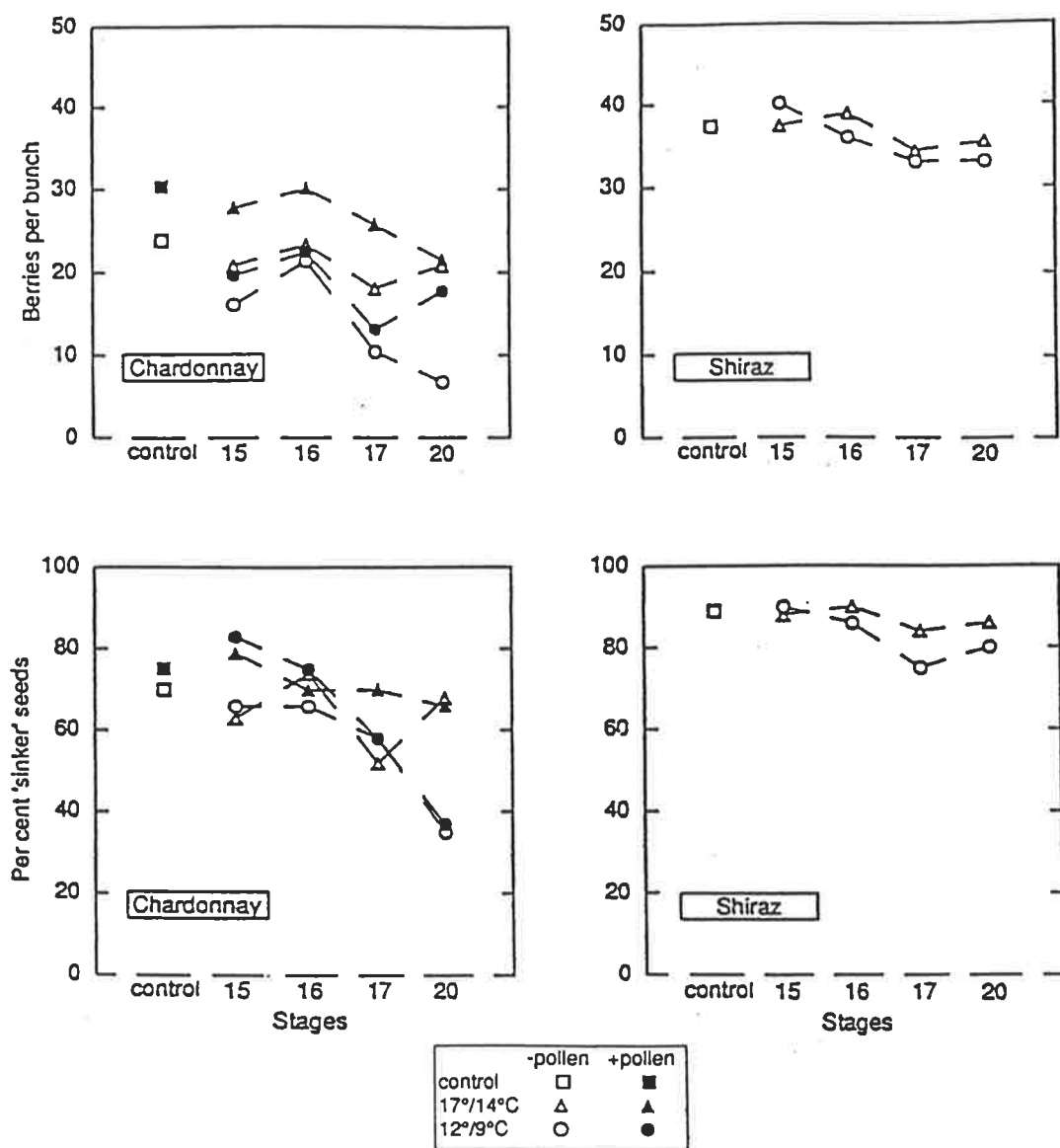


Figure 9.6: Comparison of the means of the treatments for number of berries per bunch and per cent sinker seeds of Chardonnay and Shiraz vines

of this sequence (Appendix, Table 8). For both seed weight and pericarp weight plotted in Figure 9.7, the increase from the smallest (1 floater) to the largest (2 sinkers) weight was about two-fold.

Two separate regressions were calculated for the means of pericarp weight as dependent and total seed weight per berry as independent variable, one for the values of treatments 25°/20°C and 17°/14°C and the second for treatment 12°/9°C (Figure 9.7). Both regressions were highly significant and showed different slopes. When extended, the regression lines met the y-axis at about the same point, i.e. at 215 and 260 mg, the approximate weight of 'chickens', the stenospermocarpic berries containing only seed traces.

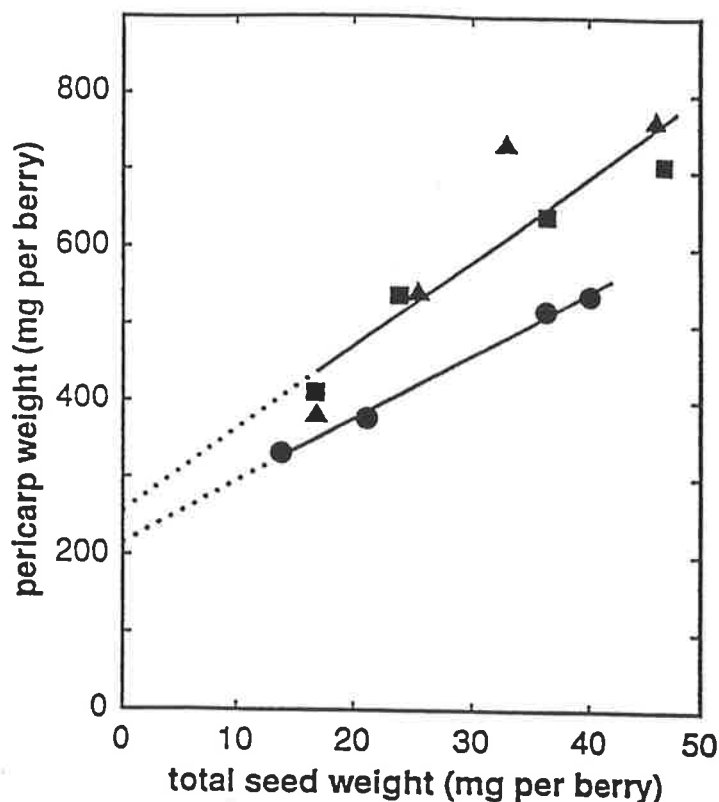


Figure 9.7. Regression of pericarp weight against total seed weight per berry for Chardonnay. ■ 25°/20°C; ▲ 17°/14°C; ● 12°/9°C

9.4 Discussion

The total number of seeds per berry was not affected by any of the treatments in either cultivar (Table 9.2, Appendix, Table 5). These seeds comprise sinkers and floaters, and in Chardonnay there were strong treatment effects both on the number of apparently functional seeds (sinkers) (Tables 9.3A, Figure 9.3A and Appendix, TABLE 6) and on the proportion of these seeds among all seeds (Appendix Table 6).

As shown in Figure 9.6, the proportion of sinker seeds in berries and the mean number of berries per bunch were both reduced by the same treatments. This suggests that treatments that were unfavourable to fruit-set were also detrimental to seed development, with greater proportions of floater and smaller proportions of sinker seeds. Perhaps exposure to suboptimal temperatures damages ovules and/or pollen of some flowers to such an extent that fertilisation cannot occur and thus per cent fruit-set is reduced. Since supplementary pollination seemed to have overcome the detrimental effect of low temperature on fruit-set pollen development seemed to be involved. In other flowers, ovule and/or pollen may be less severely damaged and thus fertilisation and conversion of the flower to a berry being achieved. However the damage may be still sufficient to prevent normal seed development, leading to a smaller proportion of viable seeds and consequently a greater proportion of floater seeds (see chapter 8). Since floater seed formation occurs only when fertilisation did (see chapter 8), damage by the less favorable temperatures to ovules rather than to pollen must have led to the reduced proportion of sinker seeds. Their increased presence after unfavorable pre-flowering conditions supports the statement by Staudt (1982, here translated): "The widely held opinion must be revised that pollen germination and pollen tube growth are strongly influenced by low temperature during flowering and that this leads to poor set. All other investigations indicate that the female gametophyte, the process of fertilisation and the zygote are probably much more sensitive to environmental influences".

In Shiraz, the number of 'sinker' seed per berry showed similar trends in their response to the applied treatments but less pronounced than in Chardonnay. This is not

surprising because this cultivar showed a lesser overall response to exposure to lower temperature.

The present results on seed number per berry differ from those obtained by Roubelakis and Kliewer (1976) and Ewart and Kliewer (1977). Both found that vines of several *V. vinifera* cultivars placed at 15°/10°C two or one week before anthesis had fewer seeds per berry in comparison with vines exposed to higher temperatures, but they did not differentiate between the number of filled seeds ('sinkers') and hollow seeds ('floaters'). Ledbetter and Ramming (1989) suggested that environmental conditions during the growing season may play a role in seed trace development, and that seed trace size may vary from year to year. Our results lend support to these suggestions, as environmental conditions in the form of temperature variations were shown to influence the frequency of occurrence of hollow, 'floater' seeds.

The fact that the experimental vines in experiment 1-4 did not retain up to maturity the small but green berries, or the true 'chickens' of the 'hen and chicken' syndrome (millerandage), evident from the absence of berries without seeds, is presumably due to the restricted ability of these small vines to accumulate metabolites. However, in experiment 2-4, vines could keep some of those berries. This difference between the two experiments could be the result of better conditions in the glasshouse or because soil rather than compost was used as the potting medium. Particularly in Chardonnay, 'chickens' are usually present on mature, field-grown bunches. The relationship between pollination and the subsequent development of 'chickens' has been discussed by Staudt and Kassemeyer (1984). They stated that these berries are truly stenospermocarpic, ceasing development soon after pollination; they are not parthenocarpic as was assumed previously. The relationship between these phenomena and the effect of various temperature regimes remains to be tested.

Temperature treatments had small but significant effects on seed weight, both sinkers and floaters weighing 12% less after 12°/9°C than after 17°/14°C. It is not known which tissue(s) of the seed are responsible for this weight increase. However the greatest effect

on mean seed weight per berry was attributable to the relatively larger effect of temperature on the proportion of 'floater' seeds which were two-thirds the weight of sinker seeds.

The present experiment provides a valuable opportunity to analyse the effects of treatments on pericarp weights and to explore the interrelationships of seed and berry development. The weights of pericarp per berry were greatly influenced by the treatments that had already been shown to influence both fruit-set and seed development. The average pericarp weights per berry shown in Fig. 9.5B had a three-fold range, from a low of 309 mg to a high value of 929 mg. The information contained in this figure indicates strong correlations between pericarp weight and seed number per berry. The larger amount of cells or larger size at different parts of seeds may lead to a better support of berry growth via diversion of metabolites to the pericarp.

The dependence of pericarp weight on seed number per berry is not new (Müller-Thurgau 1898, Winkler and Williams 1936, Coombe 1959). The magnitude of this relationship is influenced by seasonal and cultural conditions. However it has not been appreciated before how strongly final berry weight is influenced by the early pre- and post-flowering events which determine not only seed number but, especially also, seed developmental characteristics. There was a strong relationship between pericarp weight and seed weight per berry (calculated from seed number and mean weight per seed). Fig. 9.5 illustrates that both the increases in seed weight per berry and in pericarp weight ran parallel, were of the order

one floater < one sinker < two floaters < one sinker+one floater < two sinkers,
and occurred in all three temperature regimes.

This relationship is further supported, in a different way, by two positive and significant regressions of mean pericarp weight on mean seed weight per berry, one of lesser slope for the values of the 12°/9°C treatment and one of steeper slope for the 17°/14°C and 25°/20°C treatments (Fig. 9.7). There are two interesting aspects to this graph, both relating to the hypothesis that final berry size is considerably influenced by the number of cells in the pericarp:

- (a) The curves covering the values for the two treatments 25°/20°C and 17°/14°C were the same while that for the treatment 12°/9°C had a lesser slope. It is suggested that the best explanation for the lower pericarp weight/seed weight ratio of berries from 'cold'-treated vines is that cell division in the ovary wall was slower before flowering because of the lower temperature imposed at both E-L stages 15 and 17. Cell division is active in the pericarp of ovaries before flowering (Considine and Knox 1979).
- (b) The position of the intercepts of the two regression lines with the y-axis were close together (at 215 and 260 mg pericarp weight) when seed weight was zero. This could be explained by an absence of further cell division in the pericarp after anthesis in berries where stimulation from ovule/seed growth is lacking. This suggestion has already been proposed for the parthenocarpic cultivar Zante Currant which has berries of about 240 mg (Coombe 1965, 1973). Berries that are categorised as 'chickens' are often a little larger than parthenocarpic berries and, from the foregoing analysis, might be expected to have a brief period of cell division in pericarp tissue after flowering coincident with the brief period of seed trace growth.

As Fig. 9.7 shows, the final pericarp weight of seeded berries is closely correlated to total seed weight. Therefore, on the basis of the hypothesis mentioned above, the larger the total seed weight per berry, the larger the number of cells in their pericarp. It was shown by Harris et al. (1968) that berries of cv. Sultana have 0.2 million cells in each ovary wall at anthesis and 0.6 million cells 40 days later (as also at maturity). Coombe (1973, 1976) has pointed out that these numbers are attained by 17 cell doublings before anthesis and 1.5 doublings thereafter. However, due to the nature of geometric progression, each doubling after anthesis brings a large arithmetic increase in the number of cells. This point is made to emphasise that the increase in pericarp cell numbers during the second and third week after anthesis can lead to a further variation in cell numbers. A contribution to this may be the large variation in stimuli resulting from the array of seed developments shown in this chapter, the result of the response of male and female gametophytic development to temperature.

The foregoing suggestions about the interplay of ovule/seed development and cell division in the ovary wall might be useful for the unravelling of both the determination of

berry size and the problem of size variation between berries. Both aspects are important to the understanding of crop yield and berry composition.

9.5 Conclusions

- Total seed number per berry of Chardonnay was not affected by ~~time~~^{duration} of exposing vines to reduced ($12^{\circ}/9^{\circ}\text{C}$) temperature or to shading of three intensities, but it was ~~positively affected~~^{increased} by providing plants with healthy pollen. The proportion of floater seeds increased in the treatments in which set was reduced. In Shiraz, the treatment means of these observations showed only small, mostly non-significant differences
- There was a positive ~~relation~~^{relationship} between the number of sinker seeds per berry and the number of berries per bunch in both cultivars.
- In Chardonnay, shading did not reduce the total seed number per berry but it caused a significant decrease in the proportion of sinker seeds per berry.
- The proportion of berries with one or two 'sinker' seeds were reduced whereas that of berries with one 'floater' seed was increased by exposure to $12^{\circ}/9^{\circ}\text{C}$, but not by shading. These effects were more pronounced following treatments at E-L stage 17 ~~than~~^{than} E-L stage 15.
- Vines exposed to $12^{\circ}/9^{\circ}\text{C}$ had lower pericarp and seed weights compared to those treated with $17^{\circ}/14^{\circ}\text{C}$ or $25^{\circ}/20^{\circ}\text{C}$.
- Time of applying different temperature or shading treatments had no effect on pericarp and seed weight.
- There was a significant positive correlation between pericarp weight and total seed weight per berry.

Chapter 10

General discussion

Much of the experimental work in this thesis on the development of grape flowers, seeds and berries was based on the use of model vines, that is small fruiting vines such as those grown by the methods developed by Mullins and Rajasekaran (1981). This type of miniature vine has been adopted for research by a number of workers on a variety of topics e.g. development of inflorescences (Buttrose and Hale 1973, Khurshid *et al.* 1992), berry development (Hale and Buttrose 1974) and salinity effects (Hawker and Walker 1978). In adapting the technique for the present work, it was found that bunch development was unsatisfactory with cv. Muscat Gordo Blanco, but was successful with cvs Chardonnay and Shiraz. For this reason, and because of their suitability to this topic of investigation, these cultivars were chosen—Chardonnay because of its susceptibility to coulure, Shiraz because it sets reliably. Routinely, flower numbers were counted on all inflorescences allotted for treatment; no significant differences were found between 'treatments' thus validating the determinations made of per cent fruit-set

The timing of grapevine developmental stages, from before and through flowering, to setting and seed/berry growth, formed a key part of the investigations. It is important to bear in mind, therefore, that development is several times faster in the model vines compared with vines in the field. For example, from budburst to anthesis takes about nine weeks for Chardonnay in the Adelaide environment but only four weeks for model vines held at 25°/20°C. Another complication with timing is that this rapid development was almost stopped during the week when 12°/9°C was imposed, especially when close to flowering. For these reasons timing of events was based when possible on developmental stages using the E-L scheme (Coombe 1995). The stages quoted are E-L 15, flowers in compact groups; E-L 16, rachis and pedicels lengthening; E-L 17, single flowers separated and caps colour changing, and E-L 20, 10% caps fallen. While, as mentioned above, many of the results described here were from model vines held under controlled environment, opportunities were taken for selected work on field-grown vines to provide a comparison.

The main theme of the research in this thesis was directed to the grape flower and its fate. Morphological and anatomical studies were made of pollen, pollination, fruit setting and seed and berry development. Because of the large influence that weather conditions have been shown to have on reproductive events, the effects of varied temperature and shade were used as experimental treatments on model vines of cvs Chardonnay and Shiraz.

Pollen and pollination (Chs 4 to 6)

Scanning electron microscopy of pollen and stigmas during anthesis revealed interesting differences between the two cultivars. Chardonnay pollen grains had pores in the exine that were of smaller size and at a lower density compared with Shiraz pollen, differences that may relate to the poorer germinability of Chardonnay pollen on Chardonnay stigmas. Once lodged on Shiraz stigmas, Shiraz pollen germinated and hydrated more rapidly and the stigma surface collapsed early. The stigmas of pollinated watermelon show a similar collapse (Sedgley 1982). While most pollen grains had become hydrated by day two on Shiraz stigmata, few had done so on Chardonnay stigmata; on the contrary, many had become dehydrated.

Experiments testing the effects of periods of cold on flower development were extended to the *in vitro* germinability of the pollen taken at anthesis from the treated vines. Germination percentage was low in pollen from Chardonnay vines held at 12°/9°C for one week beginning at E-L stage 15; treatments after stage 15 did not have this effect. Shiraz pollen was unaffected by any of the treatments. The timing of the effect on Chardonnay pollen suggests an effect of low temperature on microspore development, in particular, damage to tapetum cells; several workers have suggested that the tapetum has an important nutritional role in microspore development (Laser and Lersten 1972, Warmke and Overman 1972, Iwao 1983, Saini et al. 1984, Bino 1985 and Hormazo and Herro 1992).

The progress of pollen germination on the stigma and tube growth into the style and ovary was studied in Chardonnay. Vines exposed for one week to 12°/9°C at E-L stages

17 and 20 showed slow pollen germination and tube growth rate leading to a deficiency of tubes in the lower ovary for adequate fertilisation of ovules. Kassemeyer and Staudt (1981) have shown that any delay in the arrival of the tube at the embryo sac reduces the chance of successful fertilization because of progressive degeneration of egg cells. The large influence of low temperature on pollen germination and tube growth has also been shown by Koblet (1966) and Staudt (1981, 1982).

In the flowers of both Chardonnay and Shiraz held at 25°/20°C the number of pollen tubes found in the lower ovary was about four which is the same as the number of ovules in each normal grape ovary. This congruence suggests that each ovule exerts control over the growth of at least one pollen tube towards it, a phenomenon that has been described for some other plants e.g. avocado (Tomer and Gottfried 1975, Sedgley 1976).

The majority of flowers on each inflorescence open over a period of one to two weeks (5 days in the measurements by Staudt 1986 and 10 days by Coombe 1995) and fertilization takes place 24 hours after opening (Kassemeyer and Staudt 1981). The asynchronous opening of the flowers on any one inflorescence reflects the position of flowers on it (May 1987), and demonstrates a spread of 'redness to flower' (May 1970). Flowers may vary in their development and thus their age, and/or they may have varying access to metabolite supply through differences in the size of the vascular connections. In any case, the opportunity will arise for competition between flowers in their development. The 'primigen dominance' theory developed by Bangerth (1989) from his study of hormonal control of tomato and apple fruit setting could well be operating in grape bunches; it is interesting to note that, on day one after flower opening, ovules of 'king' flowers had 18% greater volume than those of lateral flowers (Table 7.1) a finding also made by May (1987).

In plants which set poorly the contribution of pollination to the problem can be gauged by testing the effect of hand application of good quality pollen to stigmas at anthesis. The use of this approach here on Chardonnay vines treated with different temperatures at several growth stages, showed that the large reduction in fruit-set due to

12°/9°C just before and during flowering (stages E-L 17 and 20) was lessened; this signifies that some of the poor setting was due to faulty own-pollen. However, hand pollination only partially reversed the decrease in set caused by 12°/9°C and it is concluded that the detrimental effects of low temperature on grape flowers is due more to effects on the female gametophyte than on the male.

Ovule abnormalities at flowering (Ch 7)

Ovule development of cv. Chardonnay was severely affected by low temperature when applied at specific stages before flowering. For example, fruit-set was reduced to low levels—by one third to one half—by exposing vines to 12°/9°C at E-L stage 17 and less so at stage 20 (but not at stages 15 and 16). Treatment with 17°/14°C was not harmful to set. Microscopy of ovules showed that over half of the ovules of Chardonnay exposed to the low temperatures were abnormal having no embryo sacs or sacs that were small, lacking some nuclei or having cross walls; some ovules had degenerated nucellus. This suggests that E-L stage 17 may be the time of development of the megaspore mother cell into the embryo sac. Fougère-Rifot et al. (1993) also showed the same timing at what was called the 'button' stage which appears to be equivalent to E-L stage 17. The results show clearly that low temperature before anthesis can result in low fruit-set because of damage to ovule development, a conclusion that accords with Staudt (1982). Female sterility because of absent or incomplete development of the embryo sac has been reported in other fruit crops (Sedgley 1980, 1982).

Shading treatments, added in factorial combination to the temperature treatments to test the effect of varied light incidence on the fate of flowers, were found to have only slight effects on fruit-set and small significant effect on ovule development, resulting in a reduction in the proportion of sinker seeds among all seeds per berry (12°/9°C, 72% shading at stage 17; Table 9.3) which implicates later developmental events (see next section).

Seed development abnormalities (Ch 8)

The berries of cv. Chardonnay have seeds which are few in number and have a large variation in size and form. The study made here of the structure of seeds from just after anthesis through several stages up to berry ripeness has provided information on the type and rate of development of different seed parts and explains the ontogeny of the various seed forms. The material for the study was taken from field-grown vines.

In normal seeds the first division of the zygote occurred between days 14 and 28 after anthesis, coincident with cell wall formation in the endosperm thus agreeing with the results of Barrit (1970), Kassemeyer and Staudt (1983) and Ledbetter and Ramming (1989). On day 42, endosperm was expanding substantially but the proembryo was small, with 16 to 40 cells. The most advanced proembryos at this stage were globular and supported on short suspensors. By day 98 the endosperm had 'consumed' all of the nucellus out to the testa, but the mature embryo remained small; thus the mature grape seed is endospermous (Sedgley and Griffin 1989).

Abnormalities in ovule/seed development were evident at the first stage examined, day 4 after flower opening. (Note that this was three days later than the investigation of ovules described in the previous section). Ovules that were slightly smaller than others on day 4, lacked an embryo sac and had degenerate nucellus; presumably these did not develop further and thus continued to be termed ovules. Ovules/seeds with an embryo sac at day 4 were either not fertilised, in which case they grew little thereafter, or, if fertilised but with degenerating nucellus and endosperm, expanded incompletely to produce seed traces (stenospermic seeds). The later the degeneration of the nucellus and endosperm the larger the seed traces became. Other fertilised seeds contained a healthy nucellus, endosperm and embryo for a sufficient duration (until day 42) to stimulate the testa to develop normally. But thereafter the embryo and other tissues degenerated and collapsed leaving the seed partially hollow so that the seeds floated on water (i.e. floaters). The conclusions of Marasali (1993) on floater seed formation in cv. Cavus agree with these results. Thus both seed traces and floaters are the result of degeneration of various seed

tissues, the former happening early and the latter sufficiently late for the testa to be stimulated to develop fully. In normal, full-sized (sinker) seeds there was full development of all parts - testa, nucellus, endosperm and embryo.

Relationship of seed development to berry set and berry growth (Chs 4, 9)

One of the motives for this work was to learn about the control of fruit-set in grapevines. The treatments using varying temperatures on Chardonnay vines at several specific stages of development before flowering provided a wide array of fruit-set; the per cent set on hand-pollinated vines held throughout at 25°/20°C was over three times greater than that on vines held for one week at 12°/9°C starting at E-L stage 20. Similarly the same treatments gave a 2.8-fold range in the numbers of sinker seeds per berry; also, poor setting treatments had a high proportion of floater seeds. Thus, there was a direct positive relation between the number of functional (sinker) seeds per berry and the number of berries per bunch. Clearly, conditions that are unfavourable for setting (leading to greater flower abscission and non-development of berries) are also unfavourable for ovule/seed development. The proportion of Chardonnay berries with one floater seed (e.g. 22% in Table 9.1) are considered to be high and probably related to the conditions used for raising the vines in these experiments. In Shiraz, the differences between the treatments were small and mostly non-significant.

Thus it is apparent that the fate of grape pistils is intimately associated with the fate of the ovules. Similar differences between treatments were shown by the pericarp weights of Chardonnay. These were found to be closely related to the number and type of seeds that developed per berry. When the seed data was expressed by the single figure of the total fresh weight of seed tissue per berry, and plotted against fresh weight of pericarp per berry, direct positive correlations were found (see Figure 9.7). The two points made in the discussion of chapter 9 (section 9.4) on the interpretation of the figure are of fundamental importance to the unravelling of both the determination of berry size and to the problem of size variation between berries. The figure shows two regressions of pericarp weight on

seed weight for berries exposed before flowering to lower and warmer temperatures. It was pointed out that firstly the slope of the regression line was steeper at warmer temperatures, caused presumably by differences in cell number per pericarp before anthesis, and secondly that both regression lines indicate that the pericarp weight of a berry without seed would be about 220–250 mg. This is the weight of parthenocarpic berries such as found in cv Zante Currant.

Even though environmental conditions during berry growth play an important role, the dependence of final pericarp weight on pre-anthesis temperature conditions and post-anthesis seed component are aspects of importance in crop yield and berry composition. The physiological basis of these relationships still remains to be explored.

The timing of low temperature treatments

The low temperatures selected for these treatments are realistic for many Chardonnay vineyards in cool climate areas of Australia as shown by the data in Gladstones (1992) and by the measurements associated with poor set in the Adelaide Hills compared with Willunga by May (1992). It is instructive to tabulate the relative effects that result from treatment of Chardonnay for week-long periods with 12°/9°C beginning at four stages of flower development before anthesis. The comparisons below are judged against development of vines held at 25°/20°C. The following abbreviations are used: ns, not significantly different; -, --, ---, small, medium, large reduction in effect; +, ++, +++, small, medium, large increase in effect; † not applicable.

Factor	Stages			
Pollen germination <i>in vitro</i>	---	ns	ns	ns
Pollen development on pistil	†	†	---	---
Ovule development at anthesis	†	†	---	†
Fruit-set	ns	ns	---	---
No. sinker seeds per berry	ns	ns	---	---
No. floater seeds per berry	ns	ns	+++	+++
Per cent sinker seeds	ns	ns	+	+++
Weight per seed (all types)	ns	†	ns	†
Pericarp weight (with sinker seeds)	---	†	---	†

The effect of low temperature imposed at stage 15 were confined to the development of the pollen whereas pollen germination on the stigma, tube growth in the pistil, seed development and fruit-set all involved aspects of the female gametophyte and were affected by low temperatures at the later stages close to flowering when the embryo sac is forming. The effects of low temperature on pericarp weight were equal at early and late stages before flowering during both of which cell division is occurring in the ovary wall.

The difference between cultivars

The study has repeatedly illustrated the large difference between Chardonnay and Shiraz in the manner of their response to the treatments imposed. The following list summarises the differences shown by describing the response of cv. Chardonnay using cv. Shiraz as the point of comparison:

- pollen germinability low following cold at E-L 15
- small and sparse perforations in the pollen exine
- inhibited hydration on the stigma
- pollen growth slow in the style
- pollen tube numbers low in the lower ovary
- cold at E-L 17 and 20 gives poor fruit-set and abnormalities in ovules,
- numbers of sinker seeds lower
- numbers of floater seeds higher

These differences in the reaction of Chardonnay and Shiraz to cold temperature suggest the existence of genetic differences in the control of flower, seed and berry development. Further, they suggest that the opportunity exists for the application of gene manipulation.

Two fundamental questions are emphasized by this work. First, why do seeds abort, or rather, what causes specific tissues to degenerate; is degeneration caused by

deficiency or excess of particular nutrients or compounds, or inadequate nutrition generally? Secondly, how do different parts of the developing seed and fruit interact with each other? The progression of development in Chardonnay seeds, as in most angiospermous seeds, suggests a succession of influences from pollination and fertilisation to testa and nucellus, to endosperm and then to the embryo. Each sequence of seed development has the potential to influence cell division and development in the pericarp. Progress in understanding is likely to originate from recent studies on simple plants such as the crucifer *Arabidopsis*, a plant with a small genome and currently a favourite target of molecular biologists (Meyerowitz and Somerville 1994). It is encouraging to read in the conclusions of Meinke (1994), in his chapter on seed development in *Arabidopsis thaliana*, the opinion that: "more attention will need to be directed to the structure and function of the endosperm, nucellus and integument tissues throughout seed development, and to signal transduction pathways that allow seeds to respond appropriately to developmental and environmental signals."

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Appendix

Table 1: Means of the treatments for per cent fruit-set and number of berries per bunch of Chardonnay and Shiraz vines. Data from experiment 1-4 for Chardonnay and Shiraz, analysed by ANOVA type B.

Temperature	25°/20°C		17°/14°C				12°/9°C				F
	Control		15	16	17	20	15	16	17	20	
Chardonnay											
% Fruit Set											
- Pollen	8.5 ^a		6.7 ^{ab}	7.8 ^{ab}	6.0 ^{ab}	7.0 ^{ab}	7.2 ^{ab}	7.2 ^{ab}	3.4 ^b	3.5 ^b	*
+ Pollen	11.2 ^a		9.7 ^{ab}	12.0 ^a	8.6 ^a	7.5 ^{ab}	6.7 ^a	8.2 ^{ab}	4.3 ^b	5.4 ^{ab}	***
Berry Number											
- Pollen	23.9 ^a		20.9 ^{ab}	23.5 ^a	18.2 ^{ab}	20.9 ^{ab}	16.2 ^{ab}	21.5 ^{ab}	10.5 ^{ab}	6.8 ^b	*
+ Pollen	30.3 ^a		27.8 ^{ab}	30.2 ^a	25.8 ^{ab}	21.6 ^{ab}	19.7 ^{ab}	22.6 ^{ab}	13.2 ^b	17.8 ^{ab}	**
Shiraz											
% Fruit Set											
	14.2		13.1	13.6	10.9	12.1	13.9	10.8	10.7	13.8	ns
Berry Number											
	37.4		37.5	38.9	34.3	35.4	40.2	36.0	33.0	33.0	ns

Significance of F value: ns not significant; *, **, *** significant at $p < 0.05$, $p < 0.01$, $p < 0.001$

Values marked by different superscript letters differ significantly according to Tukey's test.

Table 2: Effect of treatments on per cent fruit-set and number of berries per bunch of Chardonnay and Shiraz. Data from experiment 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay, analysed by ANOVA type B.

Temperature Shading	Control	17°/14°C				12°/9°C			F
	0%	8%	40%	72%	8%	40%	72%		
% Fruit-set									
Chardonnay									
Control	10.8 ^e								
Stage 15		9.1 ^{de}	8.7 ^{cde}	9.2 ^{de}	8.2 ^{cde}	7.8 ^{abcde}	7.9 ^{bcde}		
Stage 17		6.4 ^{abcd}	5.8 ^{abcd}	4.5 ^{abc}	3.8 ^{ab}	3.6 ^e	3.7 ^{ab}		***
Shiraz									
Control	14.5								
Stage 15		12.1	10.8	9.4	10.9	12.4	10.0		
Stage 17		10.2	9.8	8.5	10.2	8.5	8.2		ns
Berry Number									
Chardonnay									
Control	27.5 ^e								
Stage 15		25.6 ^{de}	25.6 ^{de}	26.4 ^e	24.0 ^{cde}	22.6 ^{bcde}	4.4 ^{cde}		
Stage 17		17.5 ^{abcde}	15.6 ^{abcde}	13.0 ^{ab}	14.5 ^{abc}	13.3 ^{ab}	11.7 ^a		***
Shiraz									
Control	40.9								
Stage 15		47.8	49.2	48.7	42.8	46.8	44.0		
Stage 17		43.7	45.2	44.1	42.1	42.8	39.4		ns

Significance of F values: ns non significant; *** significant at $p < 0.001$

Values marked by different superscript letters differ significantly according to Tukey's test.

Table 3: Per cent berries with one seed (A) and per cent berries with one floater seed (B). Data from experiment 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay, analysed by ANOVA type B

A	Per cent Berries with one seed									F				
	Temperature	25°/20°				17°/14°					12°/9°			
	'Stage'	Control	15	16	17	20	15	16	17		20			
Chardonnay														
Experiment 1-4														
- Pollen	62.8 ^b	93.7 ^a	74.4 ^{ab}	72.3 ^{ab}	88.1 ^{ab}	78.1 ^b	66.8 ^b	76.3 ^{ab}	84.0 ^{ab}	*				
+ Pollen	72.7	81.3	65.0	74.5	63.6	65.1	54.4	73.4	81.9	ns				
Experiment 2-4														
Control	78.2 ^{ab}													
8%		70.9 ^{ab}	n.d.	86.4 ^{ab}	n.d.	68.8 ^{ab}	n.d.	65.1 ^a	n.d.					
40%		74.5 ^{ab}	n.d.	90.9 ^b	n.d.	73.2 ^{ab}	n.d.	67.6 ^{ab}	n.d.					
72%		77.2 ^{ab}	n.d.	81.6 ^{ab}	n.d.	78.5 ^{ab}	n.d.	68.3 ^{ab}	n.d.					
F										*				
Shiraz														
Experiment 1-4	70.9	64.1	69.2	66.9	88.4	53.1	68.2	66.7	75.6	ns				
B														
Per cent berries with one floater seed														
Chardonnay														
Experiment 1-4														
- Pollen	19.9 ^b	24.5 ^{ab}	27.5 ^{ab}	41.6 ^{ab}	43.5 ^{ab}	21.7 ^{ab}	40.5 ^{ab}	36.1 ^{ab}	66.0 ^a	*				
+ Pollen	23.1 ^{ab}	18.8 ^b	24.1 ^{ab}	17.0 ^b	23.4 ^{ab}	11.0 ^b	10.3 ^b	29.0 ^{ab}	58.4 ^a	**				
Experiment 2-4														
Control	9.2 ^a													
8%		9.4 ^a	n.d.	13.2 ^{ab}	n.d.	15.6 ^{ab}	n.d.	25.8 ^{ab}	n.d.					
40%		11.0 ^{ab}	n.d.	14.2 ^{ab}	n.d.	25.0 ^{ab}	n.d.	28.4 ^b	n.d.					
72%		16.2 ^{ab}	n.d.	23.2 ^{ab}	n.d.	15.4 ^{ab}	n.d.	26.4 ^{ab}	n.d.					
F										*				
Shiraz														
Experiment 1-4	7.1	2.5	7.7	7.4	10.4	8.7	6.7	12.0	10.8	ns				

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F value: ns not significant; *, **: significant at $p < 0.05$, $p < 0.01$

n.d. = not determined

Table 4: Percentage of Chardonnay berries with varying seed complement. Data from experiment 2-4 for Chardonnay are shown, analysed by ANOVA type B where sufficient occurrences of berries were recorded. Means for each temperature treatment, bulked over the three shading treatments, are shown.

No. and type of seed per berry	Percentage of berries with varying seed complement								
	One seed		Two seeds			Three seeds			
	1 sink	1 float	2 sink	1 sink 1 float	2 float	3 sink	2 sink 1 float	1 sink 2 float	3 float
Control	64.2 ^{ab}	9.2 ^a	17.0 ^{ab}	5.1	2.4	0.7	1.0	0.4	n.d.
17°/14°C –stage 15									
<i>Mean</i>	62.3	12.2	16.7	3.7	1.5	1.2	0.7	0.4	0.4
Shade 8%	61.2 ^{ab}	9.4 ^a	18.8 ^b	4.6	1.4	2.0	1.1	0.4	0.4
Shade 40%	63.4 ^{ab}	11.0 ^{ab}	16.8 ^b	3.5	1.5	0.4	0.7	0.5	0.6
Shade 72%	62.2 ^{ab}	16.2 ^{ab}	14.6 ^{ab}	3.1	1.6	n.d.	0.3	n.d.	2.0
17°/14°C –stage 17									
<i>Mean</i>	69.5	16.9	9.4	1.8	1.1	1.1	0.5	n.d.	n.d.
Shade 8%	73.2 ^b	13.2 ^{ab}	8.6 ^{ab}	1.0	1.5	1.9	0.6	n.d.	n.d.
Shade 40%	77.0 ^b	14.2 ^b	6.4 ^{ab}	1.0	0.6	0.4	0.4	n.d.	n.d.
Shade 72%	58.4 ^{ab}	23.2 ^{ab}	13.2 ^{ab}	3.5	1.2	n.d.	0.5	n.d.	n.d.
12°/9°C – stage 15									
<i>Mean</i>	54.8	18.7	12.2	5.6	2.6	2.1	2.4	1.0	1.0
Shade 8%	53.2 ^{ab}	15.6 ^{ab}	13.4 ^{ab}	6.4	3.4	2.0	3.5	1.2	1.3
Shade 40%	48.0 ^{ab}	25.0 ^{ab}	13.4 ^{ab}	5.8	2.0	3.1	1.7	1.0	n.d.
Shade 72%	63.2 ^{ab}	15.4 ^{ab}	9.8 ^{ab}	4.5	2.4	1.1	2.0	0.8	0.8
12°/9°C – stage 17									
<i>Mean</i>	40.5	26.9	9.5	8.7	7.1	1.7	1.6	1.6	2.8
Shade 8%	40.4 ^a	25.8 ^{ab}	11.0 ^{ab}	7.9	7.0	3.0	1.6	1.8	1.4
Shade 40%	39.2 ^a	28.4 ^b	11.6 ^{ab}	7.6	6.7	1.8	1.7	0.9	2.1
Shade 72%	41.8 ^{ab}	26.4 ^{ab}	6.0 ^a	10.6	7.6	0.4	n.d.	2.2	5.0
F	*	*	*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F values: ns not significant; * significant at $p < 0.05$

n.d. not determined

Table 5: Number of total seeds per berry. Data from experiments 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay (analysed by ANOVA type B)

Number of total seeds per berry										
Temperature	25°/20°	17°/14°				12°/9°				F
Stage	Control	15	16	17	20	15	16	17	20	
Chardonnay										
Experiment 1-4										
– Pollen	1.28	1.20	1.26	1.29	1.10	1.33	1.34	1.12	1.13	ns
+ Pollen	1.50	1.23	1.46	1.40	1.44	1.46	1.60	1.42	1.18	ns
Experiment 2-4										
Control	1.32									
8%		1.36	n.d.	1.12	n.d.	1.34	n.d.	1.28	n.d.	
40%		1.38	n.d.	1.12	n.d.	1.24	n.d.	1.33	n.d.	
72%		1.29	n.d.	1.19	n.d.	1.25	n.d.	1.33	n.d.	
F										ns
Shiraz										
Experiment 1-4	1.48	1.42	1.36	1.44	1.25	1.45	1.33	1.39	1.19	ns

ns not significant; n.d. not determined

Table 6: Number of sinker seeds (A) and floater seeds (B) per berry. Data from experiments 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay, analysed by ANOVA type B

A Temperature	Number of sinker seeds per berry									F
	25°/20°	17°/14°				12°/9°				
'Stage'	Control	15	16	17	20	15	16	17	20	
Chardonnay										
Experiment 1-4										
-Pollen	0.89ab	0.75ab	0.93a	0.67ab	0.75ab	0.88ab	0.88ab	0.65bc	0.40c	***
+ Pollen	1.12abc	0.97cd	1.02abcd	0.98bcd	0.95cd	1.21a	1.20ab	0.82d	0.44e	***
Experiment 2-4										
Control	1.16 ^d									
8%		1.15cd	n.d.	0.96abcd	n.d.	0.98abcd	n.d.	0.82ab	n.d.	
40%		1.10bcd	n.d.	0.94abcd	n.d.	0.89abcd	n.d.	0.81ab	n.d.	
72%		0.97abcd	n.d.	0.90abcd	n.d.	0.95abcd	n.d.	0.67a	n.d.	
F										***
Shiraz										
Experiment 1-4	1.32 ^b	1.25 ^{ab}	1.22 ^{ab}	1.21 ^{ab}	1.07 ^{ab}	1.30 ^b	1.15 ^{ab}	1.04 ^{ab}	0.95 ^a	

B	Number of floater seeds per berry									F
	25°/20°	17°/14°				12°/9°				
'Stage'	Control	15	16	17	20	15	16	17	20	
Chardonnay										
Experiment 1-4										
- Pollen	0.38ab	0.46ab	0.32a	0.62ab	0.35ab	0.45ab	0.46ab	0.48ab	0.73b	*
+ Pollen	0.40	0.26	0.45	0.42	0.49	0.25	0.41	0.60	0.74	ns
Experiment 2-4										
Control	0.27 ^{ab}									
8%		0.20 ^a	n.d.	0.18 ^a	n.d.	0.37 ^{ab}	n.d.	0.46 ^{ab}	n.d.	
40%		0.19 ^a	n.d.	0.18 ^a	n.d.	0.35 ^{ab}	n.d.	0.52 ^{ab}	n.d.	
72%		0.32 ^{ab}	n.d.	0.30 ^{ab}	n.d.	0.30 ^{ab}	n.d.	0.66 ^{ab}	n.d.	
F										**
Shiraz										
Experiment 1-4	0.15	0.18	0.15	0.23	0.19	0.16	0.18	0.35	0.24	ns

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F value: ns not significant; *, **: significant at $p < 0.05$, $p < 0.01$

n.d. not determined

Table 7: Mean fresh weight (mg) per seed. Data from experiment 2-4 for Chardonnay are shown, analysed by ANOVA type B where sufficient occurrences of berries were recorded. Means for each temperature treatment, bulked over the three shading treatments, are shown.

Mean fresh weight per seed (ng)									
No. and type of seed per berry	One seed		Two seeds			Three seeds			
	1 sink		2 sink	1 sink		3 sink	2 sink	1 sink	
		1 float		1 float	2 float		1 float	2 float	3 float
Control	24.4^{ab}	16.7	23.3	21.8-14.6	14.6	15.3	n.d.	n.d.	n.d.
17/14°C –stage 15									
<i>Mean</i>	24.1	16.4	23.0	23.5-15.2	15.9	n.d.	n.d.	n.d.	n.d.
Shade 8%	23.0 ^{ab}	15.4	21.8	22.8-14.8	15.5	n.d.	n.d.	n.d.	n.d.
Shade 40%	24.6 ^{ab}	16.1	23.7	23.0-15.7	16.5	n.d.	n.d.	n.d.	n.d.
Shade 72%	24.8 ^{ab}	17.6	23.8	24.8-15.2	15.8	n.d.	n.d.	n.d.	n.d.
17°/14°C –stage 17									
<i>Mean</i>	25.2	16.5	23.2	20.9-11.8	17.0	n.d.	n.d.	n.d.	n.d.
Shade 8%	25.0 ^{ab}	15.8	23.0	17.0-12.0	21.5	n.d.	n.d.	n.d.	n.d.
Shade 40%	24.0 ^{ab}	17.0	21.3	21.5-10.0	12.5	n.d.	n.d.	n.d.	n.d.
Shade 72%	26.6 ^b	16.0	25.3	24.2-13.3	n.d.	n.d.	n.d.	n.d.	n.d.
12/9°C –stage 15									
<i>Mean</i>	21.6	13.5	20.7	19.7-13.5	13.9	n.d.	n.d.	n.d.	n.d.
Shade 8%	20.8 ^{ab}	13.2	20.0	20.4-13.2	14.5	n.d.	n.d.	n.d.	n.d.
Shade 40%	21.6 ^{ab}	13.8	21.0	17.3-11.5	12.5	n.d.	n.d.	n.d.	n.d.
Shade 72%	22.4 ^{ab}	13.6	21.1	21.4-15.8	14.8	n.d.	n.d.	n.d.	n.d.
12/9°C –stage 17									
<i>Mean</i>	21.1	13.8	20.0	20.6-15.6	13.7	n.d.	n.d.	n.d.	n.d.
Shade 8%	20.8 ^{ab}	14.4	21.3	20.3-18.7	16.0	n.d.	n.d.	n.d.	n.d.
Shade 40%	22.0 ^{ab}	12.6	21.4	20.9-13.9	12.6	n.d.	n.d.	n.d.	n.d.
Shade 72%	20.6 ^a	15.6	17.7	20.6-14.1	12.3	n.d.	n.d.	n.d.	n.d.
F	***	ns	ns	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F values: ns non significant; *** significant at $p < 0.001$

Standard errors for seed weight were of the order of 10% of the mean or less

n.d. not determined due to insufficient occurrences

Table 8: Mean pericarp fresh weight (mg). Data from experiment 2-4 for Chardonnay are shown, analysed by ANOVA type B where sufficient occurrences of berries were recorded. Means for each temperature treatment, bulked over the three shading treatments, are shown.

No. & type of seed per berry	Fresh weight of pericarp (mg)								
	One seed		Two seeds			Three seeds			
	1 sink	1 float	2 sink	1 sink	2 float	3 sink	2 sink	1 sink	3 float
Control	537 ^c	416	705 ^{bc}	638	579	884	729	694	n.d.
17°/14°C –stage 15									
<i>Mean s</i>	516	407	682	639	579	825	805	746	675
Shade 8%	523 ^c	402	687 ^{bc}	656	642	804	921	896	n.d.
Shade 40%	529 ^c	441	718 ^{bc}	620	487	846	720	596	n.d.
Shade 72%	496 ^{bc}	377	641 ^{abc}	641	608	n.d.	773	n.d.	675
17°/14°C –stage 17									
<i>Mean</i>	538	387	754	732	616	929	896	n.d.	n.d.
Shade 8%	545 ^c	392	797 ^c	852	712	929	887	n.d.	n.d.
Shade 40%	492 ^{bc}	359	671 ^{abc}	572	520	n.d.	877	n.d.	n.d.
Shade 72%	576 ^c	410	795 ^c	771	n.d.	n.d.	924	n.d.	n.d.
12°/9°C –stage 15									
<i>Mean s</i>	361	320	515	512	520	589	389	614	622
Shade 8%	331 ^a	308	475 ^a	493	475	467	550	595	587
Shade 40%	397 ^{ab}	349	557 ^{abc}	530	483	609	631	654	n.d.
Shade 72%	354 ^a	303	513 ^a	512	602	690	586	593	657
12°/9°C –stage 17									
<i>Mean</i>	376	334	534	522	521	602	676	642	595
Shade 8%	380 ^{ab}	348	518 ^a	482	529	616	677	748	n.d.
Shade 40%	398 ^{ab}	345	553 ^{ab}	580	512	574	676	496	528
Shade 72%	349 ^a	309	528 ^{ab}	504	521	617	n.d.	682	663
F	***	ns	***	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F values: ns non significant; *** significant at $p < 0.001$

Standard errors for pericarp weights were of the order of 10% of the mean or less

n.d. not determined due to insufficient occurrences

Table 9: Per cent sinker seeds per total seeds. Data from experiments 1-4 for Chardonnay and Shiraz and experiment 2-4 for Chardonnay (analysed by ANOVA type B)

Per cent sinker seeds per berry										
Temperature	25°/20°	17°/14°				12°/9°				F
Stage	Control	15	16	17	20	15	16	17	20	F
Chardonnay										
Experiment 1-4										
- Pollen	72.0 ^{ab}	64.0 ^{ab}	74.0 ^{ab}	53.0 ^{ab}	68.0 ^{ab}	67.0 ^{ab}	65.0 ^{ab}	58.0 ^{ab}	36.0 ^a	***
+ Pollen	75.0 ^{ab}	79.0 ^{ab}	74.0 ^{ab}	71.0 ^{ab}	68.0 ^{ab}	84.0 ^b	76.0 ^{ab}	59.0 ^{ab}	38.0 ^a	***
Experiment 2-4										
Control	81.0 ^b									
8%		85.2 ^b	n.a.	85.4 ^b	n.a.	74.0 ^{ab}	n.a.	65.2 ^{ab}	n.a.	
40%		85.0 ^b	n.a.	83.8 ^b	n.a.	72.6 ^{ab}	n.a.	62.8 ^{ab}	n.a.	
72%		76.6 ^{ab}	n.a.	75.0 ^{ab}	n.a.	77.2 ^{ab}	n.a.	53.8 ^a	n.a.	
F										***
Shiraz										
Experiment 1-4	90.0	89.0	88.0	89.0	85.0	89.0	86.0	75.0	80.0	

Values marked by different superscripts differ significantly according to Tukey's test.

Significance of F values: ns non significant; *** significant $p < 0.001$,

n.d. not determined

Ebadi, A., Coombe, B. G. & May, P. (1995). Fruit-set on small Chardonnay and Shiraz vines grown under varying temperature regimes between budburst and flowering. *Australian Journal of Grape and Wine Research*, 1(1), 3–10.

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Ebadi, A., May, P., Sedgley, M. & Coombe, B. G. (1995). Effect of low temperature near flowering time on ovule development and pollen tube growth in the grapevine (*Vitis vinifera* L.), cvs Chardonnay and Shiraz. *Australian Journal of Grape and Wine Research*, 1(1), 11–18.

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