Berry set and development in *Vitis vinifera* L.

A thesis
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By
A.P. Friend

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The formation and growth of grape berries was studied in Canterbury, New Zealand on Chardonnay, Pinot noir, Cabernet Sauvignon, and Merlot. Experimentation was set up to examine: changes in yield components, the development of individual flowers, and the growth and cellular makeup of seeded, seedless, and shot berries. Vine yield components were manipulated by altering vine phenology using delayed winter spur pruning and alginate gel encapsulation. Flower development was studied by tagging flowers at capfall and describing the resulting berries at *véraison*. The set and development of the tagged flowers was altered with girdling and leaf area removal treatments to change carbohydrate availability. Finally the growth curves and cellular makeup of the different types of berries were described from a separate sample of berries.

The timing of phenological growth stages may have an important role in determining components of yield. Bunch weight increased (38%) with delayed winter spur pruning, due to a larger average berry weight. The increase in average berry weight resulted from changes in the berry population, with the proportion of large seeded berries increasing within bunches, associated with a possible reduction in the proportion of smaller seedless berries. Treatments that delayed bud break also delayed flowering date, perhaps to a time when weather conditions were more favourable for berry development. A weak relationship between the warmth of the bud break period and yield, as well as bunch weight, was found; this may be an indirect relationship.

Studying individual flowers showed that berry set and development could be altered by manipulating carbohydrate availability. Girdling changed the development of some flowers. The proportion of seeded berries that formed was unaffected by girdling, while the proportion of seedless berries increased. The response of shot berries and flowers that abscised differed between 1999 and 2000. In 1999 the proportion of shot berries decreased, while in 2000 it was the proportion of abscised flowers that decreased after girdling. Leaf area reduction on girdled shoots had an opposite effect to girdling, with extreme levels of leaf removal (75%) reducing the proportion of shot and seeded berries that formed. The percentage of abscised flowers increases

dramatically with leaf area removal, while the proportion of seedless berries was unaffected. However, a weak positive relationship between total shoot leaf area and seedless berry development exists. The data from yield components and of individual flowers suggest that the population of berries are fluid in nature. Shot berries and flowers that abscise appear to be a pool from which seedless berries can form, when carbohydrate availability allows. The antibiotic spectinomycin was applied to alter fruit set and seed development of berries, though no effect was identified. The lack of a spectinomycin effect may have been the result of incorrect timing of treatment application.

The mean overall fruit set differed between the 1999 (41%) and 2000 (71%) seasons. The greater fruit set and different behaviour of abscised flowers (c.f. shot berries in 1999) in 2000 might be a reflection of the warmer than average flowering period compared to the cooler flowering period of 1999. This suggests that environmental conditions at flowering may influence fruit set.

Flowers were found to vary in size at capfall, but neither flower size (ovary diameter) nor the time of capfall of individual flowers had any influence on berry set and development. Flower size and environmental conditions affected the progression of capfall. Smaller flowers tend to undergo capfall after larger flowers, and the progression of flowering is disrupted when rainfall and associated low temperatures occur. Temperatures above 15°C were found to advance capfall in the 1999 and 2001 seasons. With flowers undergoing capfall over an extended period of time (about 20 days), individual flowers will experience quite different environmental conditions. However, no strong relationships between daily assessments of temperature at capfall and berry set or berry development were found. The strong effect of carbohydrate availability on berry development (as found with girdling and leaf area removal) suggests that light intensity (due to its impact on current photoassimilate supply) may be more valid an environmental index than temperature.

The extent of berry growth is determined by the seed. A strong relationship between berry size and seed content was found. A minimum level of seed development (>0.5mg fresh weight at harvest) is required for double sigmoid berry growth, which occurs as a consequence of cell division and expansion. Both seeded and seedless berries exhibit double sigmoid growth curves, however when seedless berries have less than 0.5mg seed content they show a single sigmoid growth curve. Seedless berries grow only as a result of cell expansion. Failure of the ovules to develop mean that shot berries only show a small amount of growth immediately post-capfall then halt all growth.

A model of berry formation has been proposed, where flower abscission and fruit set are considered as contrasting processes and the formation of shot and seedless berries occurs when the normal process of seeded berry development fails. Once abscission has been prevented (i.e. the flower is set), the extent to which the flower develops is determined by what stage during pollination (shot berry) and fertilisation (seedless berry) that seed formation fails.

Keywords: Abscised flower, Capfall, Carbohydrates, Delayed winter spur pruning, Flowering, Fruit set, Girdling, Leaf area, Phenology, Rainfall, Seed, Seeded berry, Seedless berry, Shot berry, Sodium Alginate, Spectinomycin, Temperature.

For Nana and Poppa.

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- who the co-authors are.

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PRE-PUBLICATIONS

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Friend, A.P., Creasy, G.L., Trought, M.C.T., and Lang, A. (2003) Use of tagging to trace capfall and development of individual Vitis vinifera L. cv. Pinot noir flowers. *American Journal of Enology and Viticulture*. **54**:313-317.

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ABBREVIATIONS AND CULTIVAR SYNONYMS

ACC	1-aminocyclopropane-1-carboxylic acid
CO_2	Carbon dioxide
O_2	Oxygen
SD	Standard deviation
SH	Southern hemisphere

Clare Riesling	-	Crouchen
Muscat Gordo Blanco	-	Muscat of Alexandria
Shiraz	-	Syrah
Sultanina	-	Sultana
Thompson Seedless	-	Sultana
Traminer	-	Gewurztraminer
Ugni blanc	-	Trebbiano
White Riesling	-	Riesling
Zante Currant	-	Black Corinth

Chapter I

Introduction

1.1 Introduction

The New Zealand wine industry has evolved rapidly since 1960, from a small industry (388 Ha), producing mainly fortified wines for national consumption, to an industry totalling 22,024 Ha, which produced 139,000 tonnes of grapes and exported 51.3 million litres of table wine with a value of 434 million dollars in 2005. The cultivars grown have also changed, with American hybrids dominating (~60%) the industry in 1960 now only accounting for 0.03% of grapes produced. Quality vinifera cultivars are now predominant with Sauvignon blanc (45%), Chardonnay (21%) and Pinot noir (10%) dominating production. Production has also shifted from the upper North Island the 1960s to throughout the country with the cooler region of Marlborough (48%) dominating the national vineyard and Hawke's Bay (21%) and Gisborne (9%) also having significant plantings (Data sourced from: Dunleavy 1986; Winegrowers of New Zealand 2005).

The rapid change in the focus of the industry, its size (Figure 1.1), and the cultivars grown, has meant that a focal point on quality grape production has developed. As an industry focused on the production of high quality table wine for export, consistency of supply, in terms of vineyard yield and the ripeness of fruit from vineyards, has become increasingly important. Unexpected variation in supply creates inefficiencies in wine production and difficulties in marketing a product (Martin *et al.* 2000), while the ability to maintain or increase supply for strong demand is central to the growth of any industry.

Yield has an impact on the quality of grapes and hence the quality of wine. Crop load and its interaction with weather determine the timing of physiological events involved in ripening. The timing of ripening events, the extent to which they occur, and the weather the vines experience during these events determine the sugar, acid, flavour and aroma profile of the grapes, and hence quality of the crop. Yield is also a driver of profitability, determining the volume of grapes produced and amount of wine that can be made and sold.

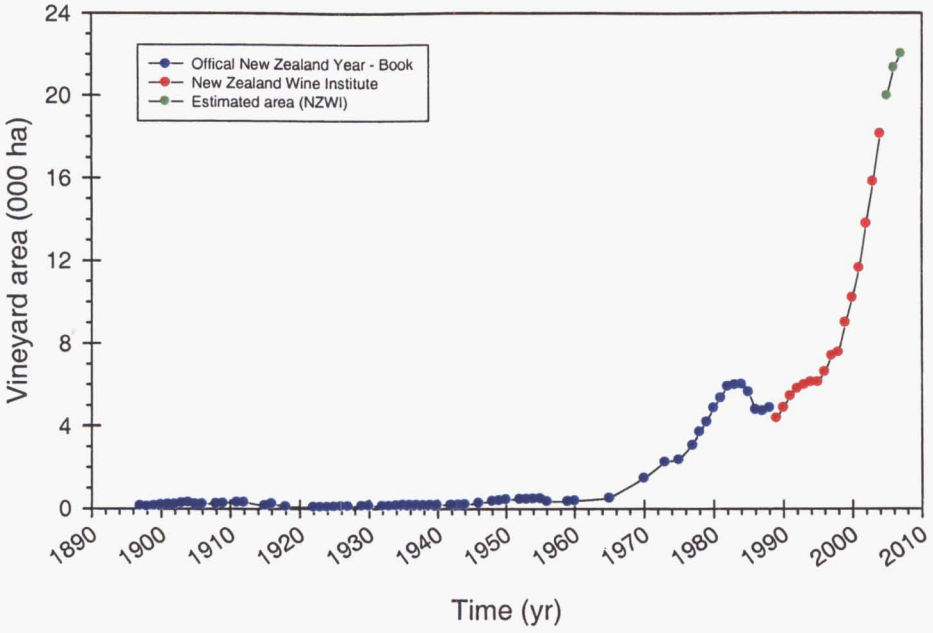


Figure 1.1 Changes in the New Zealand vineyard area 1897-2005. (Data collated from: Government Printer 1897–1988; Winegrowers of New Zealand 2002, 2003a, 2003b, 2004; Wine Institute of New Zealand 1992, 1997).

Average yields in New Zealand exhibit large variation between seasons, ranging from 4.8 to 14.4 tonnes/ha [CV = 0.228] (Figure 1.2). This variation in yield is thought to be mainly climatic in origin (Trought 2006), driven primarily by weather during two key periods: early spring and early summer. In spring, frost events can result in the death of inflorescences or whole shoots. This loss results in a decrease in bunch numbers, which in turn reduces yield (Friend *et al.* 2006). In early summer, low temperatures can reduce the initiation of inflorescence primordia and interrupt the processes leading to the set of fruit and formation of seeds within berries. The current season's inflorescence primordia develop into next season's inflorescences, while fruit-set and seed formation determine the number of berries per cluster and the size of those berries, respectively.

Recognising the impact yield has on grape quality and the importance of consistency of supply, many vineyard and winery managers endeavour to predict yield each season. However accurately predicting yield is difficult, with an average error of about 25% being common (Martin *et al.* 2000). Yield is made up of a number of components, which include: percent bud break, inflorescences or bunches per shoot, flowers per inflorescence, percentage

fruit set, berries per cluster, and berry weight (Martin *et al.* 2000, Wilson 1995). Predicting the number of bunches can be reliably achieved through normal sampling; however accurate prediction of bunch weight can be difficult (Martin *et al.* 2000). Bunch weight is determined by the number of flowers (Dunn 2005), fruit set (the number of berries per bunch), and berry size (Wilson 1995). Fruit set may be influenced by a number of factors, including temperature (Ebadi *et al.* 1996; Ewart & Kliewer 1977), light intensity (Ferree *et al.* 2000), and carbohydrate supply (Caspari *et al.* 1998), while final berry size is known to relate to the seed content of the berry (Olmo 1946), and is likely to be a reflection of the success of fertilisation resulting in seed set. Little information is available on the formation of berries from individual flowers. A greater understanding of the physiology behind fruit set and berry development of individual flowers may aid the development of more accurate yield prediction models.

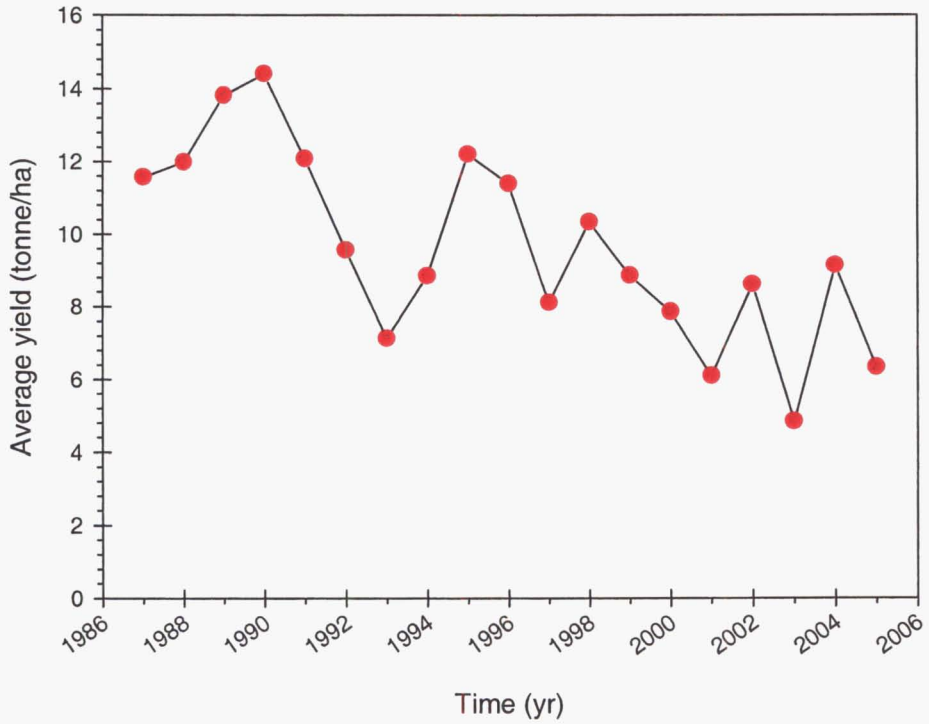


Figure 1.2 Variation in the average yield from New Zealand's producing vineyards 1988-2005. (Collated from: Wine Institute of New Zealand 1989, Winegrowers of New Zealand 2003a, 2004, 2005).

This thesis aims to investigate factors that influence berry size in *Vitis vinifera* L. It focuses mainly on how carbohydrate supply and temperature affect the fruit set and transformation of flowers into berries.

Experimentation has been based around a model of berry development (Figure 1.3). The circle surrounding the process represents the environment and management regime under which a berry develops; temperature is of primary interest. The flowers, which will develop into berries, must first develop in this environment until they reach a point of maturity, where anthesis and capfall occur. The flower can now be pollinated. If pollination is successful, fertilisation may occur, triggering berry development. Successful fertilisation results in seed formation, which in turn sets the upper limit of berry growth. The success of this process is also dependent on the physiological status of the vine, with plant-growth hormones and carbohydrate supply and demand likely to play major roles.

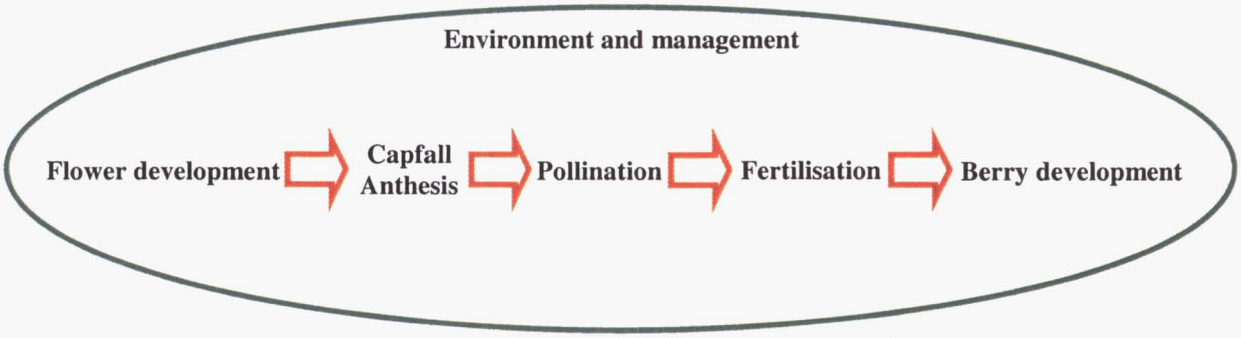


Figure 1.3 Hypothetical model of berry development.

The presentation and discussion of results from experimentation have been split into four chapters:

Chapter Three examines how viticultural manipulations of whole vines can modify the development of berries. Treatments were applied to manipulate the timing of bud break and hence shoot and flower development, thereby altering the temperature environment in which flowering and fruit-set occurs. The effects of this on berry development and yield have been discussed.

Rather than observing berry development at the whole vine level, as in Chapter Three, Chapters Four to Six move to that of individual flowers and the berries that result from them. Using data from three separate experiments Chapter Four examines the capfall behaviour of individual flowers. The behaviour of flowers is discussed in relation to flower development and environmental conditions.

Chapter Five uses the experiments described in Chapter Four to examine the fruit set of individual flowers, and discusses the effects of temperature, flower development, and carbohydrate supply on fruit set. In the experiments used, various treatments, including cincturing of shoots, application of antibiotics to inflorescences, and removal of leaf area were applied to modify fruit-set, seed formation, and hence berry development.

Finally, Chapter Six undertakes an analysis of the growth of individual berries with different seed content. This chapter aims to link the relative success of fertilisation of individual flowers, as discussed in Chapter Five, to the different growth that berries can exhibit.

Chapter II

Review of literature

2.1 Phenology

Phenology is the study of natural phenomenon that recur periodically in plants and animals and of the relationship of these phenomena to climate and changes in season. It aims to describe the causes of variation in timing by seeking correlations between weather indices and the dates of particular growth events and the intervals between them (Coombe 1988; Mullins *et al.* 1992).

In perennial plants, the timing of growth stages determines the availability of stored materials, periods when stored materials will be consumed or accumulated, the environmental conditions under which economic yield will be produced, the effects of periodic harvests on re-growth, and the beginning and end of periods of dormancy. The timing of growth stages also determines whether a species will survive in a region with periods of unfavourable weather conditions (Hodges 1991a).

Most relationships between phenological stages of development and accumulated thermal time are empirical rather than based on underlying processes. Many crop phenological and growth processes proceed in direct relation to the accumulated temperature or thermal time experienced by the crop. Below a base temperature, no thermal time accumulates and crop development does not occur or ceases. The rate of thermal time accumulation and the crop growth or development rate increase with increasing temperature up to an optimum temperature value or range of values. Above that temperature value or plateau, the rate of thermal time accumulation and the crop response decrease with further increases in temperature until no further accumulation occurs and crop development ceases (Hodges 1991b).

Degree-days are used to correlate ambient temperature with a plants growth stage. The daily summations of temperature above a base, typically 10°C, are calculated. Thermal time may be

calculated on different time scales (e.g. daily or monthly), which can give very different assessments of heat accumulation.

Correlations can be made between dates and durations, but only indicate an association. The particular growth event being measured may itself be correlated with another event, which may be the one being influenced by the weather factor (Coombe 1988). Coombe (1988) gives an example of the correlation of node formation on new shoots with the development of flowers, as described by Pratt and Coombe (1978). The question is posed, whether a correlation between flowering date and a temperature index indicates that temperature influences flowering, or is it node formation that is influenced. Caution is necessary in drawing conclusions about the role of temperature *per se* in determining the composition and quality of wine grapes (Coombe 1988).

In grape production, phenological considerations are very important for selecting cultivars that will mature their fruit within a certain time frame. Knowledge of phenological stages of vine growth is important when performing various cultural practices (Mullins *et al.* 1992). Grapevine development rates can be influenced by climatic variability. Individual grape cultivars tend to develop at consistent rates relative to others regardless of seasonal conditions, and at each stage of development are located in regular positions within the whole population studied (McIntyre *et al.* 1982). Nevertheless temperature is clearly a key environmental factor for grapevines (Coombe 1988; Pouget 1988). Individual cultivars tend to develop at consistent rates relative to others, regardless of seasonal conditions, and at each stage of development, are located in regular positions within the populations of cultivars studied. Broad seasonal climatic variation can cause a whole population to be earlier or later than usual (Coombe 1988).

All of the developmental events during the life cycle of a vine are subject to variations in timing according to variety, region, and season, many of which are inter-correlated. In Australia, cultivars show a range within each district of about two weeks for bud break, one week for flowering, but seven weeks for harvest. Flowering dates within a year or district are surprisingly constant between cultivars. Because of this, variation in the interval from bud break to flowering are attributable more to varying date of bud break, the shorter periods being years of late bud break (Coombe 1988). Between districts, for a range of cultivars, bud break occurs for about three weeks in September, flowering during the whole of November

and into December, and harvest occupied a wide period from late February to late April. Regions show a wide range in flowering date. In individual vineyards, bud break and flowering dates may vary between years by several weeks; the greatest range is shown by varieties that ripen early or late rather than mid-season. The harvest date shows considerable variation, of up to two months (Coombe 1988), though this may be a reflection of target harvest maturity. Much of the variation in harvest date may be attributable to the date of *véraison* (Coombe and Iland 1987), with early maturing cultivars having a short lag phase, and vice versa. Within any one year the stages do not necessarily vary in the same direction. Varieties vary in the stability of their phenology under different conditions (Coombe 1988).

2.1.1 Bud break

Bud break is the first visible manifestation of the onset of vegetative growth in grapevines, however, mitotic activity begins 1-3 weeks before bud break (Carolus 1970). Bud break begins with a swelling of the buds, followed one or two days later by the spreading of the bud scales and the appearance of a more or less globular tip. This pushes out the bud down (brown hair), exposing a green tip (Galet 2000; Huglin 1958). At this stage the bud is said to have burst, and when 50% of the buds on a single vine have reached this stage, the vine as a whole is said to have burst (Galet 2000).

As a liana, new shoots tend to grow from the outer extremities of the previous years foliage. On long un-arched canes, distal buds grow out first, owing to a phenomenon of apical dominance. This phenomenon prevents or delays bud break of proximal buds through correlative inhibition (Bessis 1965), thus ensuring that apical buds burst out first. Buds on a grapevine do not all burst at the same time. Generally, the distal bud on a spur or the uppermost buds on a long cane develop first. Also, the vines of a single variety do not all break bud at the same time, even in a single vineyard, since the time of bud break depends on the physiological state of the individual vine. Factors include the quantity of food reserves accumulated, the concept of vine vigour and vascular connections with the trunk (Galet 2000).

Temperature is the primary factor driving the onset of bud break (Calò *et al.* 1996; Pouget 1988). Temperature influences the metabolic activity of buds held under eco-dormancy. During a period of several weeks pre-bud break, increases in the size of the bud apex are subject to temperature (Carolus & Pouget 1971). It is important to note that bud break

calculations are site specific, due to the varying temperature regimes (different sum of daily temperature effects) of different viticultural regions (Baldwin 1966). The buds of any one vine burst over a short period of only a few days in climates with cold winters, but over a lengthy period in climates where winters are mild (Antcliff & Webster 1955). Unusually cold temperatures at bud break can delay bud break (Swanepoel *et al.* 1990), with the rate of bud break increasing rapidly above a minimum temperature (Figure 2.1) (Moncur *et al.* 1989).

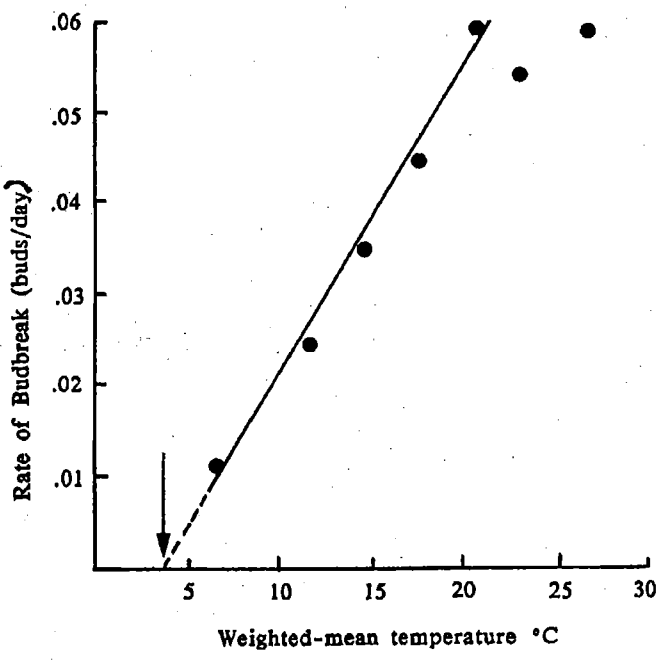


Figure 2.1 The influence of temperature on the rate of bud break (Moncur *et al.* 1989).

Following the loss of endodormancy, bud break and shoot growth have generally been thought to begin when the mean daily temperature reaches 10°C or above (Pouget 1967, Williams *et al.* 1985b; Winkler *et al.* 1974). Estimated base temperatures for bud break vary between 2-5°C for selected *Vitis vinifera* cultivars, but base temperatures for the same cultivars at different sites are comparable. The relevance of 10°C was challenged by Moncur *et al.* (1989), who suggested a base temperature of 4°C was more appropriate for grapevine bud break. Swanepoel *et al.* (1990) use a base temperature of 10°C and applied modifiers to account for cultivar sensitivity. Although an appropriate base temperature has been debated the principle remains the same; an increase in air temperature will promote bud break

(Moncur *et al.* 1989). Base temperatures may increase for successive stages within the annual cycle of a crop (Angus *et al.* 1981).

The base temperature above which bud break begins varies between cultivars, which explains the range of bud break dates for different cultivars (Calò *et al.* 1975; McIntyre *et al.* 1982; Pouget 1988). Cultivars that burst early have a lower temperature threshold for growth than later cultivars, e.g. Traminer 7°C versus Ugni blanc 11°C (Pouget 1968). Several cultivars may commence bud break at temperatures as low as 0.4°C. Pouget (1969) calculated the cultivar coefficients for a number of cultivars, while McIntyre *et al.* (1982), listed 114 cultivars in order of their relative onset of bud break, flowering, and maturity. Plotting cultivar bud break coefficients against the sum daily temperature effects allows the calculation of cultivar bud break coefficients (Swanepoel *et al.* 1990).

The progression of percent bud break is approximately linear in most cases over the range of 10 to 90%, and bud break typically occurs in this range over a short period of time – from about 4.4 to 12.8 degree days or 10 to 19 days. (Figure 2.2) (Williams *et al.* 1985b). Different sites show latitudinal differences in calendar timing, with the locations closer to the equator having relatively early bud break and the more polar locations relatively later. Using degree-day calculations Williams *et al.* (1985b) were able to predict the date of 50% bud break within one to three days over a range of sites in California.

Factors such as clone, rootstock, vigour, trellising system (Baldwin 1966), and time of pruning (Baldwin 1966; Williams *et al.* 1985b), are important in determining the time of bud break of a cultivar (Baldwin 1966). Chilling during dormancy (Calò *et al.* 1996; Williams *et al.* 1985b), soil temperature (Morlat 1989), and the status of vine carbohydrate reserves (Baldwin 1966; Pouget 1966; Williams *et al.* 1985b) may influence the timing of bud break in a particular year. Late pruning generally results in a delay in bud break (Williams *et al.* 1985b). An increase in root temperature reduces the days until bud break (Kliewer 1975; Kubota *et al.* 1987), just as an increase in air temperature does. Higher root temperatures also increase the total percentage of buds that break (Kliewer 1975; Kubota *et al.* 1987). A similar effect is not described for air temperature although the results of Antcliff and Webster (1955) indicate it may be the case. Bud break in vines relies on stored reserves and is temperature-dependent (Moncur *et al.* 1989).

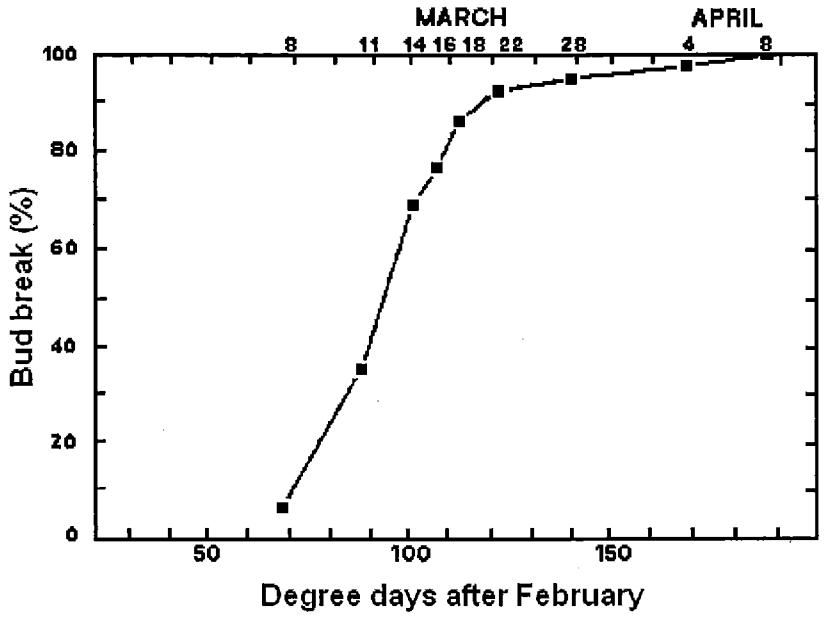


Figure 2.2 Average percent bud break (n = 30 canes) plotted on degree days (base 10°C) from a Thompson Seedless vineyard in the San Joaquin Valley, CA. Sample dates are indicated on the upper axis (Williams *et al.* 1985b).

Freezing temperatures at bud break can result in injury and/or damage to developing grapevine tissue. Unlike many other plants where freezing temperatures kills only the flowers or fruit, frost on grapes kills the whole shoot [i.e. stem, leaves, flowers, fruit] (Jackson & Spurling 1988). The perennial nature of the grapevine means that injury can affect yield, shoot development and fruitfulness not only in the season of the event but in the following season. While the compound bud of grapevines has three shoot primordia (Pratt 1974), the fertility and hence potential productivity of the primordia is lower in the secondary buds. Initial shoot growth depends on stored carbohydrate reserves within the vine, potentially affecting subsequent development of shoots, and fruit set at flowering (Trought *et al.* 1999).

The sensitivity of grapevine tissue to freezing temperatures depends on many factors, including cultivar, dew point and surface moisture, pre-freeze environmental conditions, the probability of ice nucleation events and phenological development (Trought *et al.* 1999). When dormant, grapevine buds lack free space in which ice crystals can form, and are able to tolerate freezing temperatures through super-cooling. As buds break and shoots develop the ability to super-cool is lost as xylem is formed, hydraulically connecting buds to the cane (Trought *et al.* 1999). As a consequence grapevine tissue is less able to withstand freezing temperatures (Table 2.1), and some, not all, buds will be injured during a spring frost.

Table 2.1 The influence of bud phenological development on the critical temperature at which damage and no damage occurs (From Gardea 1987).

Stage of development	Critical temperature (°C) at which damage is observed in Pinot noir	
	50% tissue death	No damage
Dormant enlarged	-14.0	-
Green swollen	-3.4	-
Shoot burst	-2.2	-1.0
First leaf	-2.0	-1.0
Second leaf	-1.7	-1.0
Four leaf	-1.2	-0.6

2.1.2 Shoot development

Shoots are formed by a combination of fixed growth and free growth (Mullins *et al.* 1992). Fixed growth refers to the elongation of internodes and leaves which were pre-formed in the dormant bud and accounts for up to the first 12 nodes of a cane. Free growth refers to the elongation of a shoot by continuous production of new leaf primordia by the apical meristem.

Shoot development is controlled primarily by environmental factors and is favoured by increasing air temperature (Figure 2.3), especially warm nights (Galet 2000; Woodham & Alexander 1966). Soil temperature, which plays a major role in the onset of bleeding, does not seem to have a direct effect on vegetative growth (Galet 2000), but influences shoot dry matter accumulation (Woodham & Alexander 1966). Variations in light intensity have little effect on shoot elongation or node appearance, but strong effects on dry weight accumulation (Buttrose 1969; May *et al.* 1967). The growth of individual shoots is influenced by within-vine competition, which is due to hormone-regulated apical dominance and correlative inhibition, causing differences in growth between shoots (May 1987). The vine regulates the growth of its shoots by adjusting the number of buds that burst and by the size of the individual shoots (May 1964, 1987). As terminal buds are not formed, growth can theoretically continue as long as climatic conditions permit (Jackson 2000).

Leaf appearance occurs at a mean base temperature of 7.1°C +/- 1.2°C (Moncur *et al.* 1989). The rate of leaf appearance shows a linear increase from 15 to 25°C, and does not increase above 25°C (Buttrose 1969; Guillon 1904). The rate of leaf appearance begins to drop off at

about the time flowers and fruit develop on the vine, as shoot growth and development during later phases largely depends on current photosynthesis (Moncur *et al.* 1989).

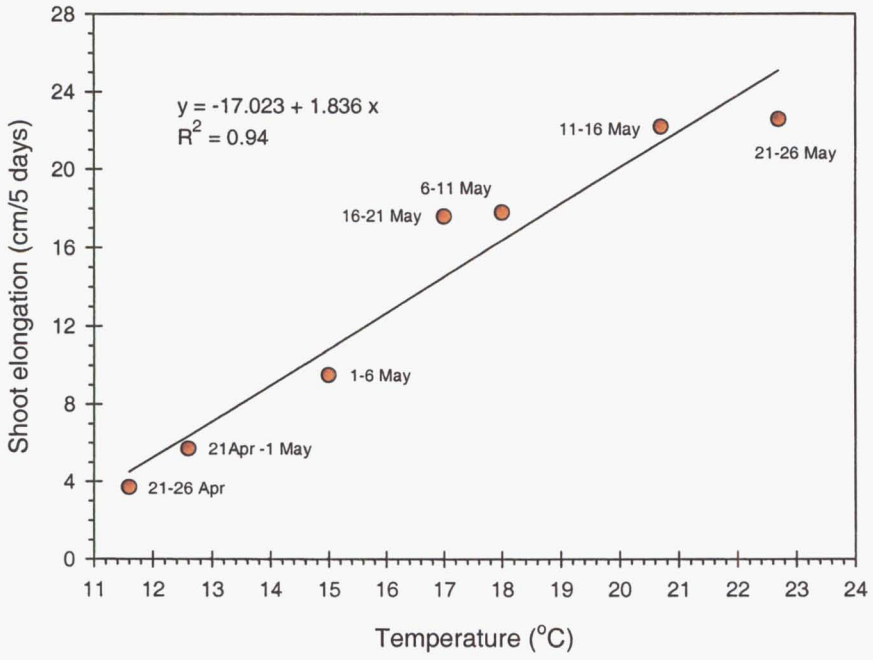


Figure 2.3 The relationship between temperature and mean shoot elongation of Aramon, over five-day-periods from 21 April to 26 May 1934, at Montpellier [Adapted from Mattras (1936) cited in Galet (2000)].

The growth of the vegetative structures derived from compound buds is close to exponential early in the growing season (Mullins *et al.* 1992). Williams *et al.* (1985a) found vegetative growth (dry matter) to be approximately linear early in the season (post bud break), when plotted against degree days. Given that shoot development is favoured by warm air temperature, it is likely that the rate increase in temperature (i.e. such as between continental and maritime climates) will influence the pattern of shoot growth. McIntyre *et al.* 1982 concluded that grapevine development rate is influenced by climatic variability. The linear development of shoots as found by Williams *et al.* (1985a) corresponds to the period of 'grand growth' (Winkler *et al.* 1974), and was followed by a second phase where dry matter accumulation levelled off. The timing of this second phase would approximately correspond to flowering, a time when sink demand shifts from vegetative to reproductive development.

Subsequent to anthesis, vegetative growth rates decrease and the growth curves for shoots of the vine under field conditions become sigmoidal. This type of growth occurs whether the time variable is calendar days or degree-days greater than 10°C (Mullins *et al.* 1992). This general reduction in vegetative growth has been attributed to the interplay of hormonal controls and of the competition for photosynthates in shoots with rapidly growing fruit, which is mediated by the availability of water (Williams *et al.* 1985a). This competition is thought to be dependent on the number of shoots and hence crop load of the vine.

2.2 The grapevine flowering and fruiting cycle

The reproductive cycle of grapevines in temperate climates occurs over a 15-18 month period, during which many factors influence the success of flowering and development of a crop (Wilson 1995) (Figure 2.4). The cycle begins during the first season, with floral induction in late spring (November-December, SH). In summer (December-February), primordium differentiation follows induction, sequentially along the shoot. By the end of the summer, primordia halt development when endodormancy occurs (May-August). In spring (September-October) of the second season the buds burst into growth and individual flowers are formed on inflorescences. This is followed by flowering and fruit set in early summer (December). Flowers that set fruit develop into berries, which grow throughout summer (January-February), and ripen in autumn (March-May).

Floral induction results in the formation of an uncommitted primordium (often described as an *anlage*) opposite a leaf primordium, on the developing shoot of a latent bud. Depending on the cultivar, the bud apex produces three to eight leaf primordia before the first uncommitted primordium is formed (May 2004). Once formed, the uncommitted primordium undergoes differentiation. The pathway of differentiation is conventionally thought of as: uncommitted primordium → tendril primordium → shoot or inflorescence or tendril (Srinivasan & Mullins 1981). However the structure of the inflorescence has been interpreted as a transformed shoot (May 1964), where the shoot axis is mostly reduced or totally absent, the first bract-apposed appendage becomes the main portion of the inflorescence, called the 'inner arm' (due to its adaxial position on the shoot), and at the second 'node' a leaf-opposed inflorescence, termed the outer arm (due to is abaxial position to the shoot) or shoulder of the inflorescence. This has lead to the interpretation of initiation as: shoot initial (mostly lost) → first inflorescence

→ second inflorescence or tendril (may be lost) → shoot apex (mostly lost). This pathway may be the consequence of a reductive process whereby the shoot portion of the apical meristem of the uncommitted primordia is overwhelmed by the more rapid meristematic development of its lateral appendage(s) (May 2000).

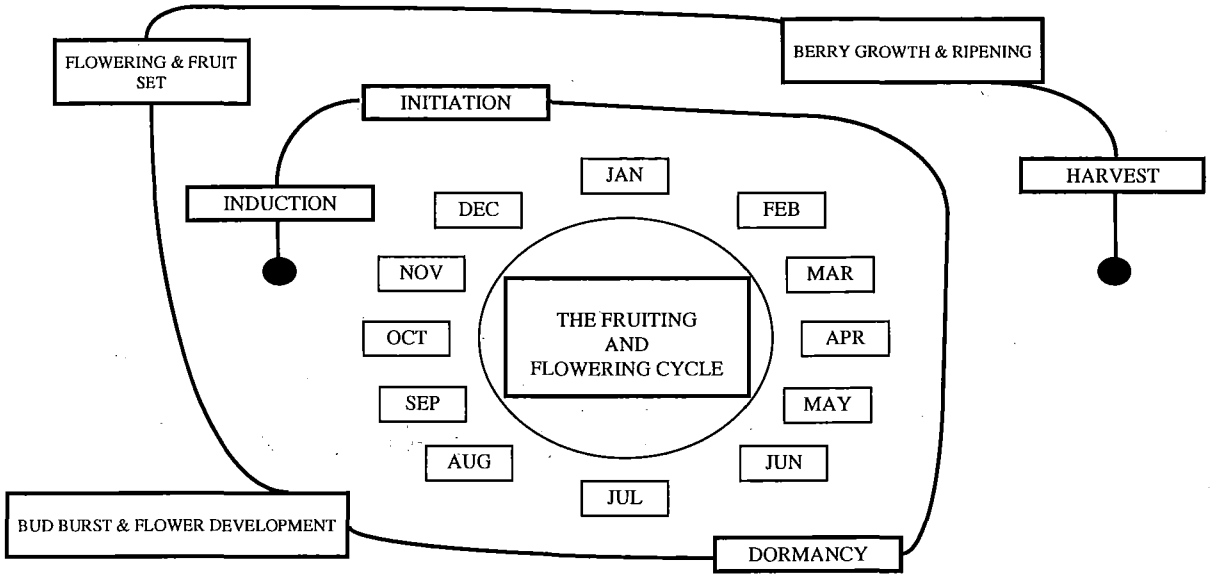


Figure 2.4 Time line of the flowering and fruiting cycle in New Zealand vineyards (Adapted from Wilson 1995).

Environmental conditions around the bud and closely associated leaves affect whether flower formation occurs or not, resulting in an inflorescence or a tendril, respectively. The environmental conditions alter the hormonal and nutrient balance of the latent bud. Cool conditions favour gibberellin synthesis, which promotes vegetative growth, limits nutrient accumulation, and favours tendril differentiation. In contrast, warm conditions (20-25°C) promote cytokinin synthesis, which favours reproductive development [inflorescence differentiation] (Jackson 2000).

Differentiation of uncommitted primordia into inflorescence primordia, within the basal latent buds of Chenin blanc begins when shoots have 12 leaves, about 12 days before the start of bloom. Initiation occurs over about eight days, and differentiation does not begin for about another week. Differentiation occurs over about seven days and is complete before 16 leaves

are present on the shoot. Once the first inflorescence primordium has been differentiated, the second is initiated (Swanepoel & Archer 1988).

Once differentiated, an inflorescence primordium undergoes repeated branching to form a conical structure. Further morphological development ceases when endodormancy commences (May 2000). When buds begin to swell before bud break, further branching, branch elongation and flower formation occur. Whether first-order branching continues after dormancy has ended is unclear. Experimentation with Sultana by May (1964) found that the inflorescence primordia of dormant buds showed first order and occasionally second order branching. On day eight, branches of the third order had been formed, while those of the fourth order had been formed on day twelve, the mean date of bud break.

There is general agreement that flower initials are not formed before the onset of dormancy, but around the time of bud break once branching is complete (Barnard & Thomas 1933; Carolus 1970; May 1964; Scholefield & Ward 1975; Snyder 1933; Srinivasan & Mullins 1981). Initiation of individual flowers is thought to be asynchronous, given the variability of flower development shown by Ezzili (1993) and Boss and Thomas (2002). The organs of individual flowers are then formed in the sequence: sepals → petals → stamens → carpel → ovules.

It is at this stage, once inflorescences have been differentiated and the individual flowers have formed, that the limit of maximum potential yield has been set. Final yield will be determined by the number of individual flowers that set fruit and the extent to which those flowers develop into berries.

Flowering occurs once the flowers have completed their development, normally within eight weeks of bud break. The precise timing of flowering varies with weather conditions and cultivar characteristics (Jackson 2000). In normal seeded berries, pollination allows fertilisation to occur, resulting in the setting of ovules into seeds. Following fertilisation, the ovary begins its growth and development into a berry. A double sigmoid growth curve is observed for berry growth (Figure 2.5). Stage I shows rapid cell division, followed by cell enlargement and endosperm development. This initial phase typically lasts from six weeks to two months. Stage II is a transitional period in which growth slows, the embryo develops, and the seed coasts harden. Stage II is the most variable in duration (1-6 weeks) and largely

establishes whether the cultivar is early or late maturing. At the end of phase II, the berry goes through *véraison*, signalling the physiological shift to ripening. Stage III is associated with this change and the final enlargement of the berry. Ripening is associated with tissue softening, a decrease in acidity, the accumulation of sugars, the synthesis of anthocyanins and the acquisition of aroma compounds. Over-ripening of fruit, if harvest is delayed, is termed phase IV (Jackson 2000).

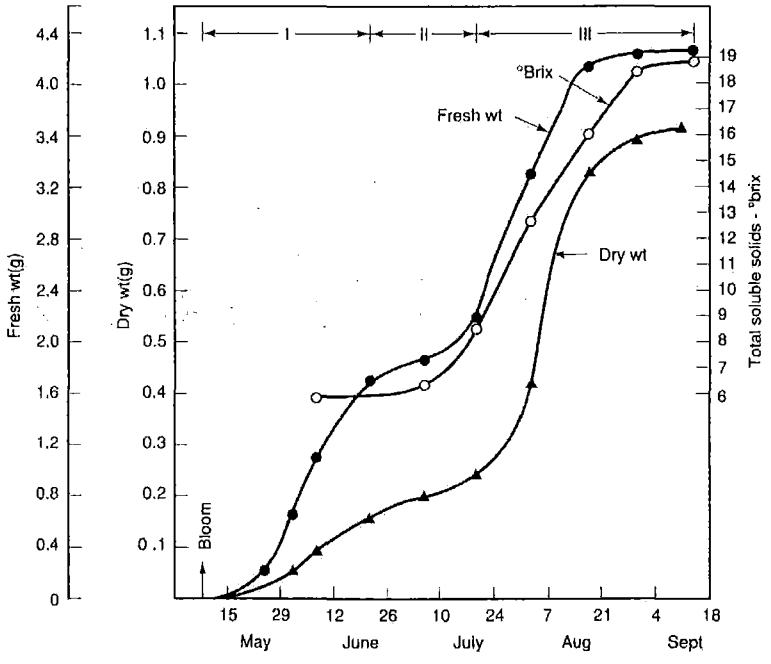


Figure 2.5 Relationship of growth phases, I, II, and III in regard to dry weight, fresh weight, and the accumulation of total soluble solids in Tokay berries. [From Winkler *et al.* 1974]

2.2.1 The grapevine flower

The flowers of the grapevine appear in groups of three, four, or five, are fragrant and green, in dense elongated panicles (inflorescences), which replace tendrils (homologous organ). Flowers are usually hermaphroditic, with a minute, 5-lobed calyx. The five petals are falsely distally connate (united) [due to interlocked papillae], falling as a calyptra post or on anthesis (Oct-Dec SH). The corolla is 1.5-2.5 mm long with valvate lobes. The disc is prominent, consisting of five osmophors or odour glands. Anthers (4-5) are positioned opposite the petals (Figure 2.6). Filaments may be longer or shorter than the corolla. The ovary has a short style, is superior with axile placentation, and consists of two carpels (cells) which are connate. Each

carpel contains two ovules within a single locule. The ovary is partially enclosed by a receptacle, and develops into a two-compartmented berry containing up to four seeds. The developing ovule has a cordlike raphe on the adaxial surface, extending from the hilum to the seed apex and onto the convex abaxial side, where it joins a round to linear, depressed to somewhat elevated, 'chalazal knot'. The three- lobed seed contains endosperm has a deep groove varying in shape and length flanking both sides of the raphe. Berries are globular, 0.7-1.5 cm in diameter, black or yellow with some glaucous bloom. The pulp is soft, watery, and sweet (Jackson 2000; Judd *et al.* 2002; Webb *et al.* 1988).

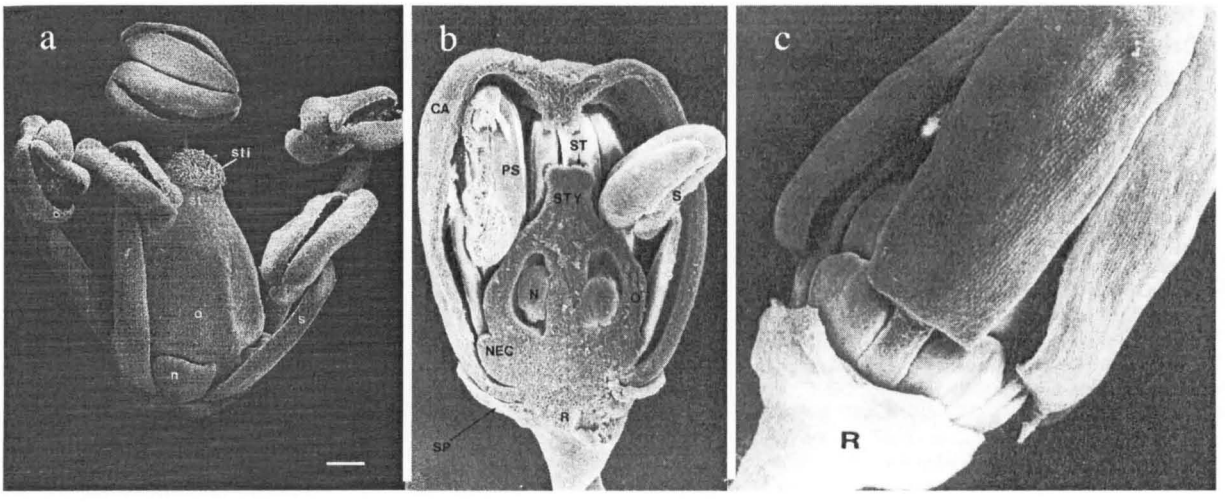


Figure 2.6 The grapevine flower. Picture a) shows five bi-lobed stamens (s), surrounding a pistil comprising a superior ovary (o) surmounted by a style (st) with a papillate stigma (sti). The base of the ovary is encircled by a whorl of osmophors (n) that in some reports are referred to as nectaries. Bar = 200μm [From Hardie *et al.* 1996]; Picture b) shows a cross-section of a flower locating an ovule (N) within the ovary (O). Other labelled organs are the calyptra (CA), sepal (SP), style (STY), stigma (ST), stamen (S) and anther (PS) and receptacle (R) [From Swanepoel & Archer 1988]; picture c) shows the calyptra separating from the receptacle [From Swanepoel & Archer 1988].

2.2.2 The grapevine inflorescence

The inflorescence and the tendril are regarded as homologous organs (Alleweldt & Balkema 1935; Barnard & Thomas 1933; May 1964; Perold 1927; Winkler & Shemsettin 1937). In a series of papers by Srinivasan & Mullins (1978, 1979, 1980a, 1980b) and Mullins (1980) it was concluded that the tendril can be viewed as a weakly differentiated inflorescence. However given that tendrils are thought of as modified stems with leaves (bracts) (Galet 2000) it may be better to consider an inflorescence a strongly differentiated tendril. The

proposed developmental sequence of the uncommitted primordium, shoot initial (mostly lost) → first inflorescence → second inflorescence or tendril (may be lost) → shoot apex (mostly lost), suggested by May (2000), supports such a notion.

The grapevine inflorescence is a complex, highly modified branch system containing reduced shoots and flowers (Jackson 2000; May 1964), in agreement with the general theory on the phylogenetic origin of angiosperm inflorescences (May 1964). The shoot axis is mostly reduced or totally absent, but may be present in various forms (May 1964). The inflorescence is described as a panicle (Perold 1927; Pratt 1971); and possesses a peduncle, at the end of which is a 'node' or swollen joint, subtended with a bract (Figure 2.7 a). At this joint two axes originate, on which subsequent orders of branches and sub-branches may be present. Branches terminate in pedicels, which give rise to the individual flowers. Flowers are borne in dichasia, a group of three flowers with two placed laterally at the base of the central one (Gerrath 1992; Jackson 2000; May 1964, 2000; Perold 1927; Posluszny & Gerrath 1986; Pratt 1971, 1974; Snyder 1933; Troll 1964).

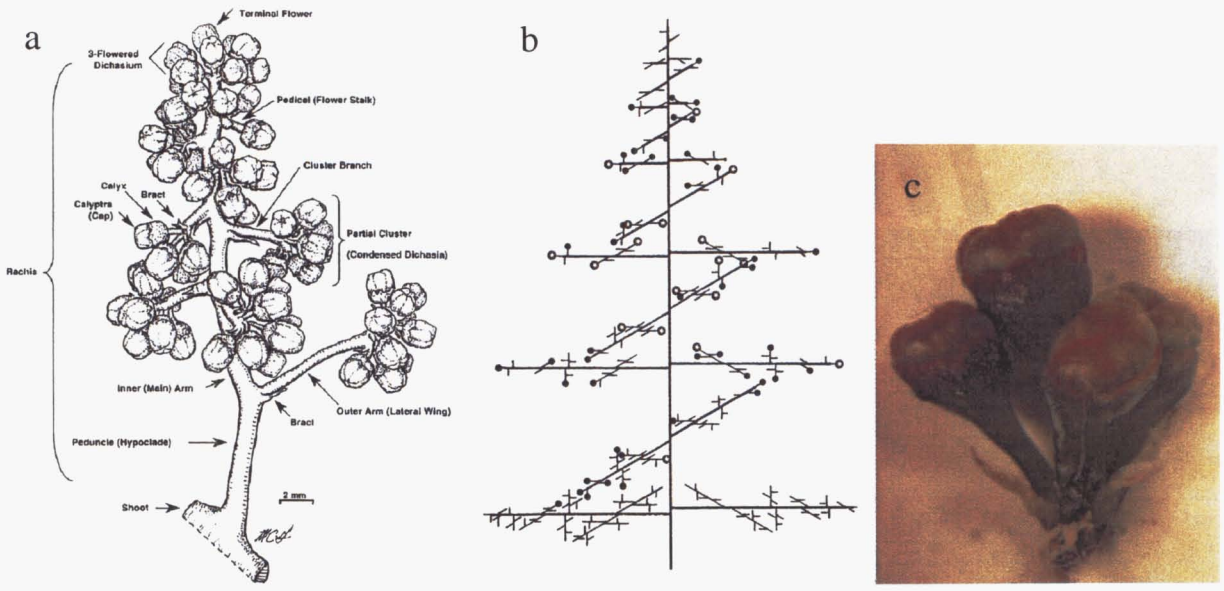


Figure 2.7 The grapevine inflorescence. Figure a) shows a typical grapevine inflorescence in near bloom condition, with the arrangement of parts and descriptive terminology [from Basiouny and Himelrick 2001]; Figure b) is a schematic of an inflorescence showing the arrangement of the main branches from the main axis [from May 1987]; Figure c) The basic floral unit, a dichasium, showing the larger king flower [from May 2004].

The inflorescence is thought of as a complex of the two axes, termed the inner and outer arms. It appears likely that the two arms are in fact two inflorescences of a rudimentary shoot, where the inner arm is the proximal, and the outer arm the distal inflorescence. In rare cases the rudimentary shoot may develop completely (May 1964). The bract opposed inner arm is adaxial to the shoot, which bears the inflorescence, and the outer arm is abaxial to the shoot and may be an inflorescence, tendril or missing altogether. If the outer arm develops into an inflorescence, it is colloquially named a wing or shoulder. The inner arm develops more rapidly than the much smaller outer arm (May 2000) with a greater degree of branching. Hence the outer arm although originating in a distal position, appears to be the most proximal part of the inflorescence prior to anthesis (May 2000). The peduncle is believed to result from intercalary growth from below the first bract (Pratt 1971).

Structures intermediate between tendrils, inflorescences, and shoots are commonly observed on grapevines (Boss & Thomas 2002; Jackson 2000; May 2000; Perold 1927). These include inflorescences where the outer arm fails to develop or develops as a tendril, tendril with a few flowers, a leafy shoot, rudimentary shoot and tendril, inflorescence or as an inflorescence with a foliage leaf instead of a bract at one or more branch points. As shoot systems, the formation of inflorescences with a tendril in place of a wing parallels the formation of main shoots with only one inflorescence and a tendril instead of a second inflorescence.

The main axis carries side branches called paraclades; these are themselves copies of the main axis, terminating in coflorescences, and may carry second-order paraclades (Troll 1964). Branches form as pairs at right angles to the previous pair, each branch being subtended by a bract. The proximal pair of branches are situated opposite each other (Figure 2.7 b). Field observations by the author found that the 'pairs' of branches, after the proximal pair, are slightly offset along the vertical axis; this arrangement forms a spiral effect.

The branching pattern of the inflorescence gives it a pyramidal shape, as the proximal branches are longer, often with a greater degree of sub-branching than distal branches. Each branch is subtended by a bract and ends in a flower (Figure 2.7a). Each terminal flower typically has at its base two flowers, each subtended by a bract; this is called a dichasium (simple cyme). The central flower is not subtended by its own bract. Either the terminal flower or one or more lateral flowers may abort. The dichasium is reduced to one or two flowers toward the top of the inflorescence (Pratt 1971). The basic floral unit is three

(dichasia) (Figure 2.7 c), though Srinivasan and Mullins (1981) found the floral unit for Syrah to be five. This may be the result of reduction of the dichasial unit, through flower abscission and a lack of elongation between dichasial units, as commonly observed at the end of the central axis of paraclades. Scholefield and Ward (1975) found in Sultana, the highest order branch primordium apex subdivided into three floral primordia, separated by approximately 120° with each flower subtended by its own bract. Growth of the inflorescence terminates with production of a terminal flower (Boss *et al.* 2003).

2.2.3 Capfall

As the inflorescence matures the flowers become visible, first in compact groups (Modified EL stage 15) [Refer Appendix A], then the single flowers separate (Modified EL stage 17). When the flowers are mature, the calyptra begin to fade from green (Modified EL stage 18), signalling that capfall, or flowering, is imminent; the calyptra separate from the receptacle, falling to the ground. Galet (2000) reports large differences in time to capfall from bud break between *Vitis* species (49 to 71 days), while cultivars of *Vitis vinifera* range over a smaller time period (56 to 63 days). In the northern hemisphere capfall usually occurs in May and June, depending on the cultivar, longitude and climate, this equates to November and December for the Southern Hemisphere.

The precise timing of flowering varies with weather conditions, cultivar characteristics (Jackson 2000) and climate type (Friend *et al.* 2003). Capfall does not require light and takes place over the course of several days, ranging from 5 to 10 days on average (Galet 2000), but can be longer if weather conditions are cold and rainy. Capfall in New Zealand's maritime climate may occur over two to three weeks, while in the continental climate of Michigan it may be less than a week (Friend *et al.* 2003).

The work of Randhawa & Negi (1965), Staudt (1999) and that of Millardet (quoted in Perold 1927, from Guillon 1905) has found that capfall follows a diurnal rhythm, beginning in the morning (0500-0700hr), peaking after approximately two hours, and finishing between four to six hours later in the early afternoon (1200-1400hr) (Refer Figure 2.8). Randhawa and Negi (1965) noted that capfall started earlier with greater air temperatures early in the morning, while Staudt postulated that the temperature at the preceding non-flowering phase probably influenced the onset of opening and the time of its maximum, with high temperatures causing an advanced rhythm and a decreased length of period and *vice versa*. Staudt (1999) found that

the diurnal rhythm was related to photoperiod and was most likely endogenously controlled, with temperature prevailing at the time of opening having no, or only a small effect, on the opening of flowers. The relationship with photoperiod may be a response to vine water status, as release of the calyptra has been ascribed to changes in the turgor of the interlocking marginal cells (Swanepoel & Archer 1988).

Millardet (quoted in Perold 1927, from Guillon 1905) described the influence of temperature on flower opening: at 15°C flowers of Chasselas opened from time to time, at 17°C flowers opened normally, and at 20°C to 25°C rapidly. Above 35°C capfall is significantly delayed (Galet 2000). Studying the course of capfall of Chardonnay in the Adelaide Hills, Australia, May (1992) described a relationship between the rate of capfall and temperature, with cold periods causing capfall to cease, with few flowers shedding their caps until the renewed onset of higher temperatures.

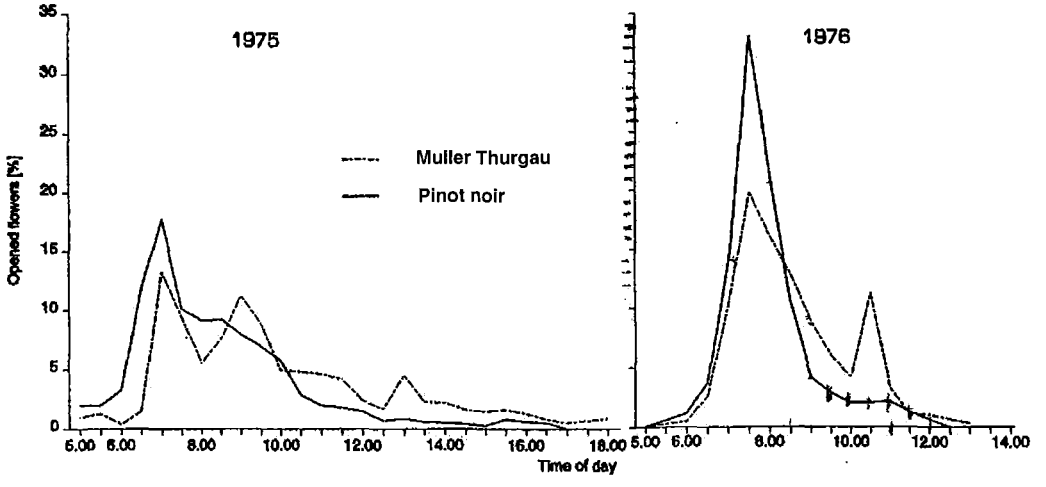


Figure 2.8 The timing of capfall events over the period of 24 hours in Muller Thurgau and Pinot noir, 1976 (Staudt 1999).

Researchers report conflicting results in regard to patterns of capfall across inflorescences; Galet (2000) and May (1987) report that flowers at the base of the inflorescence open first, and those at the tip open last, Winkler *et al.* (1974) state that grape flowers open at the base of the inflorescence first, Manaresi (1947) and Bruni (1967) report that flowers begin capfall in the centre of the inflorescence passing to the base and then the tip, while Branas (1974)

reported that capfall began in the middle of the inflorescence and proceeded to the tip and base at the same time. Examining 22 cultivars, Castelli and Pisani (1985) observed eight different patterns of capfall across an inflorescence. Given the conflicting reports on patterns of capfall and current understanding regarding the evolution of the grape inflorescence, it could be supposed that capfall may be a random event based on flower maturity.

At a finer level, the terminal flower of a dichasium, which tends to be larger, opens before lateral flowers (May 1987) and is consistent with other fruit species with king flowers [i.e. apples (Ferree *et al.* 2001) boysenberries (Trought 1983)]. Studying flower abscission in Concord (*Vitis labrusca*), Pratt (1973) suggested flowers opening early during capfall set better than those opening later, and observed that the dichasia of inflorescences only set one berry, postulating that this may be a result of the terminal flower of a dichasia opening first. May (1987) concluded that the position of the flower on the inflorescence is important for its chance of setting but that positional effects are modified by the intervention of other factors such as weather conditions. Looking at shoots with multiple inflorescences, Schöffling and Kausch (1974) found the proximal, earlier flowering inflorescences had reduced fruit set, though this may have been a result of flower number.

The opening of the flower begins as the petals become free at their bases, followed by a separation along their margins (Figure 2.7 c). The petals remain interwoven at their tip, forming the calyptra (cap), which is pushed upwards by the stamens. Upon release of the calyptra the stamens act like a spring, pushing off the calyptra. The corolla entirely detaches itself form the flower, dries out and falls off. The ovary is now exposed and the filaments of the anthers are free to extend outwards away from the style. This occurs within about 10 minutes of capfall (Galet 2000).

The calyptra is not always observed to fall from the flower after separation. If rainfall occurs during capfall, the petals may die, forming a brown cap over the flower. In this situation, self fertilisation (autogamy) is obligatory, but as the pollen sacs do not open easily, poor fruit set is frequent.

Pollen dehiscence occurs through the simultaneous opening of the pollen sacs. The sac envelope relaxes abruptly when the air is hot and dry (Galet 2000). This normally happens

after capfall, but can occur earlier, under the corolla (Heazlewood & Wilson 2004; Staudt 1999).

2.2.4 Pollination

Pollination occurs when pollen grains, released from anthers, land on a stigma and allows the transfer of the male gametes to the ovule. Once transferred to the stigma the pollen grain germinates and forms a pollen tube. The pollen tube is an outgrowth from a pollen grain and grows down through the style towards the ovule. Once the pollen tube reaches the nucellus, it enters a synergid cytoplasm, discharging the vegetative nucleus and the two sperm cells, allowing fertilisation to occur (Campbell 1993; Dickinson & Bonner 1989; Frankel *et al.* 1977).

The pistil interacts with the pollen at all stages, inducing a number of physiological changes. Pollination can coordinate the final development of the female gametophyte, facilitating reproduction by preparing the ovary for fertilisation and removing organs that have fulfilled their function in the attraction of pollinators (Woodson 2002). Pollination may activate the stylar cell to release glycoproteins and carbohydrates into the intercellular transmitting tissue for incompatibility and support of the pollen tube (Dickinson & Bonner 1989). The decision to continue floral development is dependent on pollination, with pollination being necessary for fruit set while fertilisation is not (Srivastava 2001). Pollination can induce parthenocarpic fruit development. Pollination also signals through the style, initiating the degeneration of one of the synergid cells, towards which the growth of the pollen tube is oriented. In most species the primary pollination event is associated with an increase in ethylene evolution (O'Neil 1997). The nature of these signals is poorly understood and is only generated following the arrival of pollen grains (Dickinson & Bonner 1989).

The grapevine stigma is short (Carraro *et al.* 1979; Lombardo *et al.* 1976; Staudt 1982) and of the wet type (Heslop-Harrison & Shivanna 1977). The stigmatic fluid prevents osmotic lysis of the germ tube, provides nutrition for pollen growth, varies in the extent to which it is apparent and, under certain temperature and humidity conditions, it can dry up. In the absence of pollen, the fluid persists for about a week (Galet 2000, Sharples *et al.* 1965). It is also present in the intercellular spaces of the style (Galet 2000; Jackson 2000), which may explain why rainfall doesn't significantly inhibit pollen germination (Jackson 2000). If the wind is

particularly strong, the stigma can dry out. If the air is too humid, the pollen grains swell and have difficulty dispersing (Galet 2000). Under rainy conditions, the stigmatic fluid is diluted and the wet pollen sacs have difficulty opening (Galet 2000). The presence of stigmatic fluid can be used as an indicator of stigma receptivity (Miaja *et al.* 1999), with darkening of the stigmatic surface evident 48 hours after pollination, indicating that pollination and pollen germination has occurred (Miaja *et al.* 1999), whereas non-pollinated flowers maintain stigmatic receptivity (moist stigmata) for a longer period (Carraro *et al.* 1979).

The mode of pollination in the genus *Vitis* is not entirely clear. Insect pollination has been shown to occur in *Vitis rotundifolia* (Lavee & Nir 1986; Sampson *et al.* 2001), and insects may play a role in pollination in *Vitis vinifera* (Jackson 2000; Kevan *et al.* 1985; Mullins *et al.* 1992; Pratt 1971); with *Halictus* sp. and *Apis* sp. (Randhawa & Negi 1965), syrphid flies, long-horned and tumbling flower beetles visiting grape flowers (Brantjes 1978). Many authorities state that the grapevine is primarily wind pollinated (Jackson 2000; Lavee & Nir 1986; Mullins *et al.* 1992; Pratt 1971), and the productivity of vineyards has been related to the amount of germinable pollen grains present in the air (Carraro *et al.* 1981). However the structure of the grape flower is not suggestive of wind pollination (Mullins *et al.* 1992; Pratt 1973) as it lacks enlarged stigma for more efficient interception of airborne pollen, nor do inflorescences release copious amounts of dry, buoyant pollen into the air stream (Sampson *et al.* 2001). It seems that various types of pollination can occur simultaneously. Some botanists have concluded that wind pollination may have arisen in evolution as a secondary mechanism ancillary to pollination by insects (Meeuse & Morris 1984).

There is a lack of consensus in the literature as to whether the grape is self-pollinated, cross-pollinated or both (Mullins *et al.* 1992). The review of Lavee and Nir (1986) concluded that self-pollination appears to be the rule for most grape cultivars as wind and insect pollination appears to be of little significance. Caging inflorescences, to prevent insect visitation still produced normal bunches (Lavee and Nir 1986). The presence of pollen on the stigma immediately after capfall, complete pollination, the short life of the stigma, and fruit set in flowers without calyptra drop point to the existence of autogamy (Lavee & Nir 1986). Bud-pollination (dehiscence of the anthers before capfall) is common (Miaja *et al.* 1999; Mullins *et al.* 1992; Pratt 1971). The high proportion of weak seedlings that occur in open-pollinated populations suggests that vinifera cultivars, unlike their dioecious progenitors are normally selfed (Mullins *et al.* 1992).

It is important to make the distinction that just because a plant may be self-pollinated, it does not necessarily mean that cross-pollination will not occur.

The source of pollen can influence both the efficacy of fruit set and the characteristics of the berries that result. Artificial pollination with a mixture of pollen from various cultivars is more efficient in setting fruit than pollination with pollen from the same or another cultivar (Lavee & Nir 1986). Pistillate *Vitis rotundifolia* vines set more fruit than hermaphroditic vines when cross-pollinated. Almost half the fruit set by hermaphroditic and 91% fruit set by pistillate vines is the result of cross-pollination by wind and insects (Sampson *et al.* 2001). Pollen source influences the proportions of small, medium, and large berries within bunches (NeSmith 1999; Persuric *et al.* 1998). Iyer and Randhawa (1965) reported that fruit set and berry size of bunch grapes were influenced to a degree by pollen source, but the greatest differences were between selfing and cross-pollination. The male parent always gave increased seed size and weight in crossed fruits, irrespective of which female parent was used in the cross; suggesting a direct influence of pollen on seed characteristics (Iyer & Randhawa 1965). Compatibility of pollen and cross-pollination can influence berry size and fruit set, as recognised in some table grape cultivars. Comparing bunch weights at the interfaces between two cultivars planted alongside each other, Milne *et al.* (2003) found 20% of transects show changes in bunch weight at the interface between cultivars. Comparing Cabernet Sauvignon paired with Merlot, showed a tendency for increased bunch weights at the interface, due to increased berry number and berry weights. Berry weight was related to the number of seeds present in each berry.

Compatibility mechanisms appear to be limited to the rejection of non-*Vitis* pollen (Free 1970; Galet 2000). Compatibility recognition originates from the interaction of proteins from both the pollen and pistil. The soluble pollen wall proteins of *Vitis vinifera* are genotypically determined (Cargnello *et al.* 1988), and their expression, if not the extent of their expression, is independent from external factors (Tedesco *et al.* 1989). Hybrid formation in the genus *Vitis* commonly occurs where species overlap in their distribution (Mullins *et al.* 1992).

In Thompson's Seedless, germination, and pollen tubes form 40 minutes after pollination and have passed into the style within 22 hours (Oinoue 1925). Pollen germination and germ-tube growth are markedly affected by temperature (Faust 1989; Staudt 1982), though viability is less affected (Jackson 2000). Cool temperatures just before flowering at favourably warm

temperatures, can delay pollen germinability and germ-tube growth. Similar conditions can equally reduce fertility by disrupting aspects of ovule development, with ovules showing obvious signs of degeneration after one week at 10°C (Ebadi *et al.* 1995b). Pollen germination and pollen tube growth are favoured by high temperatures (27°C day 22°C night) and pollen tubes have been recorded to appear at the micropylar end of the embryo sac within 12 hours of pollination at favourable temperatures (Rajasekaran & Mullins 1985; Staudt 1982). Below 10°C and above 35°C pollen germination is greatly inhibited or does not occur (Perold 1927). At an optimum temperature of 28°C pollen tubes will germinate within 30 minutes and reach a maximum elongation rate of 10.7μm/minute after 1 hour, after which elongation slows. Pollen tube elongation is restricted with lower temperatures. At 15°C pollen germination is restricted and after 48 hours pollen tubes stop elongating, with few pollen tubes reaching the embryo sac. At 10°C pollen germination is severely restricted and no pollen tubes reach the embryo sac (Staudt 1982).

2.2.5 Fertilisation

Once the pollen tube enters the filiform apparatus and discharges its contents into the cytoplasm of a synergid, the sperm cells fuse with their respective bodies, and their nuclei and organelles become mixed (Dickinson & Bonner 1989). Generally, nuclear fusion takes place first in the central cell and then in the egg cell (Frankel *et al.* 1977). The fusion of the male (sperm) with the female (egg and central cell) gametophyte is fertilisation (Campbell 1993; Srivastava 2001). Fertilising the egg cell initiates embryo development, while fusion with the polar nuclei initiates endosperm development. As the embryo grows, the surrounding ovule develops into a seed. The entire ovary meanwhile develops into a fruit containing one or more seeds. The wall of the ovary becomes the pericarp, the thickened wall of a fruit. As the ovary grows, the other parts of the flower generally wither away. This transformation of the flower, called fruit set, parallels the development of the seeds (Campbell 1993).

Despite large numbers of pollen landing on a stigma, fertilisation occurs in an orderly manner. Fertilisation is not straightforward process, in some species the pollen tube moves swiftly to the ovule, in others a lag phase occurs between the arrival of pollen tubes at the base of the style and fertilisation. There is a genetically based control of ovule penetration operating both at the gametophytic and at the sporophytic levels. The mechanisms are unknown (Herrero 2000).

As in many plant species during fertilisation, in the grapevine the pollen tube enters the ovule though the micropyle, crossing the cell wall of the nucellus and penetrating the embryo sac between the two synergids. Upon penetration the synergids disorganise immediately. The vegetative nucleus of the pollen grain is resorbed leaving the two male nuclei, produced by the division of the reproductive cell. One of these male gametes unites with a female gamete from the oosphere to form the egg, which is the point of departure for the embryo. The other male gamete unites with the secondary nucleus of the embryo sac, which result from the union of two polar, haploid nuclei, to form the mother cell of the endosperm, which is rapidly formed by successive division (Galet 2000).

Thus, there is double fertilisation, where the egg unites with a single male nucleus yielding an egg nucleus with 2n chromosomes; while the endosperm has a triploid nucleus, with two sets of female chromosomes and one set of male chromosomes. The union of the gametes occurs 24-28 hours after impregnation of the stigma, when the temperature is around 15 to 20°C (Galet 2000; May 2004).

After fertilisation, the integuments of the ovule develop to form the integuments of the seed. The nucellus develops rapidly in the week following capfall and is completely replaced by the endosperm 35 days later (Nitsch *et al.* 1957). The egg begins to divide two weeks after capfall.

2.2.6 Fruit set

Fruit set is the point after flowering where individual flowers are either retained (i.e. set) or abscised, and represents a change-over from the static condition of the fully developed flower to the rapidly growing condition of the young fruit (Coombe 1962; Weaver 1976). Fruit set requires positive growth signals (Gillaspy *et al.* 1993), generally, resulting from pollination that achieves fertilisation and seed development (Winkler *et al.* 1974). It is this stimulus that encourages and possibly determines the extent of berry development.

Fruit set, abscission, *coulure*, seed set (determining the extent of berry development) are often considered as separate processes, but should be considered as a whole, as they are all interlinked. Fruit set is a consequence of the prevention of the abscission process; a distinct process with its own biochemical pathways. Abscission occurs in many crop plants in

response to developmental or environmental cues. The event is highly variable according to species and cultivar and appears to be a function of endogenous growth regulator status in ovaries and with metabolic regulation during floral development. Hormonal signals and competition or depletion for photoassimilates remain primary factors influencing fruitlet abscission (Aziz 2003; Aziz *et al.* 2001).

Current theory suggests a decision to set fruit/abscise is based on a polyamine/sucrose stimulus and that consequential berry development is dependent on seed. Transition of the ovary to a fruit is dependent on nutrient availability (Moss *et al.* 1972; Gillaspy *et al.* 1993; Gomez-Cadenzas *et al.* 2000); competition among different metabolic sinks for photoassimilate and resource allocation at the whole plant level are involved (Srivastava 2001). Sucrose status in fruitlets of citrus is considered a major factor in triggering fruitlet abscission (Gomez-Cadenzas *et al.* 2000). Changes in carbon metabolism in plants are known to be associated with alterations in nitrogen metabolism (Huppe & Turpin 1994). It has been suggested that polyamines could be involved in maintenance of photosynthetic activity during the senescence process (Kotzabasis *et al.* 1993). Polyamines have anti-senescence properties (Altman 1989) and compete with biosynthesis of ethylene (Kushad & Dumbroff 1991; Turano *et al.* 1997), which has been described as a stimulator of abscission (Ruperti *et al.* 1998).

Fruit set in grapevines can be considered complete once berry fall (shatter) has occurred. The numerical loss of flowers increases after bloom, peaking about 12 days after capfall (Galet 2000). Berries fall when they are ~2mm in diameter, preceded by a cessation of growth and lightening in colour of the berry. The berries either fall along with their pedicels or else they shrivel and remain on the cluster (Galet 2000). Bunches may typically have 100-200 berries each, but inflorescences can have 300-700 flowers or even more. Galet (2000) presents a range of fruit set figures varying from 4.5 to 79%. Under normal conditions, fruit set percentages can be considered a varietal characteristic, but is subject to external and physiological factors relating to organic nutrition.

Ovule longevity is an important determinant of fruit set, but longevity can vary with species, temperature, nutritional status and plant growth substances (Basiouny & Himelrick 2001). Most environmental conditions and many management practices can indirectly influence the percentage fruit set of grapevines. Poor fruit set can result from weather, endogenous plant-

growth hormone imbalances, pollination and fertilisation problems, degeneration of the ovule, insects and disease, and competition among fruitlets (Malik & Singh 2003).

Light intensity *per se* does not seem to be a major factor in fruit set, unless photosynthesis is reduced. Shading of inflorescences has been reported to cause only a small or a nil decrease in fruit set, even in cultivars susceptible to *coulure* (May 2004). However, combining low light intensity with low temperatures can have a dramatic impact on fruit set. Roubelakis and Kliewer (1976) found by reducing light intensity under a 15°/10°C day/night temperature regime, fruit set rapidly decreased to zero (Table 2.2). However it should be noted that their calculation of fruit set was based on the number of berries per bunch and not the percent flowers set into fruit.

Table 2.2 Percentage fruit set of five cultivars exposed to different light intensities, growing under a 15°/10°C day/night temperature regime (Adapted from Roubelakis & Kliewer 1976).

Cultivar	Light intensity (foot-candles)		
	2680	750	480
Pinot noir	31%	0%	0%
Carignane	25%	9%	0%
French Colombard	25%	0%	0%
Cabernet Sauvignon	16%	0%	0%
Riesling	14%	0%	0%

Both low and high temperature can impact of fruit set in grapevines. Low temperatures pre-flowering can disrupt formation of ovules and pollen and their function during flowering. Likewise exposure to temperatures of 15° or 40°C in Pinot noir and Carignane can significantly lower fruit set (Kliewer 1977). Temperatures below 15°C and above 32°C are considered detrimental.

Rainfall during flowering can reduce fruit set by preventing shedding of the calyptra, impeding pollination and fertilisation (May 2004). Nutrient deficiencies, particularly of nitrogen, boron, zinc and possible molybdenum can reduce fruit set, by inhibiting pollination (May 2004). Shading of leaves reduces photosynthesis and carbohydrate supply to the developing inflorescences, causing flower abscission and lower yields (Jackson 1991).

Manipulating shoot carbohydrate availability by using a combination of girdling and leaf removal, Caspari *et al.* (1998) suggest that carbohydrate availability is the main factor determining fruit set in grapevines, and that environmental conditions and management techniques that reduce fruit set are a cause of reduced carbohydrate supply.

2.2.7 Seededness and Seedlessness

Since there are normally four ovules per flower, there should be an equivalent number of seeds; however, berries often contain fewer seeds: three, two, one, or even none. Ovules can abort during development or do not grow after fertilisation (Galet 2000). There are four methods by which flowering can lead to ovaries of commercially grown cultivars becoming berries: simulative parthenocarpy, stenospermocarpy and empty and normal development of seeds. Apomixis, the development of fruit and viable seeds in the absence of fertilisation, is unconfirmed in grapes (Jackson 2000).

2.2.7.1 Seedlessness

Parthenocarpy or seedlessness refers to a complete absence of seeds in a berry. It results from precocious, complete degeneration of the unfertilised ovules. A berry is said to be seedless when it has no seeds at all or when it contains only rudimentary seeds with unhardened integuments.

The ovule determines seedlessness, as the pollen of a seedless variety that fertilises the ovule of a seeded variety will not lead to stenospermocarpic abortion. Likewise, fertilisation of an ovule in a seedless variety by pollen from a seeded variety does not lead to fully a formed seed (Levadoux 1946).

There are two types of seedlessness in grapevines, stimulative parthenocarpy and stenospermocarpy.

In stimulative parthenocarpy, the flowers are perfect, but the berries contain rudimentary seeds, which are simply the remains of the ovules, and are the same size as the ovules at the time of fertilisation (Galet 2000). Black Corinth is considered an example of a stimulative parthenocarpic cultivar. After fruit set, berry development in seeded varieties occurs through

the uninterrupted action of hormones from the embryo and endosperm. In Black Corinth, as there are no embryos and thus no hormones, the berry either abscises or it develops until ripeness, but is often too small to be of commercial interest. To compensate for hormonal and nutritional deficiencies, berry size in Black Corinth can be increased by either, shoot tipping, girdling or the application of plant growth hormones (Galet 2000).

The ovules of mature parthenocarpic berries are small, with only one layer of sclerenchymatous cells in the outer integument and have no rumination. Occasionally larger hard seed forms with one or more layers of sclerenchyma, rumination develops, the nucellus enlarges and persists, but the embryo sac is non-functional. Depending on the cultivar, pollen from parthenocarpic cultivars is reported to germinate poorly or not be viable. Fruit is able to set with out pollination (Pratt 1971). Parthenocarpic fruits develop without ovule fertilisation (Ledbetter & Ramming 1989), though pollination is required to stimulate a good [*sic* high] fruit set (Olmo 1946). Many pollen tubes travel down the style and enter the locules, but very few enter the micropyle and penetrate the nucellus (Pearson 1932).

A release of hormones from pollen is thought to cause the stigma to wilt and render the ovary capable of enlarging and maturing. This occurs when the pollen comes into contact with the stigmata without germinating, either directly or indirectly though the envelope of the pollen sacs, or when the pollen tube penetrates the ovary but fertilisation does not occur (Branas 1974). Pollen from various plant species is known to contain ethylene inhibitors and auxin (Taylor and Hepler 1997); the presence of such hormones in grape pollen may be sufficient to stimulate the development of the ovule's integument, but insufficient for embryo or further seed development and subsequent berry enlargement (Galet 2000). Thus, berries are said to be parthenocarpic when the flowers have been pollinated but the four ovules are not fertilised (Branas 1974).

The formation or absence of seeds in parthenocarpic seedless berries does not depend solely on hormones from pollen, but also the time at which they act during capfall. Girdling, which modifies shoot organic nutrition carbohydrate (Weaver & McCune 1959) and plant growth regulator levels (Weaver & Pool 1965) is best applied when the stigma is still receptive. Girdling at fruit set, after bloom, reduces the probability of seed formation. Girdling only affects the size of the fruit. The cytokinin 4-chlorphenoxyacetic acid (4-CPA) acts similarly to girdling in terms of how treatment time affects seed formation (Galet 2000). Weather

conditions can affect the organic nutrition status of a vine and on the normal development of capfall (Galet 2000).

The physiological disorder 'Hen and Chicken' (termed *millerandage* in French) is thought to be a form of stimulative parthenocarpy that is observed after fruit set (Galet 2000); however Staudt and Kassemeyer (1984) found that the small berries of 'Hen and Chicken' grapes are not parthenocarpic but stenospermocarpic.

The 'Hen and Chicken' disorder is characterised by the presence of small seedless, coloured and sweet berries alongside normal, seeded berries. The presence of these small berries reduces bunch weight. Of these small berries, many intermediates stages exist, as a sort of transition state between normal berry development and abscission (Galet 2000). Girdling before anthesis can increase the incidence of small berry formation (Branas 1974), presumably by disrupting ovule formation.

The extent of 'Hen and Chicken' can depend on the variety, the clone and on the intensity of viral infection. In the 'Mendoza' clone of Chardonnay, infection with Grapevine leaf roll virus, type one (GLRaV-1) has been linked to the presence of 'Hen and Chicken' disorder (Cohen 2000). The incidence can increase when conditions are unfavourable for pollen germination, where low temperatures and rain moisten the pollen and prevent its germination. These conditions also favour the persistence of the calyptra by weakening the stamens, which is considered to be the main causes of 'Hen and Chicken' in pistillate cultivars. In perfect cultivars, low pollen germination, boron deficiency, contact between closed pollen sacs and the stigma in capped flowers, embryo sac defects and the presence of pollenicides may cause the disorder.

In stenospermocarpic seedlessness, the berries have small rudimentary seeds, with soft integuments, of various sizes, that are not noticeably crunchy in the mouth [i.e. Thompson Seedless] (Galet 2000; Pratt 1971; Stout 1921; 1936). Pollination and fertilisation of at least one ovule is required for fruit set and berry development; and the size of the mature berry is related to the number of partially developed seeds (Pearson 1932; Pratt 1971; Stout 1936). These varieties are permanently seedless, because, stenospermocarpy is a property of the gynoecium. The pollen is believed to be perfectly normal and does not provoke seedlessness

when used in cross-pollination. The pollen geminates and the first generating nucleus generally fertilises the oosphere (Galet 2000).

Female gametophyte development is identical with that of seeded cultivars, being monosporic polygonum in type. The chalazal megaspore produces eight nuclei through three mitotic divisions. Polar nuclei fuse prior to fertilisation, and the antipodals typically degenerate prior to anthesis. At anthesis the mature embryo sac contains the egg, two synergids and a fusion polar nucleus. Endosperm development has been observed to precede embryo development. The first nuclear division of the endosperm has been observed one to two days after fertilisation. The initial division of the zygote occurs 15-25 days post anthesis. Endosperm degeneration occurs from 20-25 days post anthesis depending on the cultivar. Although embryos may remain viable, embryo development is usually arrested after endosperm breakdown (Ledbetter & Ramming 1989). Not all ovules are abnormal in stenospermocarpic cultivars, with a high frequency of normal appearing embryo sacs (Pearson 1932).

Prior to the fusion of the vegetative nucleus with the secondary nucleus of the embryo sac, there are signs of alteration to the embryo sac, which becomes irregular in shape and then almost completely disappears (Oinoue 1926). The ovules abort soon after fertilisation, owing to the non-fertilisation of the secondary nucleus of the embryo sac (Pratt 1971). In Concord Seedless the ovules of stenospermocarpic seeds abort 10 to 15 days after anthesis (Nitsch *et al.* 1957).

Stenospermocarpic seeds are found in the Sultanina varieties, particularly female ones or on fanleaf-infected vines. The application of gibberellins or the use of girdling after fruit set can encourage further berry enlargement to occur in stenospermocarpic cultivars (Galet 2000).

In the cultivars 'Sultanina' and 'Russaka' the ovules form (ontogenesis) normally, but can begin degradation as early as anthesis. The female gametophyte stops development at stage of a uninuclear or binuclear megaspore. Fertilisation of the secondary nucleus and ovicell may occur but degradation of the endosperm nuclei and zygote will then occur. Embryogenesis does not proceed, meaning the resulting berries lack seed embryos and seeds (Roytchev 2000).

Research on the development and mature anatomy of seeds from a number of stenospermocarpic cultivars has been assembled by Pratt (1971) (Table 2.2), showing that by 20-25 days after flowering, the ovules have begun to degenerate. By this time the endosperm has degenerated or is lacking, and the maximum development of the embryo is a few cells to globular (Pratt 1971).

Seed trace size in stenospermocarpic cultivars is related to the relative time of embryo/endosperm breakdown; with smaller traces being present the earlier breakdown occurs. Environmental conditions during the growing season may play a role in seed trace development with traces varying in size from year to year as well as with vine age (Ledbetter & Ramming (1989).

Table 2.2 Seed development in the stenospermocarpic grape cultivars 'Concord Seedless', 'Himrod', 'Interlaken' and 'N.Y. 15302' in New York [Expressed as days after 50% capfall] From: Pratt (1971).

	Concord Seedless	Himrod	Interlaken	N.Y. 15302
Early divisions of endosperm nuclei	2	2	2	2
Cytokinesis in micropylar cell of endosperm	-	25	25	25
Rapid development of nucellus	10-11	-	-	-
Endosperm completely cellular	15-19	-	-	-
Embryo 2-7 celled	15-16	25	-	25
Degeneration of endosperm	19-22	25	25	-

Berries may form without pollination or fertilisation (Negrul 1934), being much reduced in size and termed shot berries (Ledbetter & Ramming 1989). Shot berries are small green berries that are un-pollinated ovaries. They expand only slightly (2-4 mm diameter) and remain on the cluster until picking time. They remain on the bunch due to auxin from the shoot and hormones from the flower. The literature on berry development does not provide any clear evidence that these small, green, shot berries are stenospermocarpic or parthenocarpic. However, given that shot berries form on bunches of seeded cultivars, and do not show berry development as seen in the stenospermocarpic berries of seeded cultivars exhibiting 'Hen and Chicken', it possible that they are parthenocarpic. Further work is required on this topic, especially any requirement of pollination.

2.2.7.2 Seededness

At least one seed is required to stimulate the berry to normal development (Cawthon & Morris 1982; Staudt & Kassemeyer 1984). Normal seeds have a normal, complete structure with a thin outer integument and a sclerified inner integument and possess an embryo and endosperm. These seeds cannot be crushed between two fingers and when placed in water, they do not float (Galet 2000).

In the case of normal seeds, enlargement of the berry is dependent upon fertilisation and development of the seed (Stout 1936). In Cabernet Sauvignon, berries having two or three seeds are significantly larger than those having only one (Scienza *et al.* 1978). Development and composition of berries correlated with the number of seeds per berry. A strong positive correlation exists between the concentration of gibberellin-like substances in a berry and the number of seeds (Scienza *et al.* 1978). At anthesis levels of gibberellins may be higher in seedless cultivars than in seeded cultivars (Iwahori *et al.* 1968).

A continuous variation can be observed in the development of seed traces from stenospermic grapes, of which Stout (1936) attempted to classify: ranging from papery seeds, with flexible integumenatry tissue, to empty seededness, which show normal seed, but are hollow, devoid of embryo, endosperm or nucellus.

Empty or hollow seeded berries have an identical shape to normal seeded berries, but do weigh less. They appear to be normal from the outside, but their endosperm has degenerated to a certain extent, leaving only a few traces, and they often lack embryos. These seeds float on water. The endosperm of these seeds turns brown and becomes disorganised within ten days after fertilisation, and sometimes an abnormally small embryo is visible (Galet 2000). These seeds very rarely germinate (Olmo 1935). It is thought that the state of the ovules at the time of pollination may influence the chances of an empty seed developing (Branas 1974).

Berries with empty seeds have a sufficient stimulus for maturation of the berry, but the fruit is smaller than normal seeded berries (Ebadi *et al.* 1996b). Chaouch is a cultivar that produces normal sized berries with hard empty seeds (Jackson 2000).

Examining the development of ovules in the cultivar Chardonnay, Ebadi *et al.* (1996b) found that aberrations from normal seed development can be observed four days after capfall. The

researchers identified two types of ovule with aberrant development: those without an embryo sac and those with a normal embryo sac but with no evidence of fertilisation. Ovule development, which prevented normal seed development, either had a degenerating zygote and free nuclear endosperm or the zygote had failed to divide and the free nuclear endosperm had degenerated. In other ovules, no zygote or embryo could be found. Such characteristics were observable 14 days after capfall. By 28 days after capfall, all of the third largest seeds of medium-sized berries showed abnormal development, were smaller in size than normal seeds, and some had post-fertilisation degeneration occurring. By 42 days after flowering all of these seeds had degenerated nucelli, and lacked embryos and significant cellular endosperm.

Empty seededness and seedlessness can be induced by the application of antibiotics to flowers at flowering. Treating Muscat of Alexandria and Neo Muscat with either streptomycin or spectinomycin increased seedlessness and the proportion of berries with empty seeds. Associated with the development of seedlessness/empty berries were a decrease in berry size and an increase in berry soluble solids (Widodo *et al.* 1999a). The timing of the application of antibiotics influences the proportion of emptiness that results. The antibiotics are thought to inhibit endosperm nuclei division.

Aberrant embryo sac development before flowering, lack of fertilisation at flowering, and incomplete development of zygote/embryo after flowering are seen as the causes of no or defective seed growth in Chardonnay (Ebadi *et al.* 1996b). Exposure of flowers to cool temperature conditions for seven days, two days before the onset of flowering, has been shown to increase the incidence of abnormal ovule formation and of empty seeds in Chardonnay (Ebadi *et al.* 1995b).

2.2.8 Flower abscission

Abscission is controlled by the plant. It is initiated before, and results in the shedding of plant organs at predetermined positions called abscission zones (Srivastava 2000). Abscission of flowers or young fruit allows the co-ordination of crop load to a plant's available resources (Stephenson 1981). The shedding of organs appears to be controlled mainly by the organ to be abscised (Addicott 1982). The initiating cues may originate from the plant's carbohydrate status (Gómez-Cardenas *et al.* 2000).

Fruit set and the abscission of flowers is under hormonal and metabolic regulation. In citrus, it has been suggested that after hormonal activation of initial fruit growth, subsequent development is mostly determined by nutrient supply (Talon *et al.* 1997). Once mineral and water requirements are satisfied, competition for photoassimilates is thought to be responsible for fruit drop (Gómez-Cardenas *et al.* 2000).

Carbohydrates appear to be distributed between developing citrus fruitlets according to source-sink relationships. The abscission in each fruitlet is only inhibited when a certain carbohydrate threshold is reached. The effect of complete defoliation arrests carbon build-up, and results in the continuous shedding of sucrose-deficient fruitlets. Under partial defoliation, fruit load is adjusted, by abscission, to the carbohydrate supply; abscission stops when the remaining fruitlets begin to regain normal sucrose levels (Gómez-Cardenas *et al.* 2000). The mechanisms of source-sink regulation in fruits may act as a regulatory element, providing a physiological link between the carbohydrate status and the severity of fruitlet abscission (Gómez-Cardenas *et al.* 2000).

In citrus, carbon shortages reduce the hormonal stimulators of growth, such as gibberellins, and increase stress-sensitive signals, such as abscisic acid and 1-aminocyclopropane-1-carboxylic acid (an ethylene precursor) levels. Such changes would allow, through abscission, the regulation of fruit load in accordance with the severity of a carbohydrate deficiency (Gómez-Cardenas *et al.* 2000).

Three potential sites for abscission exist in grape inflorescences, one between the peduncle and rachis, and one at each end of the pedicel (Lavee & Nir 1986). Abscission in grapevines is mainly related to young fruit (Hilt & Bessis 2000), where individual ovaries, which fail to set, abscise at the rachilla (proximal) end of the pedicel (Lavee & Nir 1986); this is termed *coulure*, or shelling (Jackson 2000). In the absence of any fruit set, the whole rachis may abscise at the peduncle. The remaining (distal) site for abscission appears to operate during the final stages of ripening, where mature fruits may abscise from the base of the pedicel, and is termed shatter or shanking (Jackson 2000). The premature shrivelling of flowers and portions of inflorescences associated with the physiological disorders, early bunchstem necrosis and inflorescence necrosis, appear to be the result of ammonia toxicity associated with protein degradation induced by carbon starvation (Keller & Koblet 1995). These should

not considered abscission processes, as the affected tissue undergoes necrosis and does not form an abscission zone.

Coulure begins at flowering and takes place for about two weeks within a population of flowers. It has been reported that *coulure* may begin 10 days before capfall in the large inflorescenced cultivar, Sultana (Bindra 1989). Abscission occurs at the base of the pedicel, once an abscission zone has formed (Bessis & Fournioux 1992); abscission zones begin to form between 4 and 6 days after capfall (Ebadi *et al.* 1996a). Berry shedding occurs when the berries are 1-2 mm in diameter. Shedding is preceded by the cessation of growth, and a lightening of berry colour (Galet 2000). Circumstances that have been linked to reduced carbohydrate supply and fecundity have been associated with an increased incidence of *coulure*: girdling, early leaf removal, dense canopies, poor weather, and vines of high vigour (competition between vegetative and reproductive organs [sinks]). Other factors such as light pruning, boron deficiency, lime-induced chlorosis, fanleaf virus infection, and poor fecundity have been associated with *coulure* (Bessis *et al.* 2000, Galet 2000). Genotype will influence susceptibility to *coulure* (Galet 2000).

Poor weather conditions (low temperature, low light, overcast weather) act mainly through reducing photosynthetic activity, leading to a decrease in supply of carbohydrates (Galet 2000). Dense canopies associated with high vigour vines often exhibit *coulure* due to competition from strong vegetative sinks for available carbohydrates (Ribéreau-Gayon & Peynaud 1971). Boron deficiency can induce *coulure* due to its involvement in carbohydrate physiology (Bindra 1989) – where deficiency limits glycolysis and favours the pentose phosphate pathway, degrading hexoses (Pilbeam & Kirkby 1983). Deficiency of zinc lowers sucrose synthetase activity limiting the conversion of starch into sucrose (Shrotri *et al.* 1980), and can induce *coulure* (Bindra 1989).

Poor weather conditions affect fecundity. Development and viability of pollen (Carraro *et al.* 1981) and ovules (Ebadi *et al.* 1995a, Kassemeyer and Staudt 1982), pollen germination (Kobayashi *et al.* 1965), pollen tube penetration into the style and growth through the transmission tissue (Staudt & Kassemeyer 1984), and fertilisation (Bindra 1989) are all reduced by low temperatures. Rain or high humidity can disrupt pollination, by causing the pollen to clump together (Galet 2000) reducing pollination and fertilisation, with abscission due to lack of fruit set. Boron plays a key role in pollen tube development [probably through

its role in the cross-linking of the pectin network in cell walls (Marschner 1995)] and is involved in regulating auxin within plants, via synthesis and IAA-oxidase activity. Zinc plays a role in the synthesis of the auxin precursor tryptophane and may have a role in regulating auxin levels. Deficiencies in these micronutrients disrupt fecundity and lead to *coulure* (Bindra 1989). If a flower is not pollinated before the stigma becomes un-receptive, *coulure* will result when all the ovules with in an ovary degenerate (Kassemeyer and Staudt 1982).

The abscission zone of a grapevine floral pedicel is consistent with what is known about the abscission zone of most plants at a morphological and physiological level (Bessis *et al.* 2000). Ethylene stimulates abscission in grapes, though its effect is dependent on ethylene concentration and the receptivity of the tissues. Tissues of individual flowers appear to be receptive to ethylene for only a few days, after which the risk of abscission dissipates (Bessis & Fournioux 1992).

The abscisic acid/ethylene ratio plays a decisive role in the regulation of fruit abscission. Recent studies in grapevines report peaks of free abscisic acid at full bloom, and a coincidence between very high levels of abscisic acid and *coulure*. Interactions between abscisic acid and ethylene may regulate fruit abscission; preliminary results indicate that abscisic acid is able to stimulate ethylene biosynthesis (Hilt & Bessis 2000). Abscisic acid has been implicated in sensing the carbohydrate status in citrus and might be able to mediate ethylene synthesis via ACC (Gomez-Cadenzas *et al.* 2000). Polyamines are thought to have an antagonistic role with abscisic acid and ethylene, intervening in abscission (Broquedis *et al.* 1996).

Leading up to anthesis, polyamine levels in inflorescences of *Vitis vinifera* cultivars are high (Aziz 2003; Aziz *et al.* 2001) and following anthesis decrease rapidly and dramatically in all fractions (Aziz 2003; Aziz *et al.* 2001; Colin *et al.* 2002; Geny *et al.* 1997; Geny *et al.* 1999). Low levels of free polyamine in inflorescences correlate with abscission, suggesting an important function in reproductive organ development and or fertility (Aziz 2003; Aziz *et al.* 2001). Application of polyamines to inflorescences at peak capfall have been found to reduce *coulure* in the field by up to 50% in Cabernet Sauvignon, Carmenère, Malbec, Merlot, Chardonnay, Sauvignon blanc, Sémillon, and Ugni blanc (Broquedis *et al.* 1996). The balance of polyamine and ethylene synthesis could be the major determinant of abscission, since they compete for a common precursor, *S*-adenosylmethionine. Disrupting spermidine pathways

increases abscission, by possibly controlling sink nutrition, and results in decreased polyamine content (Aziz 2003).

2.2.9 Berry growth and development

As described earlier, a seeded grape berry shows a double sigmoid growth curve with three phases or stages [Refer Figure 2.7] (Cawthon & Morris 1982; Coombe 1976, 1989; Jackson 2000; Mullins *et al.* 1992; Nitsch *et al.* 1960; Staudt *et al.* 1986), the same as that described for the fruit of peach (Connars 1919).

- **Phase I** (6 weeks to 2 months in length)

  Initially (0-10 days) little increase in fresh or dry weight occurs but cell division occurs rapidly (Nitsch *et al.* 1960). The nucellus grows, little endosperm development occurs, but division of the zygote is not noted. Abscission of flowers occurs during this initial period. Plant growth regulators are generally at a low level, though auxin activity is high. The growth of the developing berries then changes, with a rapid increase in fresh and dry weight. Seed and flesh growth continue in parallel to the end of phase I (Mullins *et al.* 1992). During this time the seed reaches its full size, with rapid growth of firstly the nucellus and later of the endosperm and hardening of the seed coat (Nitsch *et al.* 1960). Associated with berry growth and seed development are high levels of growth stimulating hormones such as auxin, gibberellins, and cytokinins in the flesh of the berry, while abscisic acid concentrations rapidly decrease during the same period (Cawthon & Morris 1982; Zhang *et al.* 2003).

- **Phase II** (1 to 6 weeks in length)

  The second phase is very variable in duration and is associated with a clear cut reduction in fruit growth. By this stage the embryo is at full size; the weight of the seeds reaches a maximum at the end of this period. The seed is now capable of germination. The end of phase II is a transitional period physiologically; where the berry halts growth and begins ripening. Ripening begins at *véraison*, signalled by the accumulation of colour compounds and softening of the pericarp. The concentrations of auxin, gibberellin and cytokinins in the flesh gradually decline to very low levels during the middle of phase II. In seeds, auxin concentrations are maintained at high levels, while gibberellin and cytokinins also increase rapidly, reaching their peaks during phase II (Zhang *et al.* 2003). *Véraison*, at the end of

phase II, signals the beginning of a stage of physiological development of the berry, where formation of the berry has completed and ripening commences (Mullins *et al.* 1992).

- **Phase III** (5 to 8 weeks in length)

  The final phase of growth begins at *véraison*, with an enlargement of the berry and a rapid accumulation of dry matter until maturity. Ripening is associated with tissue softening, a decrease in acidity, the accumulation of sugars, the synthesis of anthocyanins (in red-skinned varieties), and the acquisition of aroma compounds. Further growth of the berry is controlled by an interaction of an osmotically driven gradient driving sugar importation into the berry against the elasticity and growth of the pericarp (Mullins *et al.* 1992). The concentration of plant growth substances remains low. Subsequent over-ripening if harvest is delayed is occasionally termed phase IV.

In general stenospermocarpic berries have growth curves similar to those of seeded berries but the curve usually tends to appear smoothed out. The majority of growth of Sultanina berries occurs from the inner pericarp, which also responds to gibberellin treatment (Sachs & Weaver 1968). Stenospermocarpic berries appear to have similar anatomy to seeded berries. The septum enlarges to fill the locules as the seeds abort and forms with the pericarp the flesh of the berry. Cell division occurs for 25 days after anthesis, ending about one week before the lag period. Cell enlargement occurs throughout the development of the berry. Comparisons of small and large berries suggest that cell volume may determine berry size (Pratt 1971). Comparing the cellular make of Sultana berries grown in a greenhouse, with larger field grown berries showed that the smaller berries had fewer cells in the pericarp (500,000 vs. 300,000 cells respectively). Berry growth of a parthenocarpic cultivar, Black Corinth, had no consistent periodicity of growth (Coombe 1960; Sachs & Weaver 1968).

After fertilisation, the fruit enters an intensive phase of physiological activity dictated in part by stimuli originated in the developing seeds (Basiouny & Himelrick 2001). A strong relationship exists between growth of the fleshy bulk of a berry and seed development, with the weight of the flesh increasing with increasing seed mass (Boselli 1995; Ebadi *et al.* 1996a; Müller-Thurgau 1898; Olmo 1946; Petrie *et al.* 2000; Pratt 1971). The rate of a berry's cell division, during phase I berry growth, is positively correlated with the growth rates of developing seeds (Coombe & McCarthy 2000), and is presumably mediated by plant growth regulators (Basiouny & Himelrick 2001), probably gibberellins.

Differences in berry size are associated with variation in the collective weight of seeds; being a summation of the number and type of seed (Figure 2.9). Ebadi *et al.* (1996a) made detailed descriptions of the development of ovules in Chardonnay berries of various types (Figure 2.10), clarifying the work of previous workers (i.e. Stout 1936). Normal or 'sinker' seeds show typical development and possess a normal testa, nucellus, endosperm and embryo. Hollow or 'floater' seeds have an embryo sac present at an early stage, the testa is fully developed at maturity but the nucellus, endosperm and proembyro have degenerated. The abnormal development of hollow seeds means they are smaller in size and mass. Trace seeds possess an embryo sac but it is often unfertilised, if fertilised degeneration occurs around the free nuclear stage, no zygote is present and testa development is incomplete; the degree of sizing and hardening of the testa and the timing of nucellus degeneration is variable. Ovules show no embryo sacs, no integument growth and possess a degenerate nucellus (Ebadi *et al.* 1996a).

The rachis of a grape bunch supports the bunch-stem tissues of the bunch framework. The individual berries are attached to this frame work via individual pedicels. The pedicel of the berry is similar to the stem in structure; consisting of an epidermis, cortex and more or less discrete vascular bundles with cambium and pith (Pratt 1971). The thickness of the pedicel, especially of the xylem, is related to the presence of seeds (Müller Thurgau 1898). The vascular bundles of the pedicel connect the vine to each berry. The berry is nourished by the phloem sap from the vascular bundles penetrating up the centre and around the periphery in a chicken-wire-like network and are bound at the top and bottom of the central bundle (Figure 2.11). The seeds are connected to the vascular system by branches from the base of the central bundle (Coombe & Iland 2004).

The ovary wall of the flower develops into the fruit wall or pericarp (Figure 2.11). The pericarp is subdivided into the outer exocarp (consisting of an epidermis and endodermis), the middle mesocarp (with an inner and outer layers), and the inner endocarp (consisting of an endodermis and epidermis) (Figure 2.12).

The berry exocarp or 'skin' has three layers: the outer cuticle, the outer epidermal layer and a six cell deep hypodermal layer (Coombe & Iland 2004; Mullins *et al.* 1992). The skin protects the berry; the hypodermal cells are rich in the compounds, responsible for pigmentation, flavour and aroma of the fruit (Considine & Knox 1981). The bloom of the berry is composed

of over lapping platelets of cuticular or epicuticular wax, which help prevent the loss of water from the fruit (Possingham *et al.* 1967).

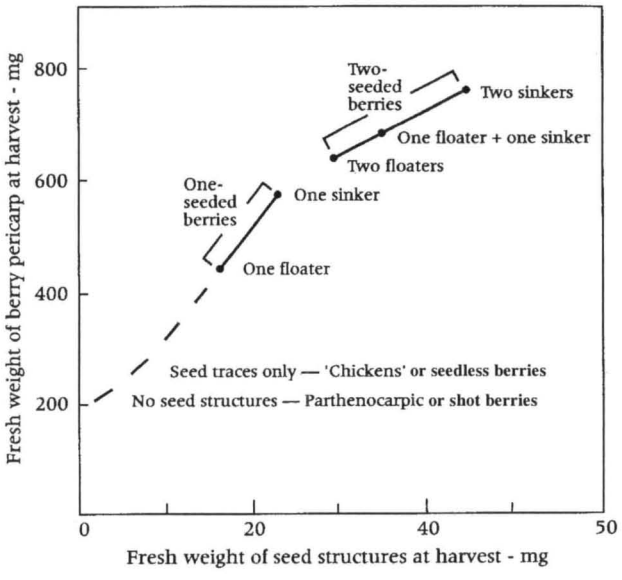


Figure 2.9 Weight of Chardonnay pericarp at harvest against total fresh weight of the combined seed structures in five categories according to the number and state of the seeds. [Adapted by Coombe and Iland 2004; From Ebadi *et al.* 1996a]

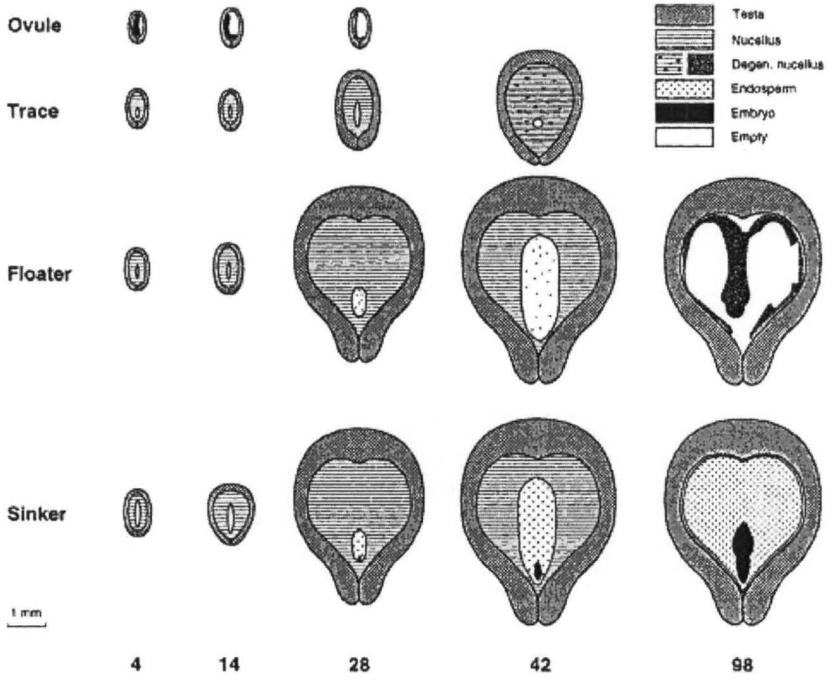


Figure 2.10 Diagrams representing the development of various seed types from Chardonnay. The numbers represent the days after flowering (Ebadi *et al.* 1996a).

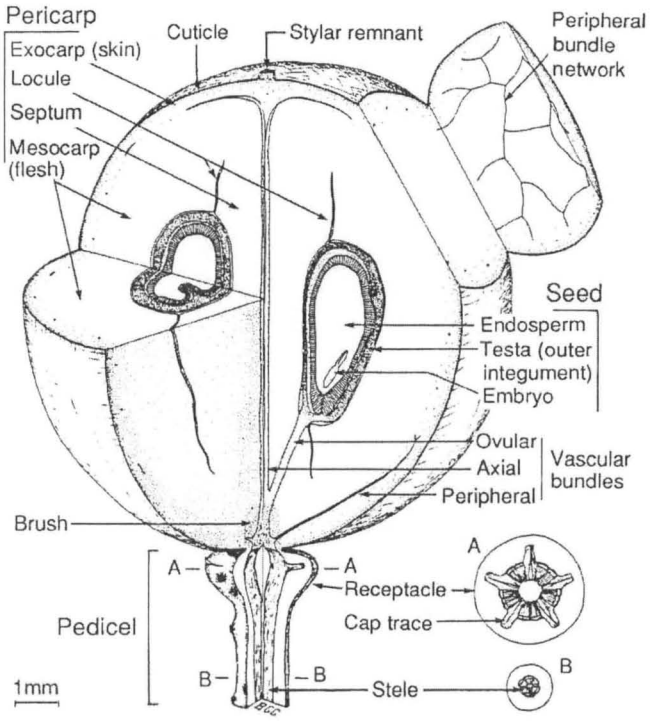


Figure 2.11 Diagrammatic representation of a grape berry. [From Coombe 1987]

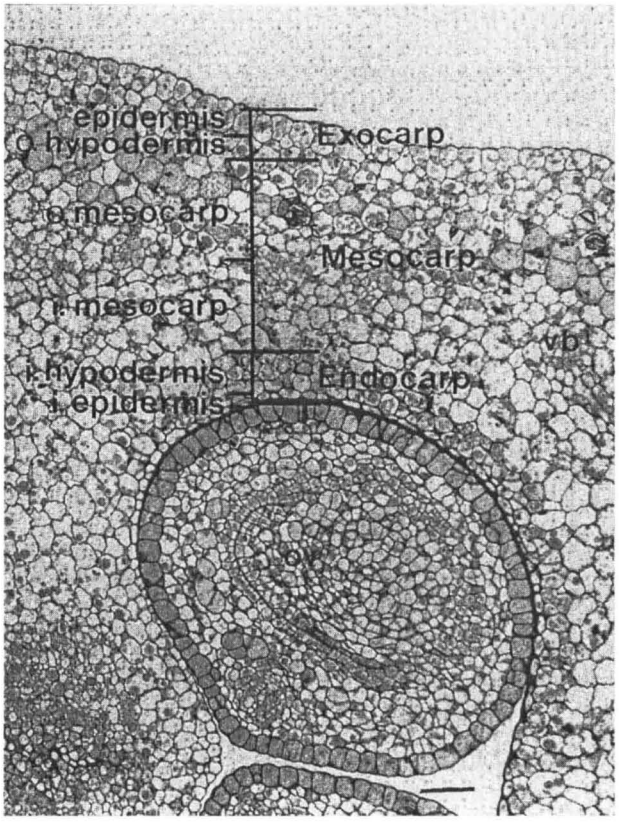


Figure 2.12 Cross section of the grape pericarp at anthesis. Bar = 20 μm. [From Hardie *et al.* 1996]

The epidermis, of the exocarp, consists of a single layer of thin-walled cells in the pre-anthesis period, arranged in longitudinal files, with each cell elongated in long axis of the pistil; during anthesis these cells divide anticlinally and enlarge. Mature berry cells are tabular and wider tangentially than radially (6.5-10μm in radial diameter) (Alleweldt *et al.* 1981). Cells remain thin during the early stages of ripening. The outer tangential walls of the epidermis are thicker than those of the interior cells. Deposits of suberin are thought to be present at 52 days after anthesis, between the epidermis and cuticle (Hardie *et al.* 1996).

The outer hypodermis is anatomically and physiologically distinct from the interior tissues of the berry (Hardie *et al.* 1996). This layer becomes distinct when the fruit is about 3.25mm in diameter, and completes its differentiation in 2 weeks, containing between 1 to 17 cell layers [107-246μm], depending on cultivar (Alleweldt *et al.* 1981). These cells are tabular, wider tangentially than radially with thick primary non lignified walls (Hardie *et al.* 1996; Pratt 1971). At anthesis the hypodermis is two cell layers thick, formed by one round of periclinal cell divisions within cells of the original layer. Rounds of asynchronous periclinal divisions occur over the next 10 days, establishing 6-7 cell layers. Anticlinal divisions accompany this growth up to day 27, keeping tangential width under control, though their tangential width is several-fold greater than radial diameter, which in turn is greater at the berry equator than poles. Cell size and wall thickness gradually increase until the berry is mature and there are no intercellular spaces (Pratt 1971). In the parthenocarpic cultivar, Zante Currant, no cell division occurs in the exocarp for up to 38 days after flowering (Hardie *et al.* 1996). During phase I berry growth, the cells of the hypodermis differentiate into collenchyma, typically supporting soft growing tissues (Esau 1965). With the onset of berry growth in phase III, the outer hypodermal cells swell, which is correlated with a marked increase in fruit plasticity (Considine and Knox 1979b).

The cuticle is thought to be built up by accretion from within of monomeric lipids that are oxidised to form a cutin polymer. It comprises an outer layer, the cuticular lamellae, an inner layer, the pectic lamella, and an interposed layer, a reticulate region comprising cutin and wax transversed by cellulose fibrils (Hardie *et al.* 1996). The grape berry cuticle is a wax-coated, translucent, acellular, multi-layered secretion which ranges in thickness from 1.6-3.8μm (Alleweldt *et al.* 1981) and is approximately 65% cutin. The cuticle begins to form about three weeks before anthesis and within two weeks covers the entire surface of the ovary with tightly-appressed ridges. Cuticular thickening in the berry commences at about day 16 after

anthesis and by day 26, three layers can be distinguished (Considine & Knox 1979b). By *véraison*, the cuticle is thickened relative to earlier stages, and the thickness of the outer layer begins to decrease, though the total thickness remains approximately constant (Considine & Knox 1979b). At maturity of the berry, the cuticle is thin, continuous and relatively smooth, containing only scattered remnants of the cuticular ridges. Epicuticular wax, the 'bloom', appears first on the surface of the cuticle at about anthesis, initially as small, individual upright wax platelets that occur both between and on the cuticular ridges. The platelets increase in size and number, completely obscuring the cuticular ridges 21 days after anthesis. They reach their highest density in the Stage II; then the distance between the platelets becomes greater probably because their number does not increase while the berry surface increases (Hardie *et al.* 1996).

The mesocarp, commonly referred to as the flesh or pulp of the berry, consists of 25-30 layers of highly vacuolated parenchyma cells that lie between the hypodermal layers of the exocarp and the layers of the endocarp (Figure 2.11) (Hardie *et al.* 1996). Tissues exterior to the peripheral vascular bundles of the pericarp are the outer mesocarp, those inside them are the inner mesocarp (Considine & Knox 1979b). At maturity, cells of the inner mesocarp make up about 64% of a berry's final volume regardless of berry size, and parallel the overall changes in berry volume (Harris *et al.* 1968). The cells of the mesocarp are more or less rounded and tend to be larger and more radially elongated in the middle of the wall than toward the hypodermis or inner epidermis, except for smaller cells around bundles (Pratt 1971). Intercellular spaces are present (Pratt 1971). The septum grows to fill any locule where the seeds have aborted. The cells are irregular in size and shape, and those of the inner epidermis are tangentially elongated (Pratt 1971). Cells of the inner mesocarp cease dividing 3-4 weeks after anthesis (Harris *et al.* 1968).

The endocarp is the innermost tissue of the pericarp, surrounding the locules. At anthesis the endocarp consists of 2-3 layers of druse-containing cells forming the inner hypodermis and the inner epidermis (Considine & Knox 1979a). The inner epidermis comprises a single layer of cells which have thickened walls and are smaller than those of the mesocarp. They are elongated tangentially (Hardie *et al.* 1996). The druses of the cells of the inner hypodermis are formed from calcium oxalate. The cells are free of polyphenols (Hardie *et al.* 1996).

A berry's final shape, size, and texture are strongly influenced by the number, shape and size of cells and the cell wall properties of the exocarp and mesocarp cells (Coombe & Iland 2004); genetic and environmental factors could influence berry size by affecting either of these quantities. The shape of any organ is determined normally by the relative growth rates of its cells in three planes at right angles to one another. However, the shape of the component cells is determined almost exclusively by the pattern and frequency of the accompanying cell divisions. Thus, if a cell layer ceases division earlier than another layer and both are experiencing the same relative growth rate, cell size will increase in the layer where partitioning stops first (Hardie *et al.* 1996).

During the first two weeks after flowering, a three-fold increase in radial cell number of the inner and outer mesocarp and a seven fold increase in the hypodermis occur. Expansion of the pericarp in the subsequent four weeks is predominated by cell enlargement in the inner mesocarp, positioning vascular bundles towards the skin (Figure 2.13). Cell division is active in the pericarp 5 to 10 days pre-anthesis, subsides during anthesis and then resumes (Coombe 1960). Post anthesis cell division in the mesocarp occurs for approximately two weeks, is especially active during the first week (Coombe & Iland 2004) and is generally complete within three weeks (Mullins *et al.* 1992). The plane of cell division is largely or wholly periclinal in mesocarp cells (Pratt 1971). Cell enlargement is continuous during the development of the berry except during the period of maturation. After division ceases, cell enlargement is mainly responsible for the increase in berry size (Pratt 1971). It is assumed that berry size in parthenocarpic shot berries is determined by the number of cells in the pericarp at anthesis, since there is no cell division after flowering and in large-seeded berries there is much.

Cell numbers across the pericarp and cell volume increase linearly during early berry development, while beyond the lag phase there is only a change in cell volume (Harris *et al.* 1968). The total number of pericarp cells attains its maximum about one week before the onset of the phase II berry growth. Working with Sultana berries grown in the field, Harris *et al.* (1968) found approximately 17-18 cell divisions occur within the flower pre-anthesis resulting in approximately 0.20 to 0.38 million cells, and between 1-2.5 cell divisions occur post-anthesis (40 days later) resulting in between 0.55 to 0.6 million cells per berry.

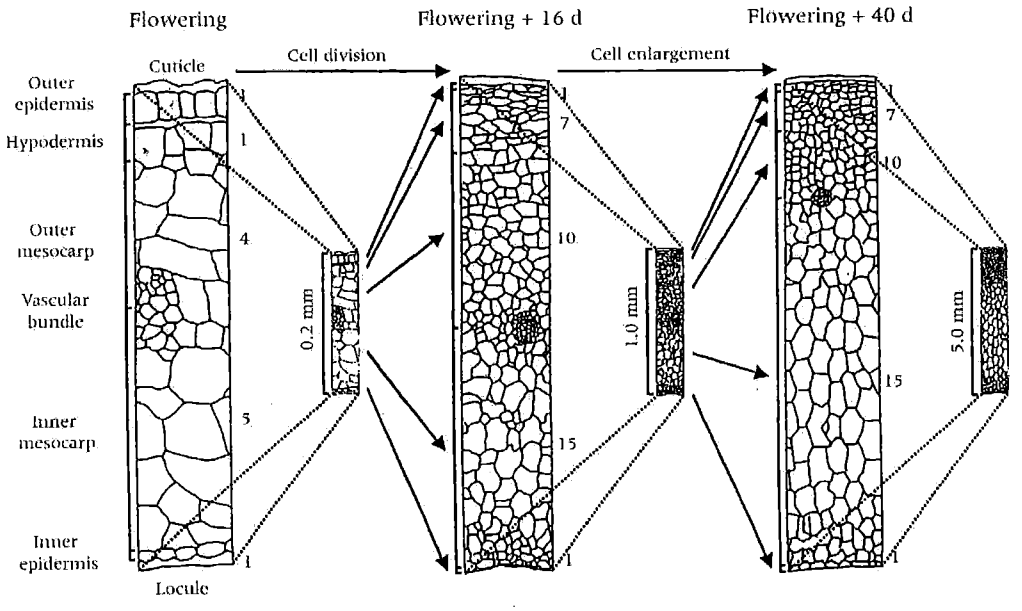


Figure 2.13 Median cross section of the pericarp of Muscat Gordo Blanco berries at flowering, 16 and 40 days later showing changes in cell size and number; the number of cell layers in each tissue type are indicated. [From: Coombe and Iland 2004].

Pre-anthesis events and the extent of flower development are likely to have an influence on cell number of mature berries; comparisons of Sultana berries grown in the field and within a glasshouse, exhibit very different cellular makeup, with glasshouse grown flowers and berries having half the number of cells that field grown flowers do (Harris *et al.* 1968). Ojeda *et al.* (2001) have suggested that the reduced berry size following post-anthesis water deficit is due more to inhibited cell wall extensibility than lessened cell division. Further, Colin *et al.* (2002) observed that the cell walls of 'Chicken' berries have high amounts of wall-bound diaminopropane; they suggest that the presence of this polyamine may inhibit cell wall development leading to the small size of these berries (Coombe & Iland 2004).

The following processes are required for growth to occur: loosening of cell walls, uptake of water, synthesis of new wall materials, maintenance of turgor, and regulation of microtubule orientation. Water uptake for turgor provides the force for cell expansion, while chemical modification of cell walls and the formation of new cell wall material prevent the expanding cells from bursting. The expansive force of turgor and loosening of cell walls is in balance with cell wall synthesis, which is controlled by the strict regulation of plant growth regulators. Application of exogenous hormones can shift the balance to turgor, creating a burst in growth.

Auxin induced growth is accompanied by an increase in water uptake, but osmotic pressures remain similar. Auxins do not affect water uptake directly, neither increasing solute uptake by cells nor water conductance. Auxins cause wall loosening which in turn reduces turgor and increase water potential difference. The capacity for growth correlates with extensibility in young tissues. Auxins loosen cell walls, by acidification of the walls, enhancing plastic extensibility in responsive regions. They may also modify the hemicellulose networks. Gibberellins soften the cell walls. They increase the rate of elongation and extend the elongation zone. Brassinosteroids are thought to acidify the cell wall increasing extensibility, but can cause an excessive build up of turgor (Srivastava 2001).

Chapter III

Manipulating phenology: Yield component response

3.1 Introduction

Bud break of grapevines is typically considered from the point of view of ensuring uniformity (Intrieri & Poni 1998) and reducing frost susceptibility (Dami *et al.* 2000; Howell & Wolpert 1978). Various techniques may be employed to modify bud break; these include the application of plant growth regulators (Ezzili & Bejaoui 2000), hydrogen cyanamide (Shulman *et al.* 1983), alginates and oils (Dami *et al.* 2000), the use of evaporative cooling (Nir *et al.* 1988), or late winter pruning (Antcliff *et al.* 1957; Loomis 1939). It has been observed that the use of delayed winter spur pruning on grapevines to delay bud break can increase yield (Barnes 1958; Bouard 1967b; Coombe 1964; Friend *et al.* 2000; Malan 1961; Whittles 1986), a response that appears to occur regardless of cultivar, region or season. The literature has only reported on the magnitude of the changes in yield, with only Coombe (1964) suggesting that yield increases arose from improved fruit set, but not specifying details about yield components. It has been speculated that inhibiting the development of buds delays anthesis to a period of time where climatic conditions enhance fertilisation of flowers (Friend *et al.* 2000).

This chapter examines the changes in berry development and vine yield with changes in the timing of vine phenological development. The working hypothesis is that by delaying bud break, shoot growth and flowering will occur under warmer environmental conditions, closer to their optimum temperatures, enhancing the fertilisation of flowers, resulting in larger berries and improved vine yield.

Note: The contribution of Dr. Cecil Stushnoff and Mr. Gilbert Wells towards the experimentation described in this chapter is gratefully acknowledged.

3.2 Experimentation

3.2.1 Experimental aims

In order to test the hypothesis that delaying bud break will enhance the fertilisation of flowers, resulting in larger berries and improved vine yield, two experiments were undertaken:

- Experiment One, carried out in 2001, aimed to establish whether delaying the date of bud break results in a delay of other vine phenological stages, as this information had not been collected in Experiment Two.
- Experiment Two, carried out in 2000, aimed to examine the yield component response to two methods of delaying bud break: sodium alginate gel encapsulation and delayed winter spur pruning, and to relate changes in yield components to environmental conditions

3.2.2 Site and vines

The vines used for experimentation in both experiments were orientated North:South and grew in the Lincoln University experimental vineyard, located in the province of Canterbury, New Zealand (43°39' S, 172°28' E). The experiment in 2001 used six-year-old vines, grafted (Riparia Gloire), field grown, *Vitis vinifera* cv. Merlot, clone unknown. These vines were pruned to 24 buds on 12 spurs; shoots were trained vertically, and industry standard canopy manipulation and disease control methods were undertaken. Different vines were used in 2000, which were ten-year-old, ungrafted, field grown, *Vitis vinifera* cv. Chardonnay clone 'Mendoza', which typically exhibits the physiological disorder *millerandage* (Hen and Chicken) in Canterbury. The occurrence of *millerandage* in 'Mendoza' Chardonnay in New Zealand is associated with Grapevine leaf roll virus, type one (GLRaV-1) (Cohen 2000). These vines were pruned to 32 buds on 16 spurs; shoots were trained vertically, and industry standard canopy manipulation and disease control methods were undertaken.

3.2.3 Experimental design and treatment application

Experiment One (2001) was laid out in a factorial design with six-vine replicates blocked six times along a single row (n = 36 vines), to account for variation between vines along the row. Two alternative methods of delaying bud break were employed: delayed winter spur pruning and sodium alginate gel encapsulation. The treatments consisted of two dates when pruning

was undertaken, each with either a control or one of two alginate gel treatments, applied to each vine (Figure 3.1).

Experiment Two (2000) was also laid out in a factorial design with nine, three-vine replicates, blocked across three rows (n = 27 vines). Again both delayed winter spur pruning and sodium alginate gel encapsulation were used to delay bud break. Vines in each replicate were spur-pruned on one of three dates. A control and three alginate gel treatments were randomly applied across the cordon of each vine (Figure3.2).

In Experiment One, spur pruning was carried out on two separate occasions: 16 August 2001 (Early), and 25 September 2001 (Late). A sodium alginate gel was applied on two occasions to pre- and post-pruned spurs, depending on treatment (Figure 3.1). Buds were either not treated as a control, or treated with a single application on the 16 August 2001, or on the 25 September 2001.

Individual vines were used as complete blocks to allow comparisons of any interaction between delayed winter spur pruning and alginate gel encapsulation. Though the mode of action of these treatments is unknown, it was anticipated that an additive effect would be found. Though the development of mature shoots on a vine is not totally independent of each other, Howell and Wolpert (1979) found that bud break and early shoot development were independent of each other along canes.

In Experiment Two, spur pruning was carried out on three separate occasions: 28 July 2000 (Early) [typical time of pruning], 18 August 2000 (Mid) [late winter pruning], and 8 September 2000 (Late) [very late winter pruning, at bud-break]. Sodium alginate gel was applied on three occasions to pre- and post-pruned spurs, depending on treatment; the spurs were separated into groups along the cordon (Figure 3.2). Groups of four spurs were either, not treated as a control, treated with a single application on the 28 July 2000 or the 18 August 2000, or treated with multiple applications on the 28 July 2000, 18 August 2000, and 8 September 2000. The gel treatments were randomly applied across the cordons.

Sodium alginate is reported to delay bud break in grapevines, though its mode of action is unknown (Dami *et al.* 1996). The Sodium alginate was applied, as per Dami *et al.* (1996), as a

4% sodium alginate (anhydro-β-D-mannuronic acid sodium salt; Sigma, St Louis, MO, USA) gel, dissolved in a 0.75M solution of sucrose ($C_{12}H_{22}O_{11}$ New Zealand Sugar Company, Auckland, New Zealand). The gel was made up in a food processor several days beforehand to allow the alginate to hydrate completely. To aid application of the alginate gel, spurs were sprayed by hand with a 0.2 M calcium chloride ($CaCl_2.2H_2O$ BDH Laboratory Supplies, Poole, England) solution. Using a 30mm paintbrush, alginate gel was liberally applied to these spurs, ensuring complete coverage of the buds. A final application of 0.2M calcium chloride was sprayed over the alginate gel to set the gel (Figure 3.3). Calcium chloride provides positively charged calcium ions, which form a solid matrix with the alginate gel. Over the next two days the gel dried forming a thin but solid casing around the buds and spurs. During rainfall events the alginate gel would re-hydrate, with some loss of gel due to gravity.

Alginate treatment	Application dates 16 Aug	25 Sept
Control	-	-
Early	✓	-
Late	-	✓

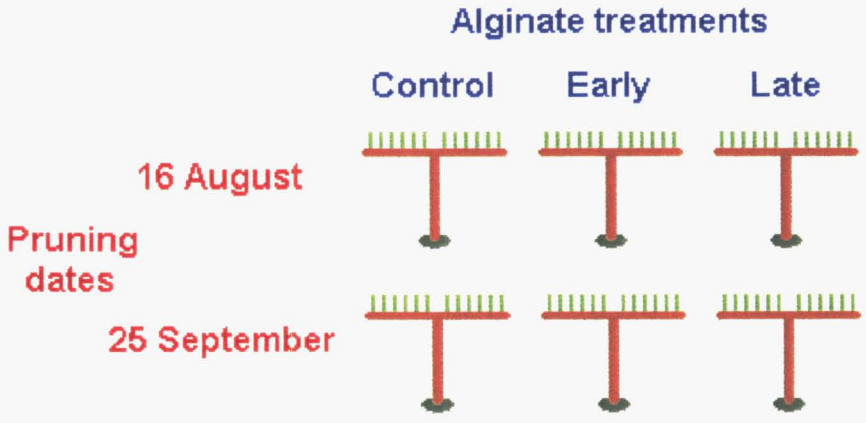


Figure 3.1 Treatment structure used in Experiment One (2001).

Alginate treatment	Application dates 28 July	18 Aug	8 Sept
A – None	-	-	-
B – Early	✓	-	-
C – Mid	-	✓	-
D – Repeat	✓	✓	✓

Figure 3.2 Treatment structure used in Experiment Two (2000).

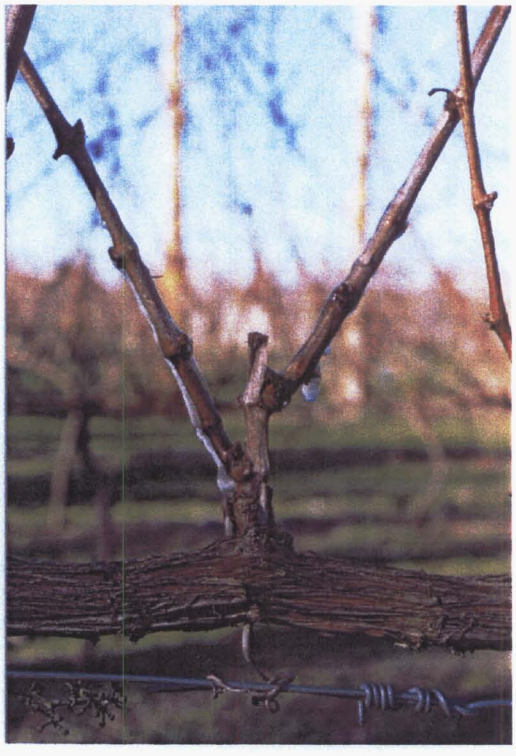


Figure 3.3 Recently applied sodium alginate, set with calcium chloride, on an un-pruned spur.

3.2.4 Phenology assessment

Assessments of bud and shoot development were made using the Modified E-L system (Coombe 1995) [Appendix A] in both experiments. Assessments of bud development were made on 2 October, 15 October, and 1 November 2001 in Experiment One. The apical and basal buds of six randomly selected spurs were tagged and rated. In Experiment Two, a snapshot of bud development was collected on 26 September 2000. The apical and basal buds of two randomly selected spurs, from within each alginate treatment, were tagged and rated.

For Experiment One, at approximately three-day intervals, from 11 December 2001, eight assessments of the percentage of flowers that had under gone capfall were made on the basal inflorescences of the shoots from tagged spurs. Assessments were made visually using the scale in Table 3.1. At approximately five-day intervals from 5 March 2002, five assessments of the percentage of berries that had under gone *véraison* were made on the basal inflorescences of shoots from tagged spurs. Assessments were made visually using the scale in Table 3.1, with *véraison* defined as the beginning of colour accumulation.

Table 3.1 Rating scale used for assessing the progression of capfall and *véraison*.

	Rating scale								
	1	2	3	4	5	6	7	8	9
Percent flowers open / berries at *véraison*	0	5	15	30	50	70	85	95	100

3.2.5 Frost

In Experiment Two on 26 September 2000 a radiation frost occurred, where air temperatures dropped to –1.5°C at 1.2 m high, causing damage to a proportion of the developing buds (Figure 3.4a, b). An assessment of primary bud and shoot death was made on 5 October 2000 on tagged buds. Subsequent development of secondary shoots (Figure 3.4c), from nodes on the tagged spurs, was noted on 1 November 2000.

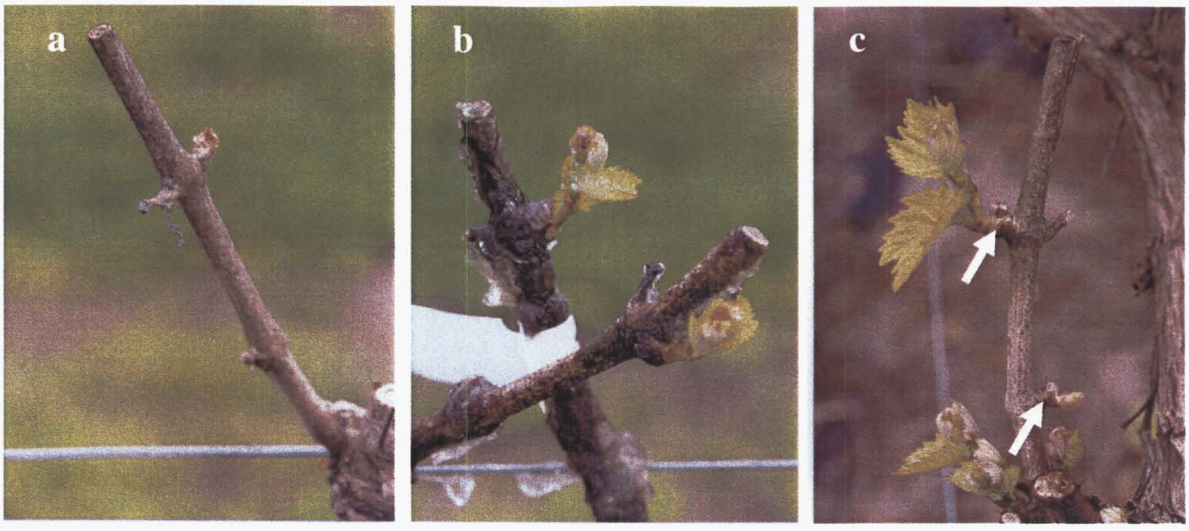


Figure 3.4 Frost affected buds. a) A frost killed primary bud, from a spur treated with 'Early' pruning and without an alginate application, b) Buds surviving the spring freeze event from spurs treated with 'Late' pruning and a 'Late' alginate application, c) A frost damaged spur showing development of secondary shoots from where primary shoots (arrows) were killed by the spring freeze event.

3.2.6 Assessment of yield components in Experiment Two

Harvest date of the experiment was on 10 April 2001, when the fruit could be considered to be at a minimum quality for winemaking (17.3°Brix sugar concentration, 12.7g/L titratable acidity, 3.26 pH).

The apical and basal bunches from the shoots of tagged spurs were collected, counted, weighed, and dissected to assess bunch yield-components (A record was made of whether the bunch came from a frost damaged induced secondary shoot). Bunch yield-components include the total number of berries per bunch, the number of seeded, seedless and shot berries per bunch (Figure 3.5), the calculated average weight of all berries and the calculated average weight of seeded, seedless and shot berries. Bunch yield-components were counted and weighed to an accuracy of ±0.01g. The remaining bunches were counted and weighed according to treatments for vine yield-component assessment. Due to the experimental design, yield and bunch number assessments are values of the four groups of alginate treated spurs, along the cordon of a vine.

If any of the seeded or seedless berries were shrivelled they were counted separately. Shrivelled berries were included in berry counts, and excluded from calculations of average

berry weights. Seeded and seedless berries were identified by looking through the berry with a light behind it. If present the shadow of any seeds could be clearly seen.

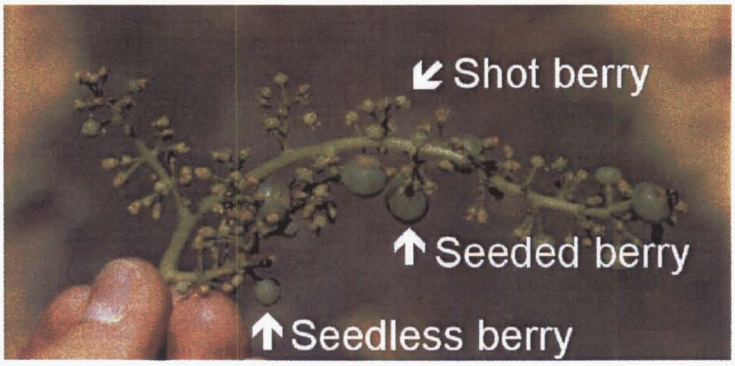


Figure 3.5 An immature bunch exhibiting poor fruit set showing examples of seeded, seedless and shot berries (Photo G. Creasy).

3.2.7 Growing degree-days

Growing degree-days were calculated using a base temperature of 10°C and daily maximum and minimum temperatures. Temperature data were collected from the Lincoln weather station, H32574 (43°34'S 172°43E), part of the New Zealand Meteorological Service network. Air temperature was measured using thermometers in exposed screens at 1.3m height.

3.2.8 Statistical analyses

Statistical analyses using ANOVA and regression were completed using Genstat 5 (Release 4.1; Lawes Agricultural Trust. Rothamsted, England). Coefficients of determination were calculated manually.

For Experiment Two, the data analysis of phenology was completed using ANOVA and included an input matrix, testing for linear and quadratic relationships within treatments [Refer Appendix B]. Frost affected shoots (identified by their brown colouration as in Figure 3.4a) were excluded from analyses of bud development in Experiment Two. Data analysis of vine and bunch yield-components was completed using ANOVA, with frost damage incidence included as a covariate for vine yield-components. Accumulated growing degree-days were summed from 7 Jul 2000 (3 weeks before start of experiment) as a starting date,

during bud ecodormancy, until an estimated date of fifty percent bud break, estimated from means of bud phenological data.

3.3 Results

3.3.1 Phenology

In Experiment One alginate gel encapsulation had no effect on bud phenological development (Data not presented). Delayed winter pruning delayed bud phenological development, with the 'Late' pruning treatment exhibiting a lower stage of bud development (Figure 3.6a). The delay in phenological development, due to of the time of winter spur pruning, was still evident at flowering (Figure 3.6a) and *véraison* (Figure 3.6b).

By comparing the point in time at which 50% of the shoots are at the stage of bud break [modified E-L stage 4, Appendix A], for the two pruning dates, an accurate assessment of the extent to which bud break has been delayed can be made. Postponing the date of winter spur pruning by 40 days resulted in a six-day delay in bud development (Figure 3.7a). The actual proportion of 55% was used to estimate the delay in bud break, due to the extent to which the buds had developed by the time of the first assessment.

This delay in phenological development was still evident at flowering, with a three-day delay in the progression of capfall (Figure 3.7b), when comparing the time at which half of the inflorescences had reached 50% capfall. By *véraison* the delay in the progression of *véraison* was four-days (Figure 3.7c), when comparing the time at which half of the bunches had undergone 50% berry-colour development. In 2000, phenological development of buds was delayed by both delayed winter spur pruning (~6 days), and alginate gel application (~2 days).

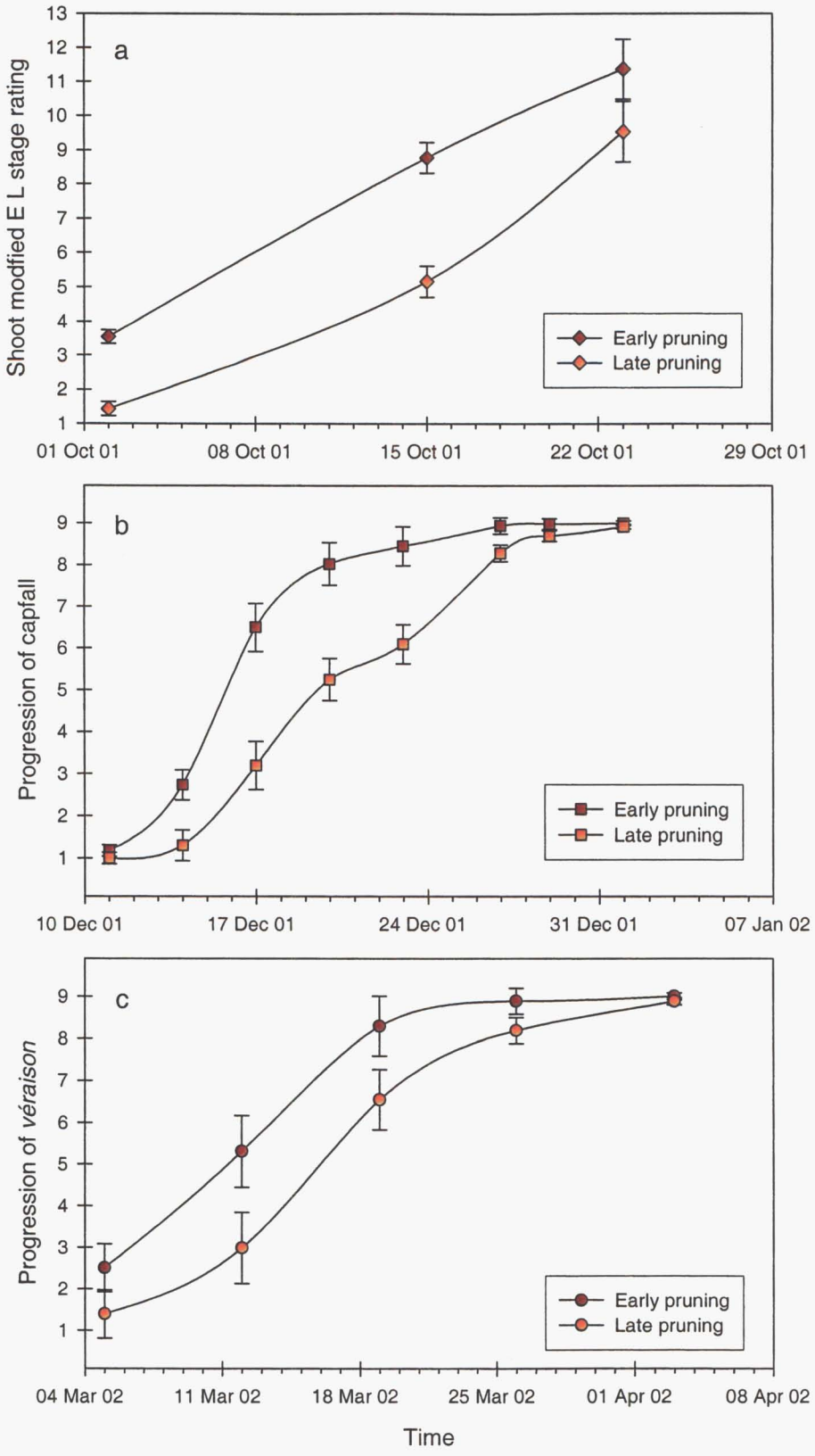


Figure 3.6 The effect of delayed pruning on shoot development (a), flowering (b) and *véraison* (c) in Experiment One. Error bars indicate LSD (5%).

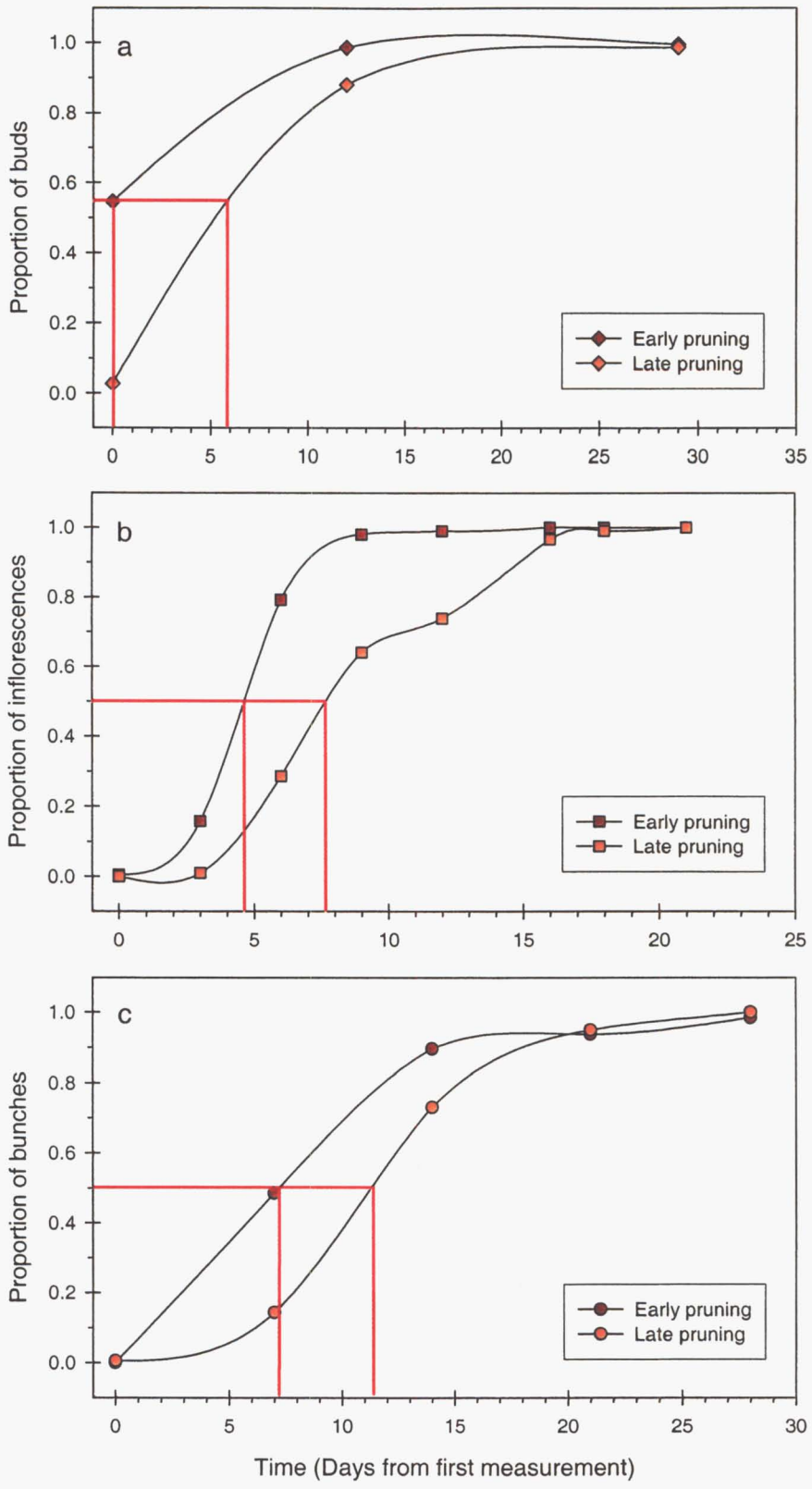


Figure 3.7 The influence of delayed pruning on the proportion of nodes at bud break (a), inflorescences at 50% capfall (b), and bunches at 50% *véraison* (c) in Experiment One, (data calculated from Figure 3.6, n = 6 vines) .

Treatments showed an interaction (P = 0.020), with the early gel treatment showing an improved response when combined with the late pruning treatment (Table 3.2). Bud development showed a linear response to delayed winter spur pruning (P = < 0.001). Application of the alginate gel caused a delay in phenological development of buds (P = 0.003), with there being no difference between single and multiple applications (P = 0.292), while the mid application was more effective than the early application in delaying bud development (P = 0.010).

Table 3.2 Phenological development (Modified E-L scale) of Chardonnay buds and shoots in Experiment Two on 26 September 2000.

	Bud phenological development			
Sodium alginate gel treatment	Control	Early	Mid	Repeat
Time of pruning				
Early (August)	3.2	3.9	2.8	3.2
Mid (September)	2.8	2.7	2.7	2.3
Late (October)	2.6	2.2	2.1	1.8
			LSD (at 95%) = 0.3	
Except when comparing means at the same level of pruning = 0.5				

3.3.2 Vine yield-components

Delaying bud break with alginate gel encapsulation had no effect on vine yield or yield components [Refer Appendix B]. The use of delayed winter spur pruning had no effect on yield or bunch number (Table 3.3), though an increase in yield (P = 0.091) is evident with an increasing delay in the time of pruning. Average bunch weight is greater between the mid and late pruning dates. It is of note that despite having the same number of bunches and an increasing average bunch weight as winter pruning is delayed, no significant increase in yield occurs. The occurrence of a covariate effect on average bunch weight (Table 3.4) suggests that the September frost may be influencing vine yield [Refer Appendix C].

Table 3.3 Vine yield-component responses to delayed winter spur pruning in Experiment Two; co-variate is of presence of frost damage to primary buds.

	Early	Mid	Late	*P* value	L.S.D.	Co-variate *P* value
Yield (kg)	0.60	0.70	0.81	0.091	0.177	0.371
Bunch number	16	17	17	0.778	0.778	0.798
Average bunch weight (g)	35.3	38.2	49.0	<0.001	6.64	0.048

3.3.3 Bunch yield-components

Delaying bud break with alginate gel encapsulation had no effect on bunch yield-components [Refer Appendix B]. Bunch weight, sampled from tagged spurs, increased between the 'Mid' and 'Late' pruning treatments (Table 3.5), confirming the pruning treatment effect on the calculated average cluster weight (Table 3.4).

The increase in bunch weight appears to have resulted from an increase in the calculated average berry weight (Table 3.5), with average berry weight increasing between the 'Mid' and 'Late' pruning treatments. No treatment effect was evident on the calculated average weight of seeded, seedless, or shot berries, suggesting that a change in the population of berries lead to the increase in the calculated average berry weight.

Delayed winter pruning had no effect on the total number of berries within a bunch (Table 3.5). However, the number of seeded berries within a bunch increased between the 'Early' and 'Mid' pruning treatment. The number of seedless and shot berries within bunches was not affected by the date of pruning; but a non-significant trend to fewer seedless berries was evident. The importance of seeded berries in determining total berry number is shown in Table 3.6 with multiple linear regression revealing that the number of seeded berries within a bunch accounted for most (87%) of the variation in bunch weight. Including the number of seedless berries within bunches accounted for little more of the variation, while the number of shot berries per bunch accounted for none.

A change in the proportion of the berry types within bunches supports the notion that the increase in calculated average berry weight is a result of changes in the berry population of bunches. The proportion of seeded berries is greater in the 'Late' pruning treatment than the 'Early' (Table 3.6). Corresponding with this is a decrease in the proportion of seedless berries within bunches, which tends towards significance. An increase and decrease in the number of

seeded and seedless berries, respectively, would account for the increase in calculated average berry weight.

Table 3.4 Bunch yield-component responses to delayed winter spur pruning in Experiment Two.

	Early	Mid	Late	*P* value	L.S.D.
Bunch weight (g)	28.93	32.96	47.51	0.001	8.706
Total berry number	51	54	61	0.265	11.88
Seeded berry number	26	37	42	0.001	6.61
Seedless berry number	19	17	13	0.310	7.73
Shot berry number	6	5	6	0.443	1.73
Average berry weight (g)	0.85	0.87	0.96	0.047	0.086
Average seeded berry weight (g)	1.02	1.03	1.08	0.318	0.087
Average seedless berry weight (g)	0.19	0.19	0.19	0.660	0.035
Average shot berry weight (g)	0.010	0.010	0.017	0.264	0.009

Table 3.5 Simple and multiple (+) linear regression coefficients of determination (R^2) and probability values from relationships between bunch weight and berry types in Experiment Two.

Variate	R^2	*P* value
Bunch weight:		
Number of seeded berries	0.87	< 0.001
Number of seeded + seedless berries	0.88	< 0.001
Number of seeded + seedless + shot berries	0.88	< 0.001

Table 3.6 The response of the proportion of berry classes within bunches to delayed winter spur pruning in Experiment Two.

	Early	Mid	Late	*P* value	L.S.D.
Proportion seeded berries	0.58	0.62	0.68	0.045	0.072
Proportion seedless berries	0.31	0.29	0.22	0.056	0.074
Proportion shot berries	0.11	0.09	0.10	0.347	0.037

Table 3.7 Simple and multiple (+) linear regression coefficients of determination (R^2) and probability values from relationships between bunch weight and berry characteristics in Experiment Two.

Variate	R^2	*P* value
Bunch weight:		
Total berry number	0.87	< 0.001
Total berry number + average berry weight	0.88	< 0.001

3.3.4 The relationship between yield and environment

Correlation analysis of the delay in bud break against the growing degree-days that had accumulated since the beginning of spring, revealed a strong linear correlation (Figure 3.8). Accumulated growing degree-days have been used as an index of the warmth of the environment in which shoots develop. The relationship suggests that by delaying the onset of bud break shoots develop under warmer climatic conditions.

The climatic conditions in which shoots develop relate to yield. There is a tendency for treatments, whose shoots develop under warmer temperature conditions, to yield higher (Figure 3.9), with a significant exponential relationship ($P = < 0.001$) and growing degree days accounting for a fair portion of the variation in vine yield. Bunch weight also shows a similar relationship to the climatic conditions in which shoots develop, with increased average bunch weight for treatments developing in warmer environmental conditions (Figure 3.10).

Bunch weight is driven by the number of seeded berries within bunches (Figure 3.11) and not the calculated average berry weight (Figure 3.12). However, the use of a boundary line in Figure 3.12 shows an upper limit regarding average berry weight and bunch weight. Regardless, the extent of the scatter in this correlation suggests that other factors are also determining bunch weight. Multiple linear regression revealed that the total number of berries within a bunch accounted for most (87%) of the variation in bunch weight. Including the average berry weight of berries within bunches accounted for little more of the variation (Table 3.7).

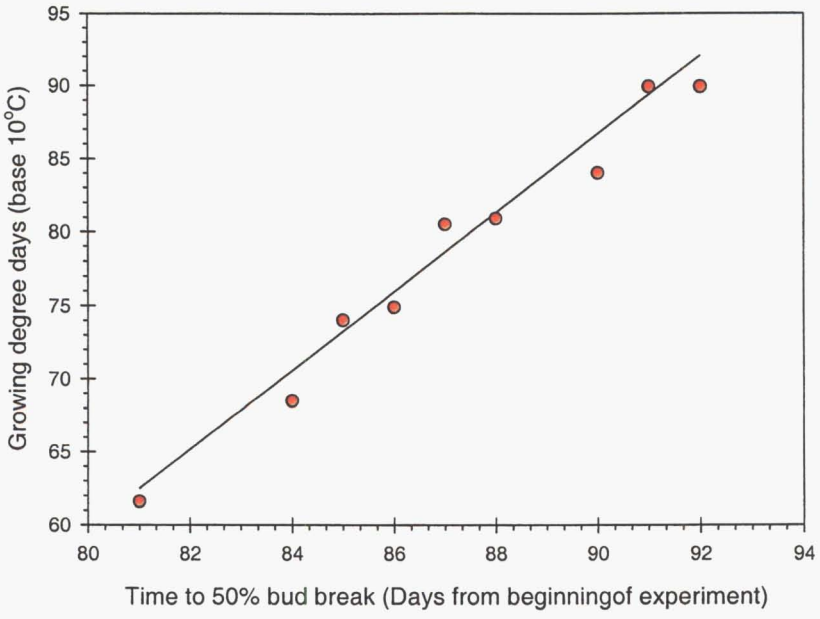


Figure 3.8 The correlation of the warmth of the environment with the timing of fifty percent bud break; y=-155.1+2.686x, r^2=0.978, P = < 0.001 (• Replicate means).

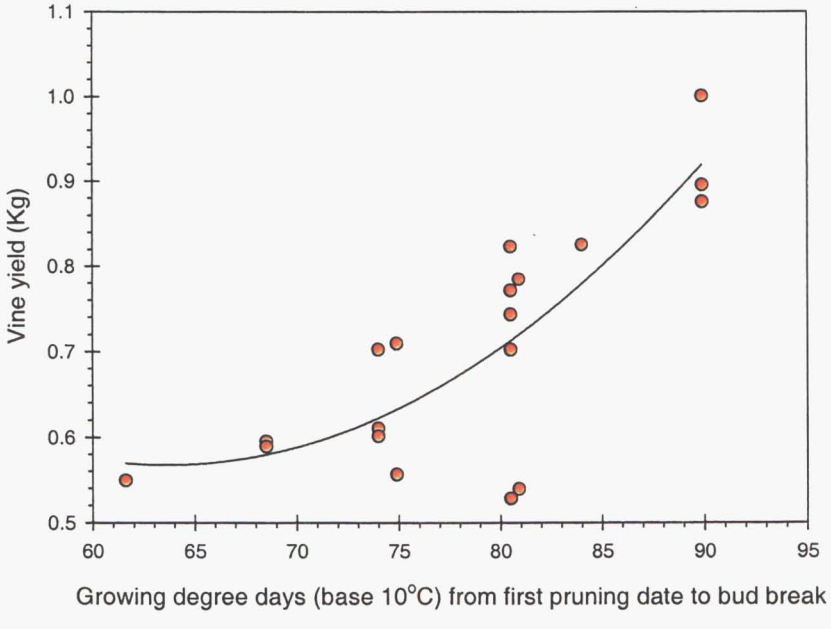


Figure 3.9 The relationship between the environment that shoots develop in (as a consequence of delayed bud break) and vine yield; y=0.516+0.00027*1.0848^{2}x, R^2=0.62, P = < 0.001 (• Vine treatment means).

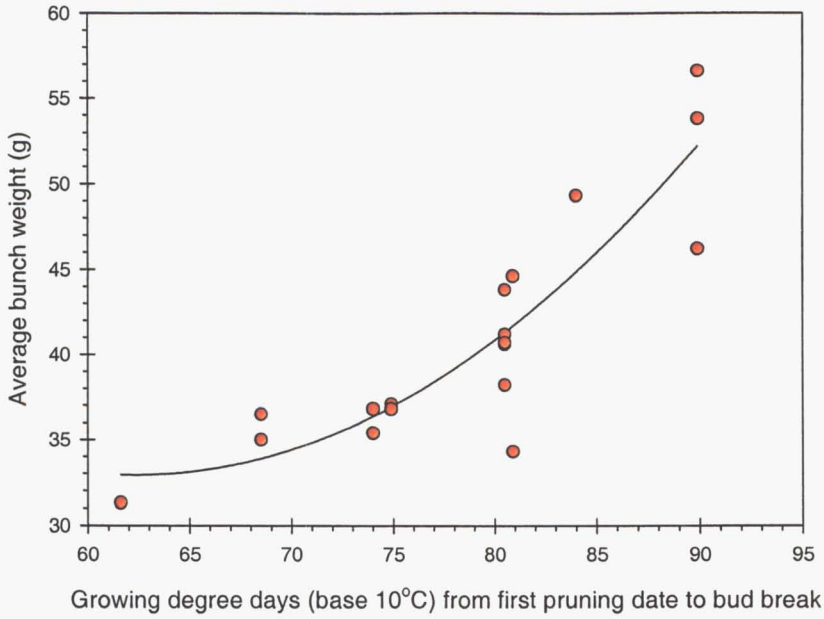


Figure 3.10 The relationship between the environment that shoots develop in (as a consequence of delayed bud break) and average bunch weight; $y=29.39+0.033*1.0754^{2}x$, $R^{2}=0.77$, $P = < 0.001$ (• Vine treatment means).

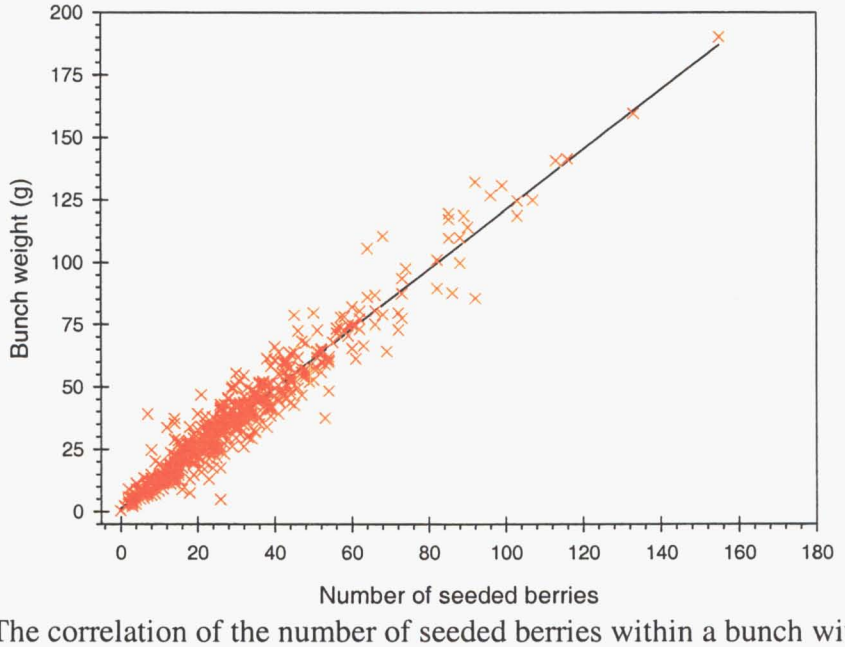


Figure 3.11 The correlation of the number of seeded berries within a bunch with bunch weight; $y=1.1985+1.277x$, $r^{2}=0.96$, $P = < 0.001$.

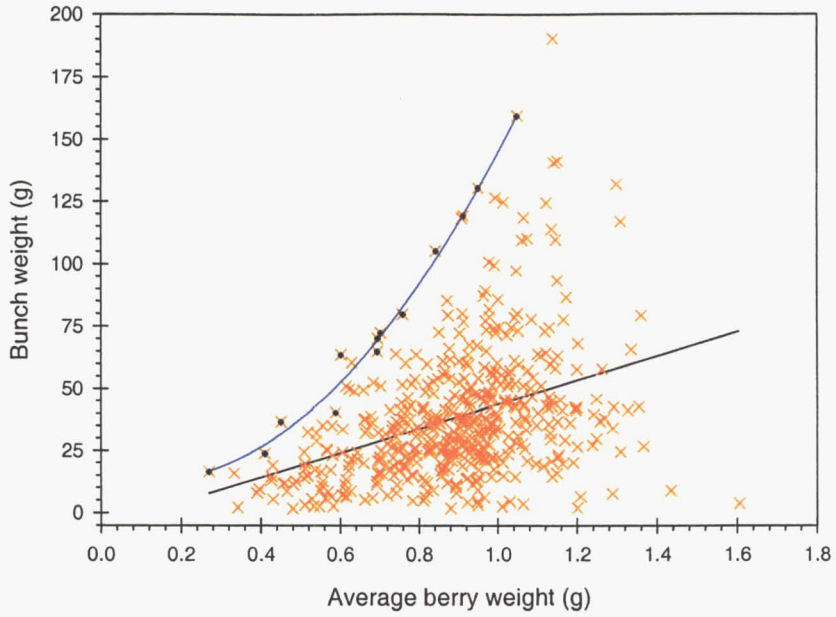


Figure 3.12 The relationship between the average weight of berries within a bunch and bunch weight; y=-5.59+49.11x, r^2=0.37, P = < 0.001; the blue line represents the boundary line; the black points mark the values used to calculate the boundary line as per Webb (1972).

3.4 Discussion

3.4.1 Bud break

In Experiment One (2001), only delayed winter spur pruning resulted in a delay in bud break, with the alginate encapsulation being ineffective. Delaying the date of winter pruning by 40 days resulted in an six day delay in time taken until 55% of the buds had reached bud break [modified E-L stage 4, Appendix A] (Figure 3.7a). Importantly, a delay in other important phenological stages was also evident with delayed pruning. The proportion of inflorescences that had reached 50% capfall had been delayed by three days (Figure 3.7b) with the 'Late' pruning treatment, and the proportion of bunches of which 50% of the berries had begun colour change, was delayed by four days (Figure 3.7c).

In Experiment Two (2000), both alginate gel encapsulation and delayed winter spur pruning delayed the date of bud break (Table 3.2). Approximations of the delay in bud break from the treatments imposed suggested that the time of pruning (~7-day delay) had a greater effect than alginate gel application (~3-day delay).

The delay in bud break found with the time of pruning was within the range quoted in the literature: delaying the pruning of Cabernet Sauvignon from early June to mid August at Dookie, Australia gave a 4 to 5 day delay in bud break (Martin & Dunn 2000), delaying the pruning of Sultana from June to August in Merbein, Australia, delayed bud break by about 3 days (Antcliff *et al.* 1957). A 10 and 13 day delay in bud break for Gewürztraminer and Cabernet Sauvignon, respectively, was reported when pruning in early September as apposed to Mid July, at Te Kauwhata, New Zealand (Whittles 1986). The delay in bud break found by Antcliff *et al.* (1957), varied considerably between seasons and sites, with warmer and drier conditions favouring an earlier bud break.

The delay in bud break with alginate gel treatment was small, compared to what has been reported in the literature, e.g. application of alginate on peaches can delay bloom by 4 to 7 days (Larsen *et al.* 2000). Applying alginate gel to grapes resulted in a 5 to 9-day and 5-day delay in bud break at Grand Junction, Colorado and Winchester, Virginia, respectively (Dami *et al.* 2000).

The possible nature of the means by which a delay in bud break is achieved may result in the differing magnitudes of effect.

The delay in bud break experienced through delayed winter spur pruning may result from a form of apical dominance. The upright position of last season's canes and the influence of the 'end point principle' and 'highest point principle' ensure that apical buds develop ahead of basal buds. After spur pruning is completed, the advanced apical buds have been removed, leaving only the less advanced or dormant basal buds from last season's canes, effectively delaying the date of bud break. Observations by Howell and Wolpert (1978) support this speculation into the mode of action of delayed winter spur pruning. The 'end point' and 'highest point' principles state that the shoots at the end of a cane and at the highest point, respectively, tend to have a vigour advantage (Jackson 1997). With the grapevine being a liana, it is likely that apical dominance will be strong.

The mode of action of alginate gel encapsulation is less clear. Dami *et al.* (1996) found that canes treated with alginate gel have lower water content, and postulated that this could slow bud metabolic activity. They also speculated a mechanical effect, in that encasing the bud within the dry matrix physically hinders bud opening and shoot growth. The placement of

spring loaded pegs around buds can delay their development (Huglin and Schneider 1998), supporting the view to a mechanical effect. Another possible mechanism may be the development of an O_2 deficiency or CO_2 accumulation within buds, due to the low gas permeability of alginate (Dami *et al.* 1996). Without detailed knowledge of the mode of action of alginate gel correct application to maximise its effect is difficult.

The consequence of delayed bud break on other phenological stages, as found in 2001 are consistent with those found in the literature: May (2000) states that the date of bud break is an important determinant of subsequent seasonal development. Delaying the pruning of Gewürztraminer and Cabernet Sauvignon from mid July to mid September delayed bud break by 10 and 13 days, and flowering by 7 and 2 days, respectively (Whittles 1986). Loomis (1939) reported a delay in flowering by delaying bud break with the time of pruning. Martin & Dunn (2000) found similar delays in the course of capfall (5 days) and *véraison* (4 days), to that of bud break that had been delayed with late winter pruning (4-5 days). Coombe (1964) found that delayed spur pruning resulted in later bud break, shorter shoots, and later flowering of Grenache. Work by Coombe (1995), noted that the growth curves of internodes per shoot, of four cultivars (Cabernet franc, Flame Seedless, Muscat Gordo Blanco, & Sultana) growing in Mitcham, Australia, regardless of length, varied little, until day 35 whereupon they diverged; and that time 50% capfall typically occurred, at 19 internodes. This is in agreement with Pratt & Coombe (1978) and Koval' & Mart'janova (1963), who found the number of internodes at anthesis was fairly constant, and McIntyre *et al.* (1982) who found that individual grape cultivars tend to develop at consistent rates, regardless of seasonal conditions. The existence of a synchrony in the rates of development of shoots (internodes) and inflorescences supports the concept that a significant delay in bud break will delay flowering, and possibly other phenological stages.

There are no reports in the literature on whether a delay in bud break, resulting from alginate encapsulation, can delay the date of flowering; however, should the delay be great enough, it would seem reasonable to expect a delay in other phenological stages. Experience with alginate encapsulation on Chardonnay in Colorado, USA supports such a notion (Stushnoff, C. *Pers. Comm.* Colorado State University; 14 August 2002).

Without knowing the mode of action of the alginate gel it is difficult to identify reasons for its variable effectiveness in delaying bud break. The effectiveness of the alginate treatment, in

experiment one, may have been reduced as a result of the time of application. Observations at the time of gel application suggested that ecodormancy might have already been released, which could have reduced the effectiveness of the alginate treatment. At the time the bud break assessment was undertaken in 2001, replicate six of the experiment showed a more advanced stage of bud development (Data not shown). As different seasons may be warmer or cooler than average, and ecodormancy is maintained by environmental conditions (Lang *et al.* 1986), the release date from dormancy will vary from year to year. Alternatively rainfall causing hydration of the gel could remove any physical restriction to bud development, and potentially allow hydration of the bud and a change in the CO_2 and O_2 concentrations.

3.4.2 Yield response

No effect on any vine or bunch yield-component was evident with alginate encapsulation treatment; this may relate to the period by which the date of bud break was delayed. The objective of late pruning is to delay flowering, and if this occurs it is possible that normal and late pruned vines will undergo fruit set under quite different climatic conditions (El-Zeftawi & Weste 1970). The two-day delay in bud development, associated with alginate encapsulation, may not necessarily result in shoots experiencing significantly warmer growing conditions, as the weather systems in Canterbury typically occur at approximately three-day intervals.

Delaying the time of winter pruning resulted in a 38.8% increase in average bunch weight, but did not increase yield (Table 3.3). The number of clusters were unaffected by the pruning treatment (Table 3.3). No change in the number of clusters was expected from the treatments used to delay bud break, as inflorescences are initiated and differentiated in the previous season (Barnard 1932; Barnard & Thomas 1933; May 2000; Perold 1927).

It was unexpected not to see a yield increase with delayed winter pruning. The occurrence of a covariate effect from the incidence of frost damage (Table 3.3) suggests that the September frost event may have confounded a treatment effect on vine yield, possible explaining the non-significant nature of the yield increase. Secondary shoots, which develop after death of the primary shoots [Refer Appendix C], are less fruitful (Hu *et al.* 1999; Wallace 1973), and their incidence reduces yield (Wallace 1973). Sampling of bunches for a bunch yield-component assessment confirms the time of pruning effect on bunch weight (Table 3.4)

providing further support to the confounding effect of the incidence of primary shoot death on yield (Table 3.3).

Several authors have reported yield increases with delayed pruning. Malan (1961), and Ravaz (1912), reported that some cultivars exhibited yield increases with delayed pruning. Barnes (1958), found increased yield with delayed pruning, but the response was complicated by mite damage and boron deficiency. Woodfin (1938) remarked that late pruning at bud break reduced shoot vigour and resulted in improved setting of the crop. Winkler *et al.* (1974) observed that pruning after 15 March in Southern California resulted in marked increases in yield.

The increase in average bunch weight associated with delayed winter pruning (Table 3.3) is comparable to the yield increases described in the literature with techniques that delay bud break. Increased yields of Perlette were reported when bud break was delayed with evaporative cooling and the use of hydrogen cyanamide. The yield increases found were variable between seasons and treatments, ranging from 6% to 46%. No cause for the increase in yield was discussed (Nir *et al.* 1988). The winter spur pruning of Gewürztraminer and Cabernet Sauvignon was delayed until September at Te Kauwhata, New Zealand, resulting in yield increases of up to 122% and 53%, respectively. Pruning from August to September gave consistently higher crop than pruning before mid August. Yield increases were reported to have resulted from improved bunch weights (Whittles 1986). Delaying the pruning of Merlot, growing in Marlborough, New Zealand, from July to September resulted in a 93% yield increase. This yield increase resulted from increased average bunch weight (Friend *et al.* 2000). Coombe (1964) found a trend towards increased yield with late spur pruning of Grenache in the Barossa Valley, Australia. The yield increases were proposed to result from improved fruit set. The yield increases ranged from approximately 20% to 60% over three seasons when pruning was undertaken in September as apposed to June, however in one season maximum yield was obtained at the August pruning date and not September as in two previous seasons. However the yield of Ugni blanc, growing in Bordeaux, France, was almost doubled, through increased bunch number, when the date of cane pruning was delayed from leaf fall until bud break (Bouard 1967). This may be the result of an increase in the number of shoots that developed, but was not discussed in the paper.

Contrasting effects of the effect of delayed bud break on yield was found on Sultana at Merbein, Australia. Delaying spur pruning from mid winter till developing shoots were 5cm long decreased yield by 24% in first season and had no effect in the second season. The decrease in yield was a result of reduced average berry weight (El-Zeftawi & Weste 1970). Very late pruning (end of November) [The length of the developing shoots would probably be well in excess of 5cm, though shoot length was not measured] of Syrah vines in Griffith, Australia, drastically reduced yield to about one quarter of a normal crop (Parkin 1980).

The literature generally supports the concept that yields are increased if bud break is delayed (Barnes 1958; Bouard 1967; Coombe 1964; Friend *et al.* 2000; Malan 1961; Nir *et al.* 1988; Ravaz 1912; Whittles 1986; Winkler *et al.* 1974; Woodfin 1938) over a range of latitudes, climate types, production systems and cultivars. Several authors have reported decreased yields (El-Zeftawi & Weste 1970; Parkin 1980), but the extent to which bud break had been delayed may have been too great. It is possible that delaying bud break for too great a period will negatively affect shoot and crop development, perhaps due to exhaustion of storage carbohydrates. At the time of bud break the vine is solely dependent on stored carbohydrates, for growth and development, until sufficient a leaf has been established to restore a positive carbon balance; by the time of flowering, storage levels of carbohydrates are at their lowest (Bennett 2002). The nature of delayed winter pruning is likely to place a carbohydrate stress on the vine, through the removal of developing shoots. If pruning is completed too late, carbohydrate storage may be inadequate to ensure a high percent bud break, inflorescence development, and/or fruit set.

The latest time that pruning can be delayed to, without an impact on stored carbohydrates, is likely to be determined by the date at which ecodormancy is released. In seasons of early bud break delayed winter pruning should be completed before shoot development becomes too advanced; further experimentation is needed to determine this developmental stage.

The literature has typically only reported on the magnitude of the yield increases experienced with delayed bud break, and not the cause. Bouard (1967) found the yield increase resulted from an increase in the number of bunches on the vines, which is surprising given that inflorescences are initiated in the previous season. An increase in the percentage bud break was reported with delayed spur pruning by Cirami and Furkaliev (1991), which would lead to an increase in bunch numbers per vine. In another study, using mini-greenhouses to enhance

air temperature around buds at break, Petrie and Clingeleffer (2005) found no change in inflorescence number per shoot but did see a decrease in the number of flowers per inflorescence. An improvement in fruit set was proposed by Coombe (1964), to account for the yield increases experienced in his experiments, but no data were collected to support this supposition. The yield increases experienced by Whittles (1986) were found to result from an increased in bunch weights. Friend *et al.* (2000) found their yield increase was a product of greater average bunch weights and examined bunch yield components to describe the cause of increased bunch weights.

Substantial increases in vine yield, resulting from delayed pruning, could potentially impact negatively on vine carbohydrate status. When vine yield exceeds vine capacity, particularly in cool climates, competition for photoassimilates can limit the partitioning of carbohydrates to storage reserves (Howell 2001). Given the role that stored carbohydrates play in bud break and early shoot development (Hale & Weaver 1962; Koblet 1969), and inflorescence development (Botti & Sandoval 1990), repetitive use of delayed pruning, resulting in yield increases exceeding vine capacity, could reduce the effectiveness of this technique, even reducing vine yields.

An increase in average bunch weight was found with delayed winter spur pruning in Experiment One (Table 3.3). The increase in average bunch weight was confirmed when bunch samples were collected to assess bunch yield components (Table 3.4). Bunch weight has two components: the total number of berries and the weight of those berries. The increase in bunch weight in experiment two was ascribed to an enhanced average berry weight with no change in the total number of berries (Table 3.4). However, no treatment effect was evident on the calculated average weight of seeded, seedless, or shot berries, suggesting that a change in the population of berries had led to the increase in the calculated average berry weight. Although the was no change in the total number of berries within bunches, the number of seeded berries increased with delayed winter pruning (Table 3.4). Linear regression suggests that the number of seeded berries is driving the total number of berries, with seedless and shot berries contributing relatively little (Table 3.5).

Calculating the change in the proportion of the various berry types within bunches (Table 3.6) confirms a change in the population of berries within bunches due to delayed winter pruning. The data suggest that average berry weight had increased, because there were more of the

larger seeded berries, leading to increased bunch weights. This is in support of the findings of Friend *et al.* (2000), who found an increase in average berry weight resulting from a change in the berry population of bunches. Working with Merlot, the number of seeded berries increased with a corresponding decrease in the number of seedless and shot berries, with no change in the total number of berries. The authors speculated that delayed winter pruning may improve the fertilisation rate of ovules by deferring anthesis to a time when climatic conditions enhance fertilisation.

It was interesting that there was no increase in the average weight of seeded berries, suggesting that even though the success of fertilisation (i.e. whether either a shot, seedless or seeded berry forms) may have been enhanced, the extent (i.e. mass of berry seed contents) was not.

3.4.3 Yield and environment

An attempt has been made to relate the environmental conditions in which shoots develop with yield, by using the accumulated growing degree-days that result from a delay in bud break as an index of the warmth of the environment. Figure 3.9 suggests that by delaying the onset of bud break, shoots begin to develop under warmer climatic conditions. As a concept, this makes sense, because the very nature of spring results in an accumulation of heat with the improving seasonal conditions. Soil and air temperature are closely linked and tend to increase as spring progresses (Dunn & Martin 2000).

The effect of shoot environmental conditions at bud break is considerable with both yield and bunch weight showing a beneficial response to warmth (Figures 3.9, 3.10). The exponential nature of the relationship offers a possible explanation as to why no alginate effect on yield was evident with relatively large improvements in environmental conditions required to result in a yield and bunch weight increase.

It is difficult to identify at which period temperature is having an effect. Various authors have demonstrated the importance of temperature at both bud break and at flowering on yield development. The tendency of temperature to increase as the season progresses, makes identifying the key period, at which temperature affects yield, difficult. Warmth (average and

extremes) at bud break or flowering, or more likely during both periods, may lead to improved fruitfulness.

Individual flowers are initiated during the short period spanning bud break (Barnard & Thomas 1933; Scholefield & Ward 1975; Snyder 1933; Winkler & Shemsettin 1937); cooler temperatures during this time have been linked with increased numbers of flowers per inflorescence (Dunn & Martin 2000; Ezzili 1993; Pouget 1981), but Ezzili (1993) noted that under cool temperatures, as many as five percent of those flowers are non-functional. The organs of individual flowers are formed during shoot elongation, within 10 to 15 days of the appearance of the inflorescence (Swanepoel & Archer 1988). Wilson (1996) found that during this period, flowering success was enhanced under warmer environmental conditions.

Warm temperature at flowering is a major driver of yield development. Buttrose and Hale (1973) identified 18°C as the optimum temperature from bud break to flowering for increased fruitfulness (in the current season) of Cabernet Sauvignon, Shiraz, White Riesling, and Clare Riesling. MacGregor (2000) found that bunch weight of Chardonnay increased about two-fold between an average air temperature of 13.3 and 19.4°C.

Average temperature is not the only aspect of temperature determining yield development, as 'cold snaps', and their timing, can have significant impact. Research has shown that a one-week exposure of 'cold' temperature (12°/9°C) at flowering can reduce fruit set of Chardonnay to less than that of vines held at 17°/14°C or 25°/20°C continuously from bud break to flowering. Exposure to this 'cold' temperature also lowered the number of functional seeds per berry, but not the total number (Ebadi *et al.* 1995a). A period of 'cold', earlier during inflorescence development, did not have the same detrimental effect. Both Roubelakis and Kliewer (1976) and Ewart and Kliewer (1977) found that vines, exposed to 15°/10°C day night temperatures for one or two weeks before anthesis, had fewer seeds per berry in comparison with vines exposed to higher temperatures.

The effect of cold temperature, at or on flowering, on fruit set was found to be the result of low temperature being detrimental the normal development and functioning of the ovules and the pollen (Ebadi *et al.* 1995b; Staudt 1982).

Figures 3.9 and 3.10 link temperature at bud break to yield development, but do not provide evidence as to the specific cause. Considerable variation exists within the data of Figures 3.9 and 3.10, suggesting that temperature during bud break may not be a direct or the only cause of the proposed relationships. The trend to increasing temperature during the progression of a season provides some support to the notion that, temperature during flowering may play a role in the yield increases experienced in experiment two. El-Zeftawi & Weste (1970) state that the object of late pruning is to delay flowering, and if this occurs it is possible that normal and late pruning treatments will develop their crops under quite different climatic conditions. Experiment one confirmed reports in the literature by Coombe (1964), Loomis (1939), Martin and Dunn (2000), and Whittles (1986), that delaying bud break delays other phenological stages such as flowering and *véraison* (Figure 3.5). However, it cannot be ruled out that warm temperatures during bud break and shoot development may have improved the ability of flowers to develop into fruit or that rapid shoot development could lead to carbohydrate stress.

The increase in bunch weight, reported in experiment two (2000), is being driven by the number of seeded berries, not average berry weight (Figures 3.11, 3.12, Table 3.7). Under conditions of constant bunch number, bunch weight is the main driver of vine yield. The importance of the number of seeded berries on bunch weight provides further support for temperature at flowering being the primary driver of yield development. Changes in the proportions of seeded and seedless berries resulting from delayed winter pruning (Table 3.6) imply an enhancement in the relative success of fertilisation of ovules, leading to greater berry development. However, instead of the seeds being larger giving larger berries, more ovules are developing into seeds in individual flowers, resulting in more seeded berries.

It is possible, with later flowering dates, the percentage of flowers that set fruit could be modified via changes to vine carbohydrate supply, as current vine carbohydrate supply is considered to be a major determinant of fruit set (Bennett 2002; Caspari *et al.* 1998; Coombe 1973).

Chapter IV

Grapevine flowering

4.1 Introduction

The onset of capfall (flowering) in grapevines is a crucial time, where yield potential is at its maximum and events over the following weeks determine the extent to which this potential is realised. The success of flowering, resulting in fruit set, will determine both the number and size of berries on a vine; though the number of berries per vine exerts a greater influence over fluctuating yields than does the size of berries (Dunn G.M. *Pers. Comm.* University of Melbourne; 7 April 2006). Berries are the basic unit of yield (May 2000), and it is surprising how little is known regarding the potential of flowering events to impact on fruit set and berry development.

The characteristics and behaviour of flowers in grapevines are poorly understood. Considerable variation in flower size is known to exist within but not between the branches of inflorescences, and this variation applies to both the calyptra and pistil (May 1987). Flower size differences are related to their positions on the branch: the terminal central flowers of the primary branches are the biggest, the central flowers of the secondary branches are intermediate and the lateral flowers are the smallest (May 1987). Variation in flower size is less under cool temperature conditions at bud break, but still remains (Ezzili 1993).

Flowering in grapevines follows a diurnal rhythm (Randhawa & Negi 1965), with daily capfall peaking early in the morning, soon after sunrise. Air temperature (Randhawa & Negi 1965), specifically that of the preceding day (Staudt 1999), is thought to influence the likelihood of a particular flower undergoing capfall.

The literature has only touched on the relationship between the characteristics of individual flowers and their subsequent development into berries, or their interaction with short-term weather events. Cool and wet springs, such as those experienced in New Zealand's maritime climate, appear to extend the period of flowering over a two to three week period, or greater [*c.f.* less than a week in continental USA) (Friend *et al.* 2003). The differences in

development reported among berries within a bunch (Glynn & Boulton 2001) may in part be due to differences in date of capfall within a bunch (Trought & Tannock 1996). Variation in fruit maturity, and the composition of berries within bunches, has been suggested as an important factor affecting wine quality (Long 1987; Trought 1996).

Chapter three showed how varying the timing of phenological stages can modify yield at a whole vine level. The changes in yield were found to be a consequence of altered berry development, and might be associated with temperature conditions under which shoots develop. The next two chapters aim to describe the development of individual flowers into berries. This chapter uses data from three experiments to describe the characteristics of a population of flowers, and to examine the influence of flower characteristics and temperature on the progression of capfall.

4.2 Experimentation

4.2.1 Experimental aims

Three experiments were conducted over three seasons, to follow capfall within grapevine inflorescences and to relate the behaviour of flowers and the extent of berry development to weather conditions. In addition treatments were applied to alter the carbohydrate balance of shoots or modify seed development within berries, manipulating fruit set and berry development. Data were collected on the characteristics of flowers and the berries that develop from them.

The aims of these experiments were:

Experiment Three: To examine the effect of shoot girdling on flower and berry development. Shoot girdling increases the carbohydrate supply (Weaver & McCune 1959) and alters plant-growth hormone levels (Coombe 1959; Weaver & Pool 1965) within the shoot by severing the phloem, preventing, below the girdle, the passage of photoassimilates to sinks, such as root and trunk growth, and accumulation of storage carbohydrates.

Experiment Four: To examine the effect of the antibiotic spectinomycin on flower and berry development. Application of spectinomycin and streptomycin

to inflorescences at peak capfall can induce seedlessness in grapes (Widodo *et al.* (1999b).

Experiment Five: To examine how modifying leaf area influences fruit set and berry development. Shoots were girdled to create a closed carbohydrate system (Caspari *et al.* 1998). Carbohydrate supply was modified by the removal of a proportion of each shoot's leaf area, to attempt to set up a range of positive and negative carbohydrate balances.

4.2.2 Site and plant material

Experimentation was conducted within the Lincoln University Experimental Vineyard, New Zealand (43°39' S, 172°28' E). Experiment Three was conducted on 3-year-old field grown, *Vitis vinifera* cv. "Pinot noir" clone AM10/5 (grafted to "Riparia Gloire") vines in the 1999-2000 season, Experiment Four was conducted on the same vines in the 2000-2001 season. Due to an earlier than anticipated start of capfall, Experiment Five was conducted on 26-year-old field grown, *Vitis vinifera* cv. "Cabernet Sauvignon" vines in the 2001-2002 season.

The vines were positioned 1.6m apart, within North-South (approximately) orientated rows, with 2.5m spacing between rows. Industry standard canopy manipulations and pesticide programmes were in place. The Pinot noir vines were spur pruned to 12 two-bud spurs and trained to a vertical shoot positioning system, with a canopy height of 1.2m, in both seasons. The Cabernet Sauvignon vines were cane pruned to 40 buds on four canes and trained to a vertical shoot positioning system, with a canopy height of 1.2m. Vines were selected to each have similar vine capacity [Capacity = (yield*0.25) + (pruning weight*0.55)].

Shoots used in experimentation were selected to each be of a similar vigour and fruitfulness, as assessed by the shoot diameter between nodes two and three, and to each have two well-formed inflorescences arising from nodes four and five. If present, the outer arm (shoulder or tendril) of each inflorescence was removed at the onset of capfall. Shoots were trained up bamboo canes to reduce the likelihood of damage to inflorescences and bunches, or breakage at the girdle, and were topped once they had reached the top training wire (some shoot may have had greater numbers of leaves as a consequence). Lateral shoots were removed as they appeared during the season. No leaf plucking was carried out.

4.2.3 Experimental design and treatment application

The experiments were laid out in completely randomised block designs, with a single vine constituting both a replicate and block. Treatments were applied to individual shoots within blocks. Thirty flowers, on the proximal inflorescence of each shoot, were tagged for monitoring and data collection.

Experiment Three was conducted on ten vines (replicates); flowers were tagged on two shoots on each vine. To encourage adequate fruit set, one of the two shoots per vine with tagged flowers was girdled. The goal of girdling was to enhance the carbohydrate supply within the shoot (Caspari *et al.* 1998). Girdles were made on the 1 December 1999, approximately 3 days before the onset of flowering, by removing a 2 mm wide strip of bark from around the shoot between nodes two and three (Figure 4.1). Girdles were cut by hand using a razor blade.

Experiment Four was conducted on ten vines (replicates); flowers were tagged on three shoots per vine, with one shoot girdled, another treated with the antibiotic Spectinomycin (Sigma; St Louis, MO, USA), and the other a control. Spectinomycin treatment results in seedless berries, because of a reduction in endosperm nuclei, causing ovule abortion before sclerification of the seed coat (Widodo *et al.* 1999a). Treatment with spectinomycin on Muscat of Alexandria resulted in smaller seedless berries (Widodo *et al.* 1999a). The girdling and antibiotic treatments were applied at the onset of capfall (9/12/2000). Shoots were girdled as in Experiment Three, while the antibiotic was applied by dipping whole inflorescences, for 10 seconds, in a 200mg per litre water solution of Spectinomycin [as per Widodo 1999a], with 5mg per litre Tween 80 (BDH Ltd; Poole, England) at 18°C.

Experiment Five was conducted on seven vines (replicates); flowers were tagged on four shoots per vine. All shoots were girdled as per Experiment Three, and the area of each leaf was adjusted once flowering had begun on the 18 December 2001. Portions of leaves were removed to reduce leaf area per shoot by approximately, 0, 25 (cutting across, between the upper leaf sinuses), 50 (cutting across, between the upper leaf sinuses and along one of the third lateral veins), and 75% (cutting across, between the upper leaf sinuses and along both the third lateral veins) (Figure 4.2), to reduce shoot carbohydrate supply like Caspari *et al.* (1998).

4.2.4 Tagging and Data Collection

Individual flowers and their resulting berries were tracked by tagging with colour-coded strands of dental floss. The tags were applied at modified E-L stage 17 (Appendix A) when flowers had separated. Flowers for tagging were selected from across the whole inflorescence. In an attempt to get an even distribution of flower diameters, large and small flowers were chosen.

Tags were prepared by dipping one end of individual 10 cm lengths of waxed dental floss (Johnson and Johnson; Sydney, Australia) into enamel paint (Humbrol; Poole, England). The dental floss was hung to dry and then a second coat of paint applied, creating two bands of colour. Thirty colour combinations were used to create unique identifiers for the 30 flowers tagged per inflorescence. The tags were pre-tied around pencils with a half hitch to aid placement around flowers (Figure 4.3). The pre-tied tags were placed over the flower and gently tightened (Figure 4.4). Dental floss was chosen as the wax coating would reduce the likelihood of knots slipping and tags falling off flowers, and the lightweight nature of the strand would prevent damage to the flower or pedicel (Figure 4.3).

Data were collected on the date of capfall of each flower. Flowers were assessed for capfall each morning (ca 10:00hr NZST) from the date of treatment. Calyptra diameter at tagging and ovary diameter at capfall were measured using a pair of digital callipers (Sylvac, Switzerland; ± 0.01mm) (Figure 4.4). Percent fruit set was calculated from the thirty-tagged flowers. Data were also collected on flower fate, with tagged flowers that set fruit classified as either a seeded, seedless, or shot berry (Figure 3.5). Seeded and seedless berries showed development of the mesocarp and had accumulated colour and sugar, differing by their size and seed content, with seedless berries being smaller than seeded berries. Shot berries were flowers that did not develop nor abscise.

Temperature and rainfall data were collected from the Lincoln weather station, H32574 (43°34'S 172°43'E), part of the New Zealand Meteorological Service network. Growing degree-days were calculated using a base temperature of 10°C and daily maximum and minimum temperatures (°C).

For Experiment Five, the correlation between the length of a leaf's mid-rib and leaf area was assessed in order to estimate the leaf area removed from the shoots (Table 4.1) and calculate

total shoot leaf area (Figure 4.5). Leaf area was measured using an area meter (Model 3100, Li-Cor, Lincoln, NE, USA) and mid-rib measured with a ruler (± 1mm). The length of leaf mid-ribs from the shoots in Experiment Five were assessed at flowering (18 Dec 01).

4.2.5 Statistical analysis

Statistical analysis by regression, linear regression, curve fitting, and ANOVA were completed using Genstat 5 Release 4.1 (Lawes Agricultural Trust, England). The mid-point of capfall was calculated by fitting a logistic curve to the accumulated capfall of individual inflorescences.

When examining the influence of temperature on the progression of capfall a range of air temperature calculations (mean, minimum and average) were compared over a range of time periods (day of flowering, and one, two, three, four, or five days preceding capfall), with mean temperature of the preceding two days providing the best results; there is no physiological basis for this. Regression analysis was run twice in years where rainfall events greater than 5mm during flower were recorded. Rainfall is reported to disrupt flowering (Galet 2000).

Figure 4.1 An example of a shoot girdled between nodes two and three.

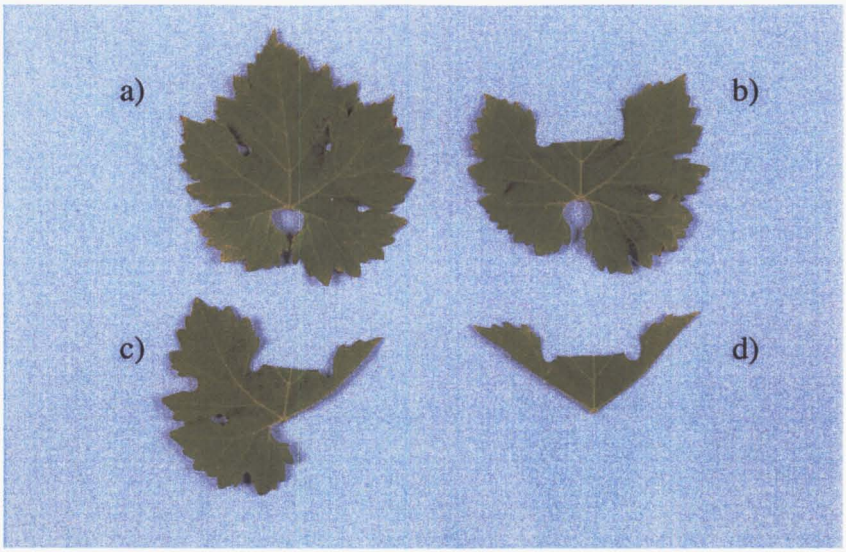


Figure 4.2 Examples of leaves in Experiment Five, cut to reduce shoot leaf area. a) 0% reduction, b) 25% reduction, c) 50% reduction, and d) 75% reduction in leaf area.

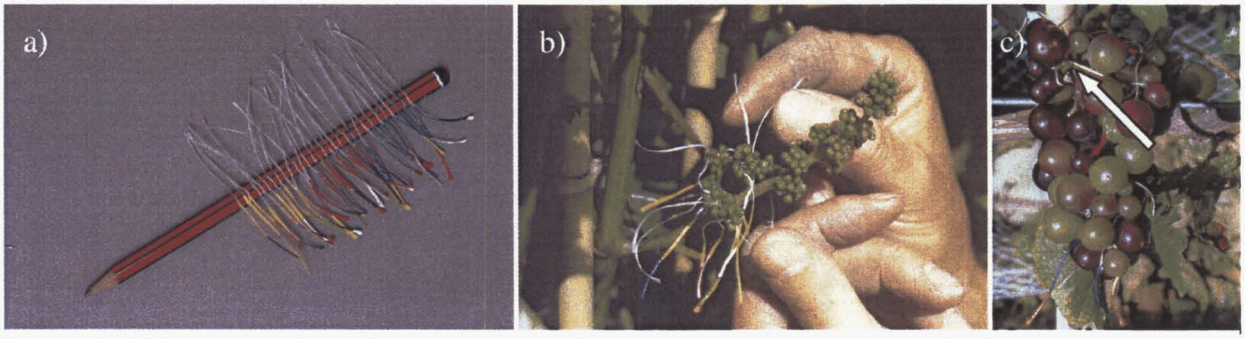


Figure 4.3 Flower tagging, a) An example of pre-tied tags around a pencil, for ease of tagging; b) Placement of pre-tied tag over a grape flower; c) Demonstration of how the tag expands with the developing pedicel, preventing damage (arrow).

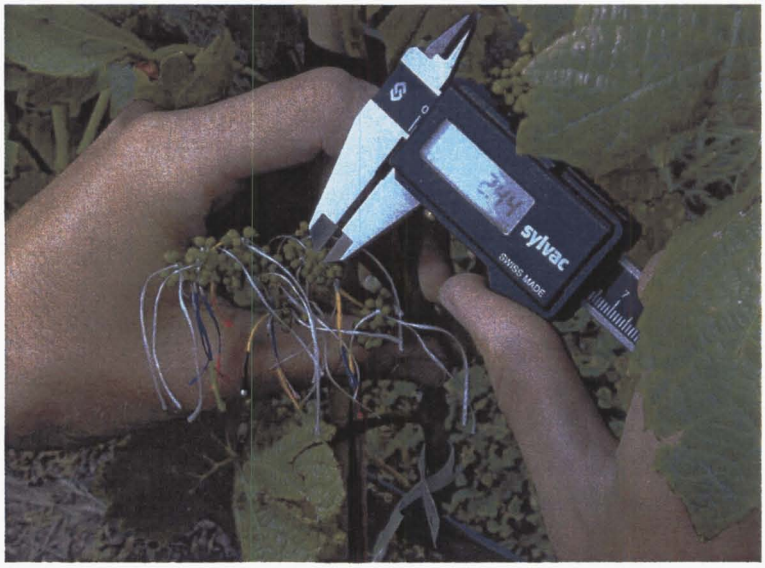


Figure 4.4 Measuring the diameter of the cap of a grape flower with a pair of callipers.

Table 4.1 Estimated remaining shoot leaf area after removal of lamina portions.

	Leaf area treatments					
	Control	1	2	3	Sig[1]	LSD (5%)
Area (%)	100	78.7	53.6	25.5	<0.001	1.09

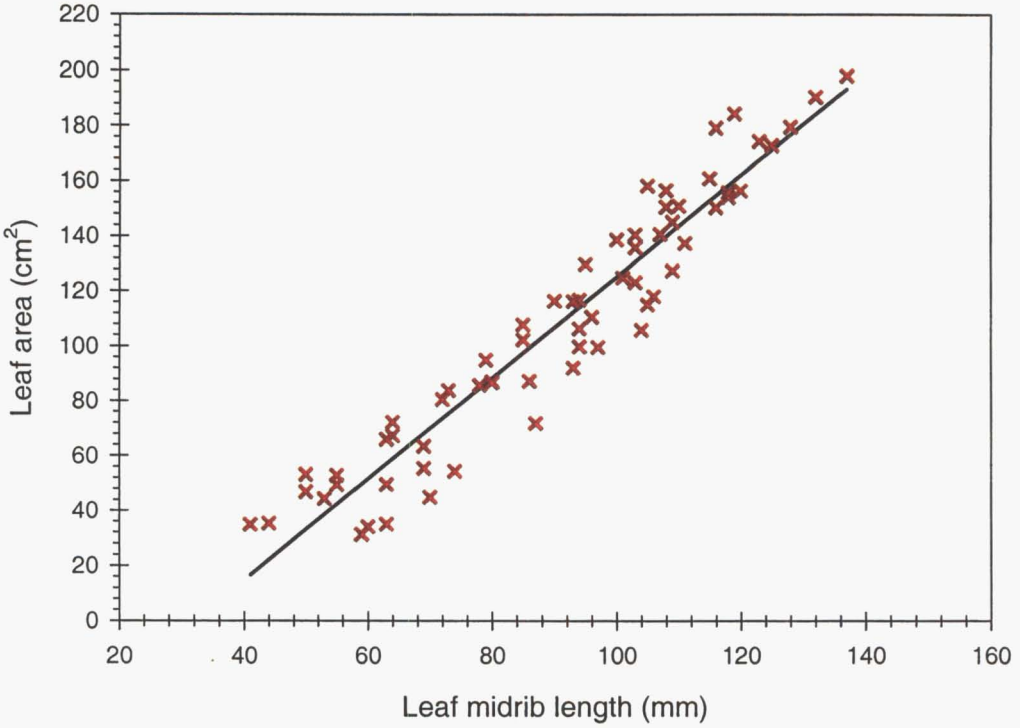


Figure 4.5 The correlation of the length of the lamina midrib with leaf area of Cabernet Sauvignon shoots; Regression: $y = -58.9 + 1.8404x$, $r^2 = 0.96$, $P = <0.001$.

4.3 Results

4.3.1 Flower characteristics

The populations of tagged flowers in Experiments Three to Five showed within inflorescence variation in the diameter of the ovary (Table 4.2). Though mean flower size alters between the three years the standard deviations are similar (± 0.027) suggesting the variation in ovary diameter is consistent between the seasons. Differences in mean flower size appear to occur with cultivar, and season, but are likely to be confounded by year of experimentation.

A considerable range in flower size is found within the populations, but the similarity of the mean and median flower size suggest that populations are symmetrical in shape. The Kurtosis values suggest that the Pinot noir populations have distribution tails greater than a normal

distribution, while Cabernet Sauvignon have distribution tails less than a normal distribution. The population distributions show a slight skewness with Pinot noir skewing to the right and Cabernet Sauvignon to the left (Table 4.2)

Few outliers are present within the sampled populations with a very small percentage of flowers in 1999 Pinot noir being less than 1.2mm in diameter; a larger but small percentage of flowers in 2000 Pinot noir being greater than 2.2mm, and a small percentage of Cabernet Sauvignon flowers in 2001 being less than 0.9mm in diameter (Figure 4.6).

Table 4.2 Descriptive statistics from the population of tagged flowers from Experiments Three, Four and Five.

Statistic	Experiment		
	Three	Four	Five
	P. noir (1999)	P. noir (2000)	Cab Sav. (2001)
Mean (mm)	1.61	1.69	1.27
Median (mm)	1.61	1.69	1.28
Mode (mm)	1.64	1.65	1.34
Standard deviation	0.124	0.144	0.117
Maximum (mm)	2.12	2.45	1.59
Minimum (mm)	1.10	1.32	0.76
Range (mm)	1.02	1.13	0.93
Skewness	0.238	0.640	-0.668
Kurtosis	1.804	1.581	0.708
Count	565	846	832

4.3.2 The progression of flowering

Neither the girdling or spectinomycin treatments, or leaf area removal in Experiments Three to Five, respectively, had any impact on the progression of flowering. The duration of flowering was not altered by the treatments. The start date of capfall was not altered by the treatments, with no advance or delay in flowering evident (Table 4.4). The mid-point of flowering, as calculated by fitting a logistic curve to the accumulated flowering of each inflorescence, was not affected by the treatments (Table 4.5). The progression of flowering of individual inflorescences is shown in Appendix D.

Flowering in the whole populations of tagged flowers in Experiments, Three (Pinot noir 1999), Four (Pinot noir 2000), and Five (Cabernet Sauvignon 2001) continued for 18, 21, and 20 days, respectively.

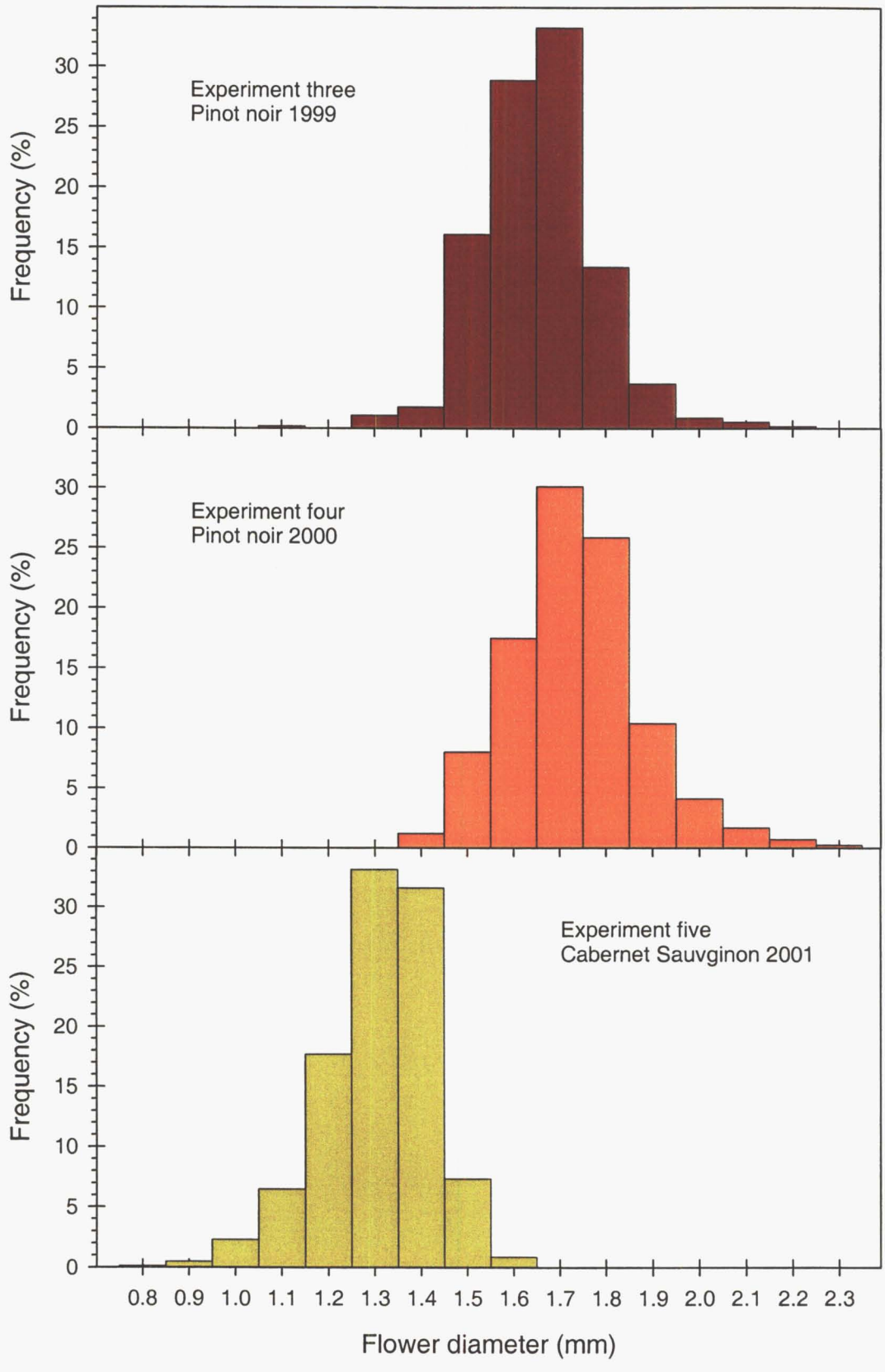


Figure 4.6 Frequency distribution of the diameter of tagged flowers from Experiments Three, Four and Five.

Smaller flowers tend to undergo capfall after larger flowers. The relationship is not strong, with only a small proportion of the total variability in ovary diameter being accounted for by the date of capfall. The relationship appears to be stronger in Cabernet Sauvignon than Pinot noir (Figure 4.9), assuming that the varieties are not influenced by seasonal differences.

A summary of the analysis of the types of relationships between ovary diameter and capfall date in individual inflorescences is shown in Table 4.6. Larger flowers show a more consistent tendency to open before smaller flowers in individual inflorescences of Cabernet Sauvignon, when compared to Pinot noir. Though the data for Pinot noir and Cabernet Sauvignon was collected in different seasons, it was not considered that season would influence this relationship. The number of individual inflorescences exhibiting a negative relationship, as seen in the whole population (Figure 4.7), varies between seasons. The mean flowering date of individual inflorescences and ovary diameter were not related in any experiment, suggesting the trends in Figure 4.9 are a result of differences within inflorescences and not between inflorescences.

In general the treatments in Experiments Three to Five have no effect on the slope (Table 4.7) or the y intercept (Table 4.8) of the treatment regression lines for the relationship between ovary diameter and the date of capfall; however in Experiment Five, shoots with 75% of their normal leaf area increased the slope of the relationship and in Experiment Four girdling increased the y intercept.

Table 4.3 The influence of treatments on the duration of capfall in Experiments Three to Five.

Capfall duration (Days)					
Experiment Three Pinot noir (1999)		Experiment Four Pinot noir (2000)		Experiment Five Cab. Sauvignon (2001)	
Control	10.3	Control	9.3	100% leaf area	11.4
Girdled	10.8	Girdled	10.4	75% leaf area	12.1
		Spectinomycin	7.9	50% leaf area	12.4
				25% leaf area	13.1
p value	0.681	*p* value	0.125	*p* value	0.828
LSD (5%)	2.66	LSD (5%)	2.432	LSD (5%)	3.88

Table 4.4 The influence of treatments on the starting date of capfall in Experiments Three to Five.

Capfall start date (Day in December)					
Experiment Three Pinot noir (1999)		Experiment Four Pinot noir (2000)		Experiment Five Cab. Sauvignon (2001)	
Control	7.6	Control	10.9	100% leaf area	16.6
Girdled	8.4	Girdled	10.0	75% leaf area	17.1
		Spectinomycin	12.3	50% leaf area	16.0
				25% leaf area	16.4
p value	0.502	*p* value	0.059	*p* value	0.785
LSD (5%)	2.59	LSD (5%)	1.89	LSD (5%)	2.56

Table 4.5 The influence of treatments on the midpoint of capfall in Experiments Three to Five; Refer to Appendix D for curve fitting to individual inflorescences.

Midpoint of capfall progression (Day in December)					
Experiment Three Pinot noir (1999)		Experiment Four Pinot noir (2000)		Experiment Five Cab. Sauvignon (2001)	
Control	13.2	Control	14.9	100% leaf area	20.0
Girdled	14.2	Girdled	14.4	75% leaf area	22.4
		Spectinomycin	15.2	50% leaf area	21.6
				25% leaf area	19.6
p value	0.981	*p* value	0.777	*p* value	0.261
LSD (5%)	2.85	LSD (5%)	2.53	LSD (5%)	3.32

Table 4.6 The type of relationship between the date of capfall and ovary diameter for individual inflorescences in Experiments Three to Five; Refer to Appendix E for individual regressions.

	Number of inflorescences showing positive or negative relationships		
	Experiment Three Pinot noir 1999	Experiment Four Pinot noir 2000	Experiment Five Cab. Sauvignon 2001
Negative	7	4	23
Positive	1	1	0
No relationship	12 (9 negative 3 positive)	25 (22 negative 3 positive)	5 (5 negative 0 positive)

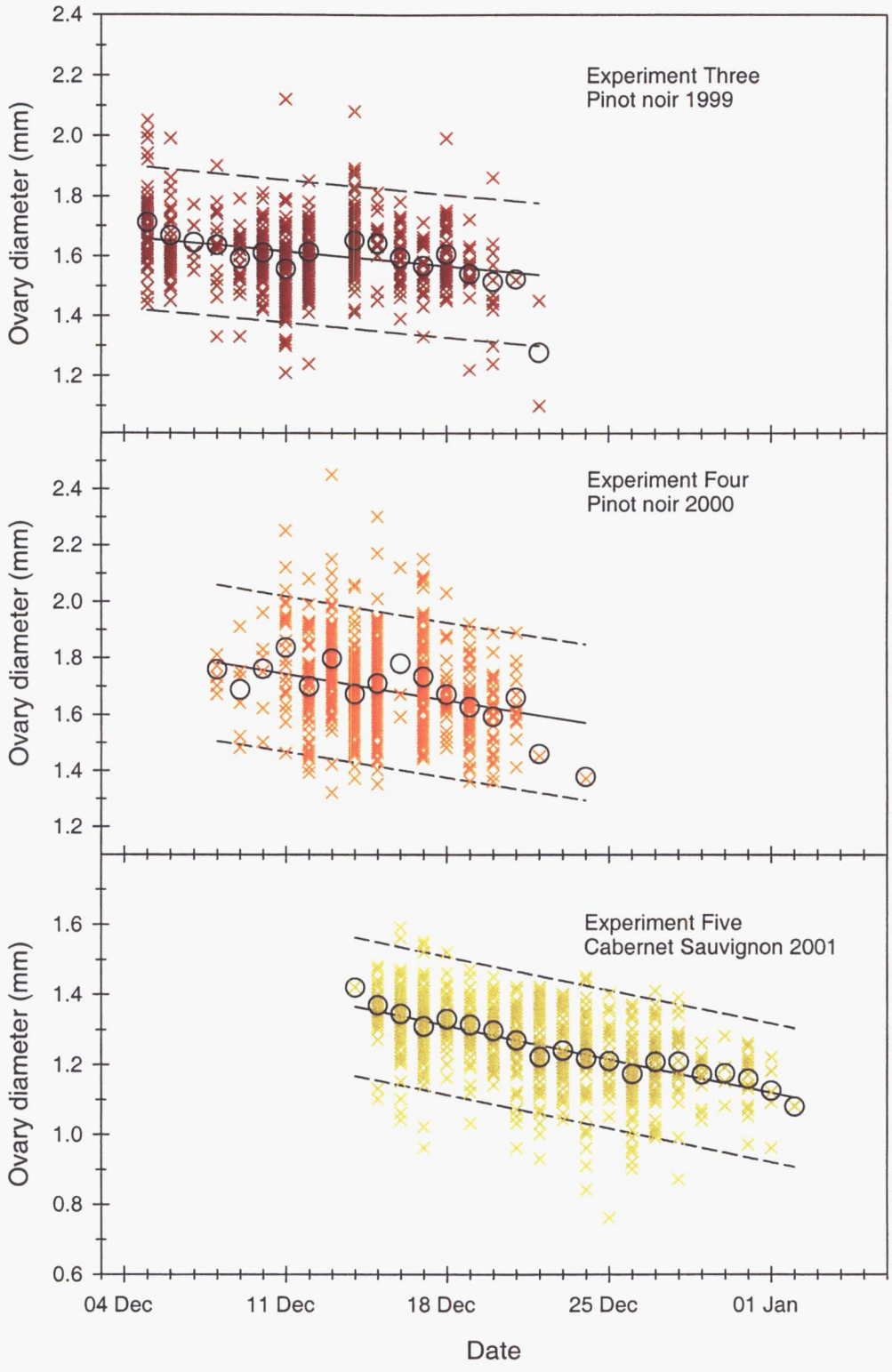


Figure 4.7 The influence of flower diameter on the date of capfall in Experiments Three, Four and Five. Note different scale on the y axes; × individual flowers, ○ mean ovary diameter, — linear regression, ---- 95% prediction interval. Calculated linear regressions of individual flowers; Experiment Three $y = -0.007x + 1.706$ ($R^2 = 0.04$) $p = <0.001$; Experiment Four $y = -0.013x + 1.914$ ($R^2 = 0.05$) $p = <0.001$; Experiment Five $y = -0.014x + 1.556$ ($R^2 = 0.25$) $p = <0.001$.

Table 4.7 The influence of treatments on the slope of the relationship between the date of capfall and ovary diameter.

Slope					
Experiment Three Pinot noir (1999)		Experiment Four Pinot noir (2000)		Experiment Five Cab. Sauvignon (2001)	
Control	-0.014	Control	-0.019	100% leaf area	-0.0152
Girdled	-0.032	Girdled	-0.026	75% leaf area	-0.0265
		Spectinomycin	0.023	50% leaf area	-0.0167
				25% leaf area	-0.0103
p value	0.462	*p* value	0.161	*p* value	0.010
LSD (5%)	0.0534	LSD (5%)	0.056	LSD (5%)	0.00886

Table 4.8 The influence of treatments on the y intercept of the relationship between the date of capfall and ovary diameter.

y intercept					
Experiment Three Pinot noir (1999)		Experiment Four Pinot noir (2000)		Experiment Five Cab. Sauvignon (2001)	
Control	1.688	Control	1.750	100% leaf area	1.449
Girdled	1.694	Girdled	2.060	75% leaf area	1.458
		Spectinomycin	1.754	50% leaf area	1.418
				25% leaf area	1.350
p value	0.923	*p* value	0.012	*p* value	0.182
LSD (5%)	0.1318	LSD (5%)	0.224	LSD (5%)	0.1089

4.3.3 The effect of temperature on the progression of flowering

The progression of accumulated capfall events, of the tagged flowers, typically followed a logistic curve (Figure 4.8, 4.11, 4.14). Although the modelled curves fit the data well, some departure of the actual capfall events to the modelled curves exists. Weather conditions during flowering in all three seasons' experienced considerable variation in air temperature and in 1999 and 2000 substantial rainfall events (≥15mm) occurred during peak flowering (Figure 4.9, 4.12, 4.15). In 2001 five days of moderate rainfall were recorded totalling 32mm at the end of flowering (Figure 4.15).

Mean air temperature of the preceding two days showed the strongest relationship with the residuals from curve fitting to the accumulated capfall events. The presence of such a relationship suggests that temperature may influence the progression of capfall. In 1999 (Figure 4.10) and 2001 (Figure (4.16) mean air temperature above 15°C may advance the

progressions of capfall in Pinot noir and Cabernet Sauvignon. This relationship is not evident in Pinot noir in 2000 (Figure 4.13) with the variance from the regression being too great to estimate the regression fit.

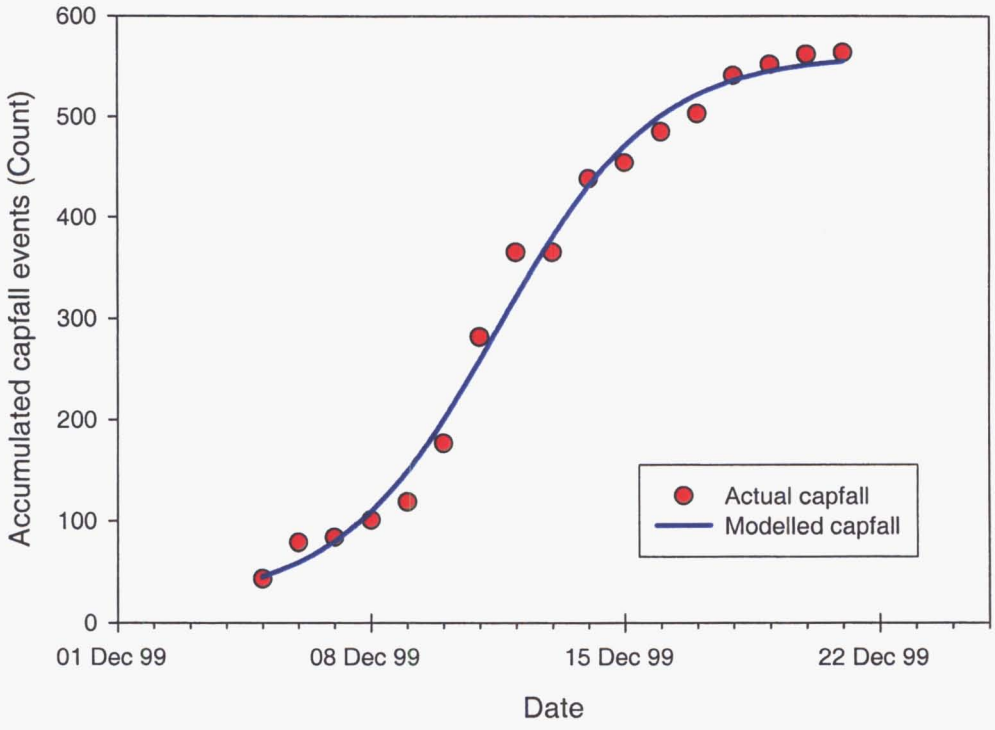


Figure 4.8 The progression of capfall of Pinot noir flowers from Experiment Three; logistic curve $y = 18.8 + 543.4/(1+EXP(-0.4597*(x - 7.508)))$, $R^2 = 0.98$.

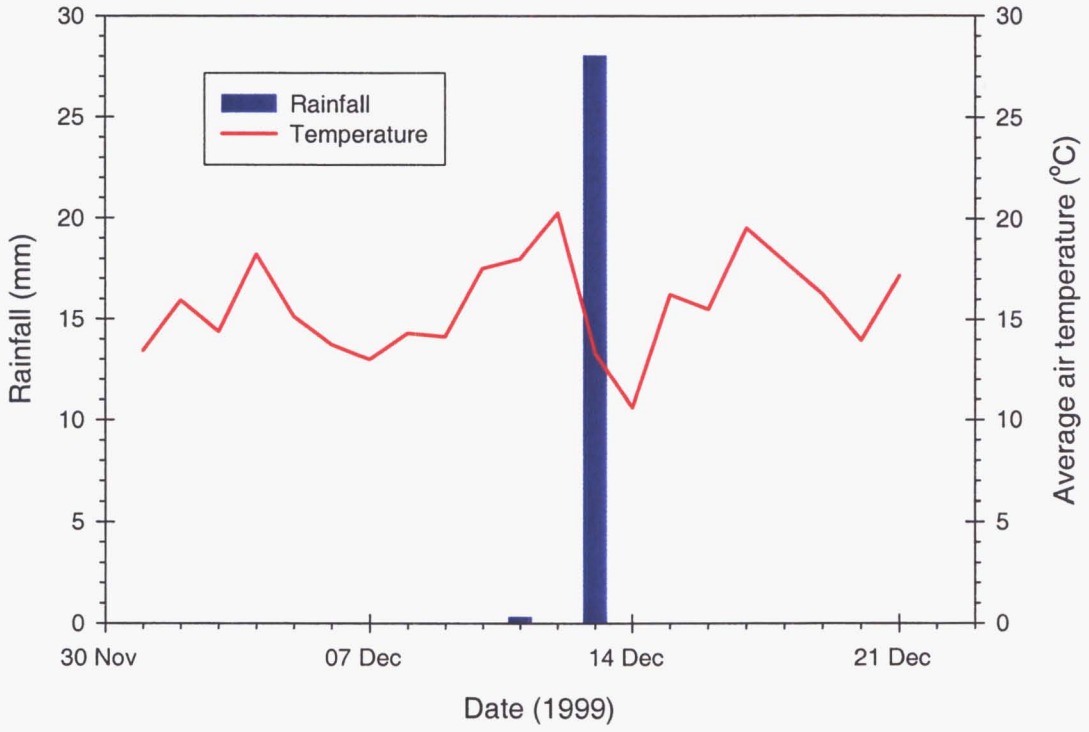


Figure 4.9 Weather conditions during capfall in Experiment Three.

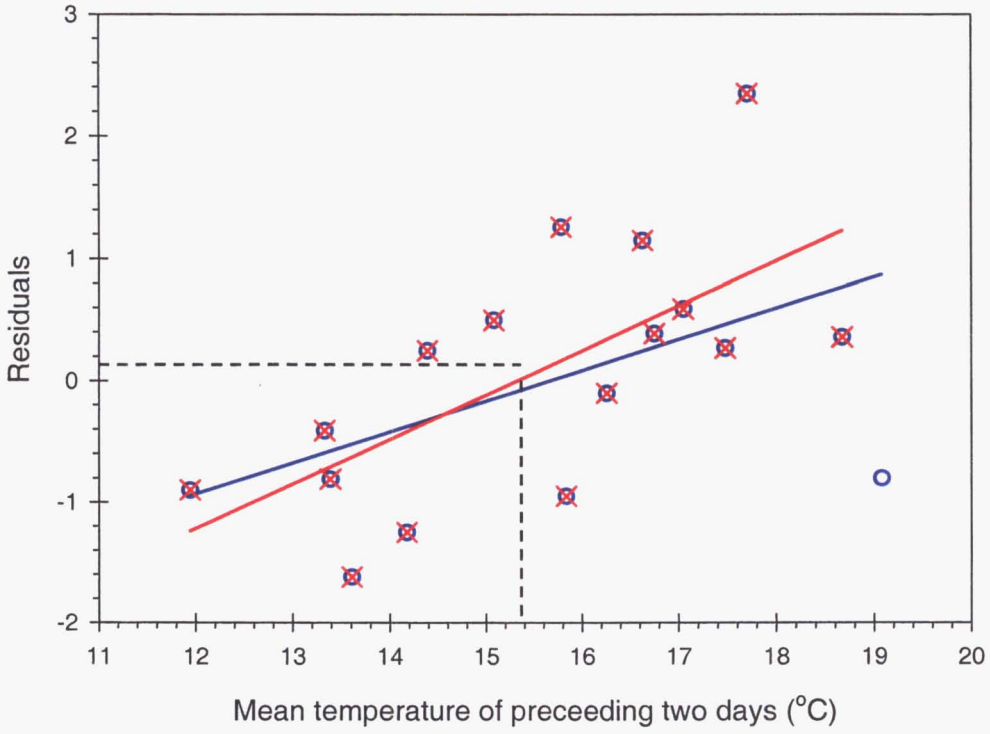


Figure 4.10 Relationship between capfall and temperature for Pinot noir in Experiment Three; all data (○) $y = -3.99x + 0.255$, ($R^2 = 0.20$), $p = 0.040$; adjusted for rainfall (×) $y = -5.62x + 0.366$, ($R^2 = 0.40$), $p = 0.005$.

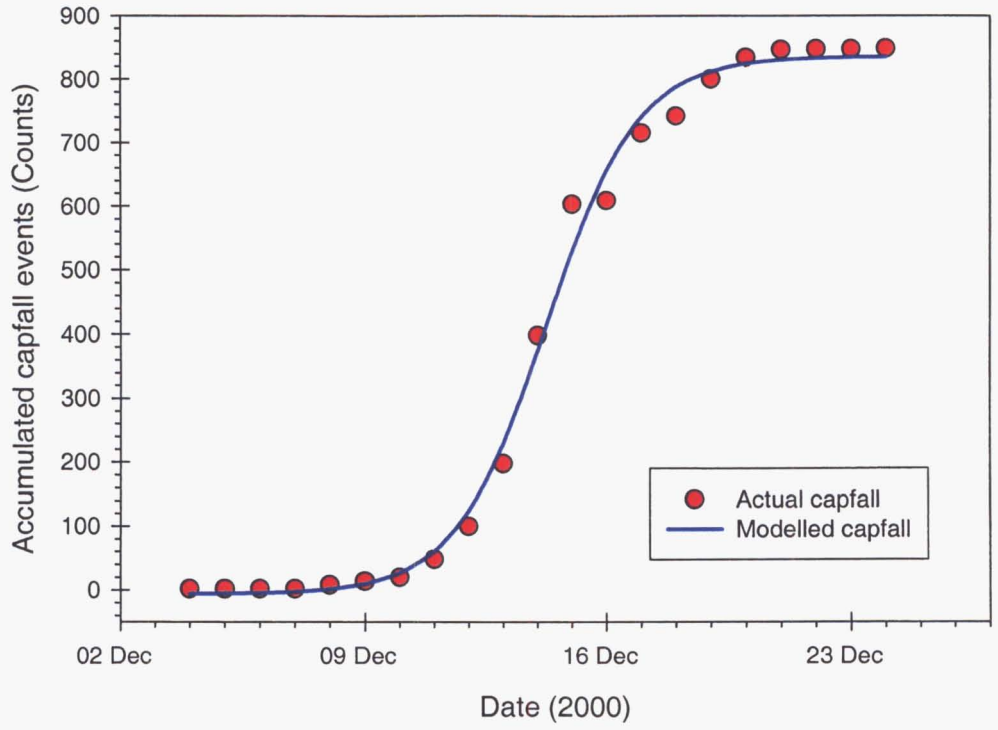


Figure 4.11 The progression of capfall in Pinot noir in Experiment Four; logistic curve $y = -5.9+840.6/(1+EXP(-0.7530*(x-14.258)))$, $R^2 = 0.99$.

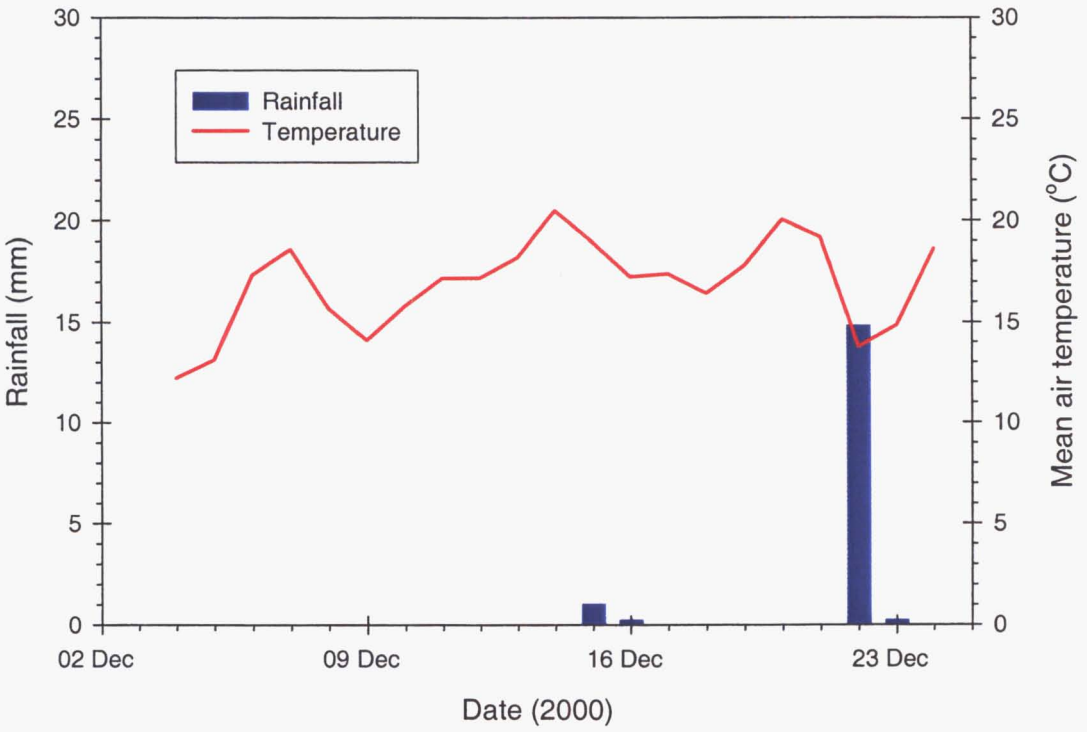


Figure 4.12 Weather conditions during capfall in Experiment Four.

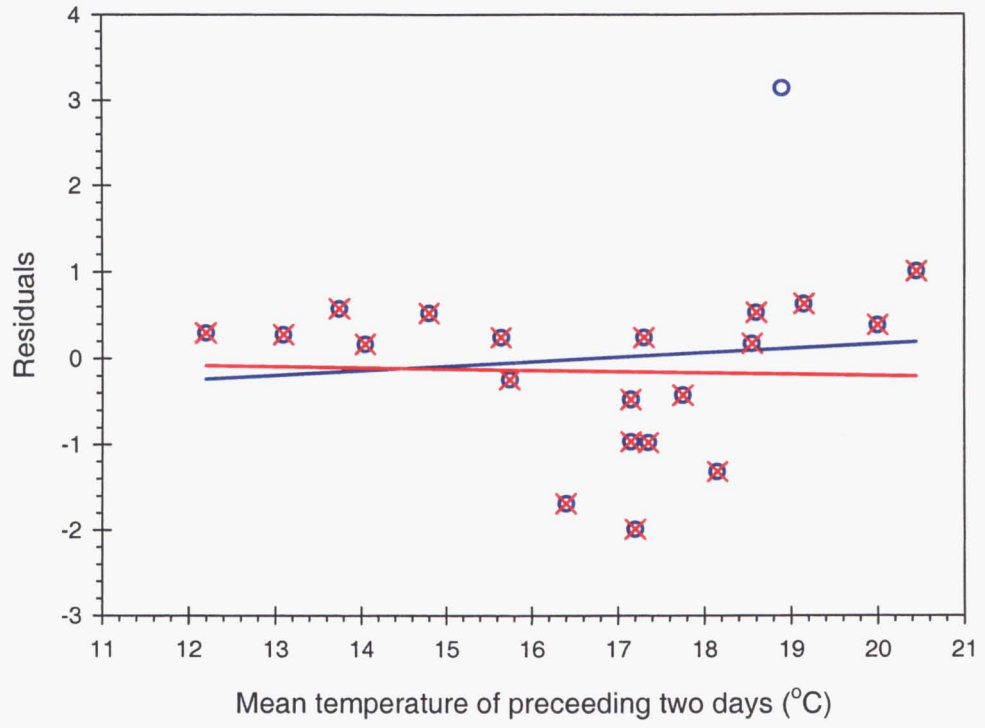


Figure 4.13 The relationship between capfall and temperature in Pinot noir in Experiment Four; all data (○) y = -0.90x + 0.053, $p = 0.631$; adjusted for rainfall (×) y = 0.08x + -0.014, $p = 0.873$.

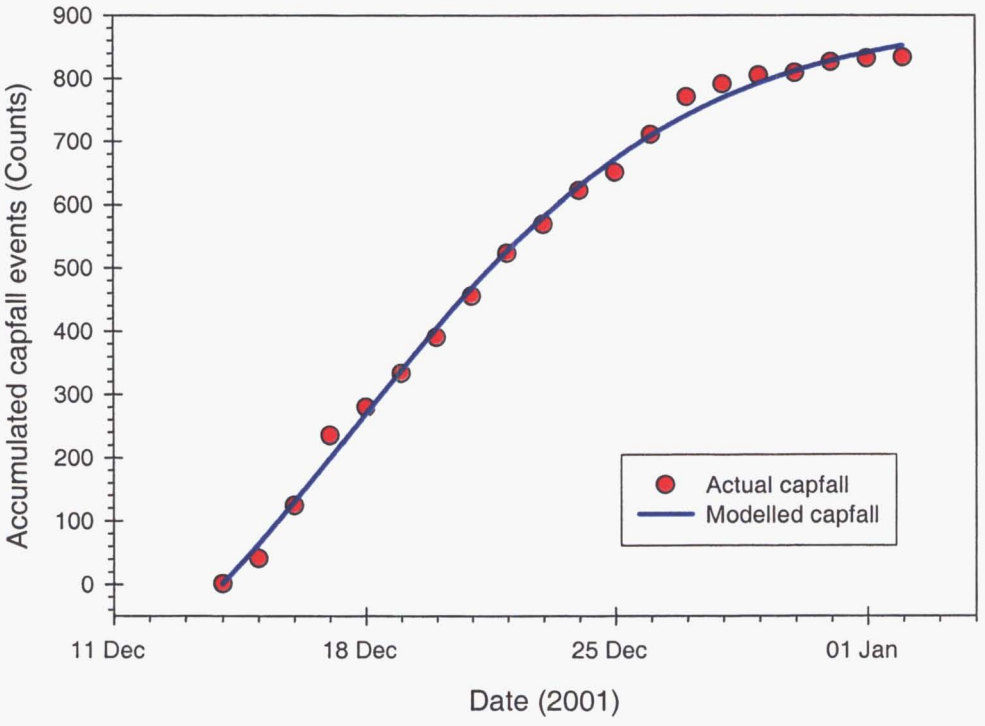


Figure 4.14 The progression of capfall in Cabernet Sauvignon in Experiment Five; logistic curve y = -415+1316/(1+EXP(-0.2118*(x-17.66))), $R^2 = 0.99$.

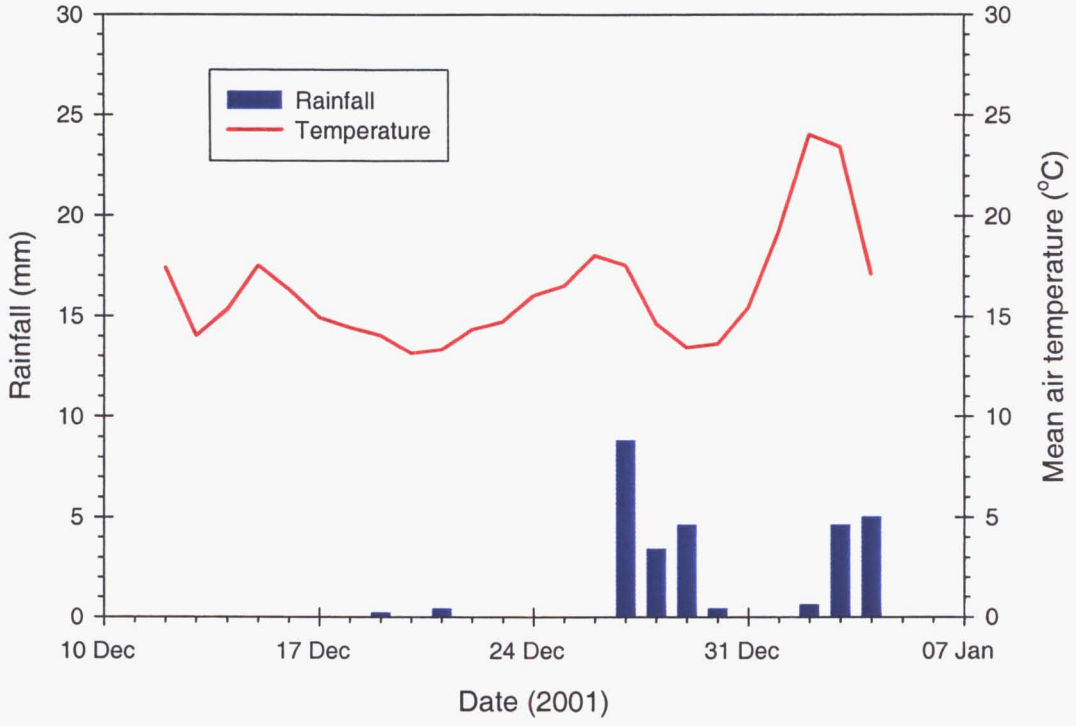


Figure 4.15 Weather conditions during capfall in Experiment Five.

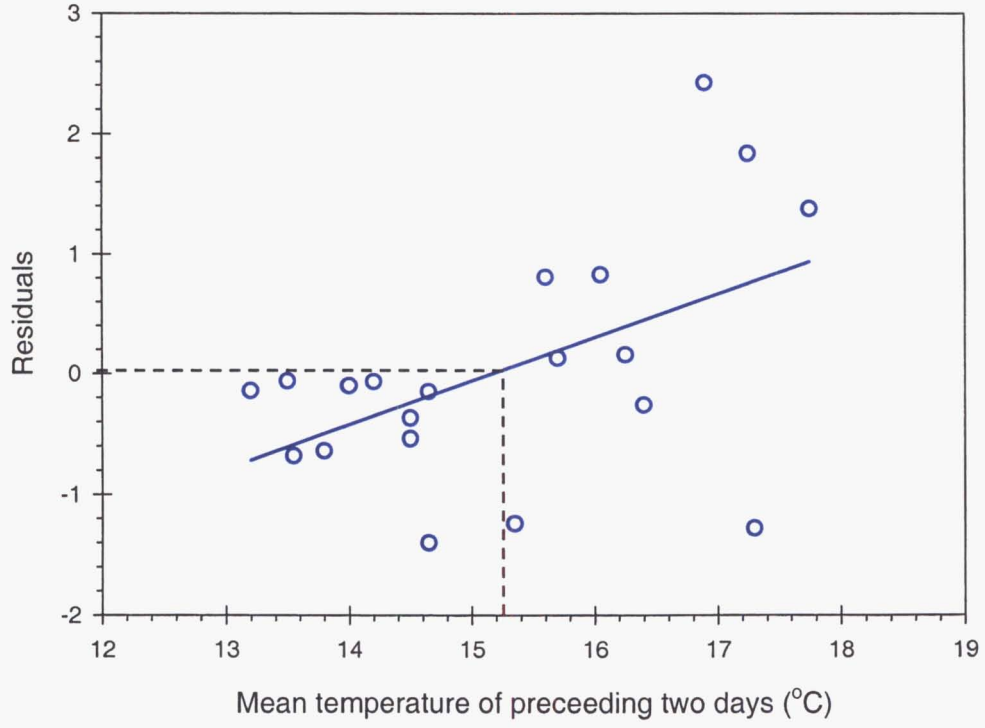


Figure 4.16 The relationship between capfall and temperature in Cabernet Sauvignon in Experiment Five; all data (○) $y = -5.52x + 0.364$, ($R^2 = 0.21$), $p = 0.022$.

4.4 Discussion

4.4.1 Flower characteristics

Ovary diameter was found to vary at capfall (Table 4.2), suggesting that any variation in flower size during flower initiation (Ezzili 1993) and the five weeks before capfall (May 1986) still remains at capfall and is a consistent characteristic of grapevines. May (1986) found the variation in flower size within inflorescences to be systematic; individual branches possess flowers of different sizes and this variation in flower size is consistent between branches. May (1986) pondered whether a flower size effect influenced persistence of the flowers at anthesis and the further development of the berries.

4.4.2 Flowering progression

The start (Table 4.4), duration (Table 4.2), or progression (Table 4.5) of flowering was not affected by girdling, leaf area removal or spectinomycin application. Treatments were applied at the very beginning of capfall and were not expected to influence capfall. Calyptrae are thought to fall when turgor of the interlocking marginal cells change in response to diurnal photoperiod rhythms (Swanepoel & Archer 1988). It is unlikely that the treatments applied will affect diurnal photoperiod detection, but girdling has been shown to reduce xylem water flow in trees by 10-15% (Zwieniecki *et al.* 2004).

The total period of flowering of the tagged flowers is longer than that reported by Friend *et al.* (2003) [9 days], Staudt [11 days] (1999) and Perold [14 days] (1927), and may be a reflection of New Zealand's maritime climate (Friend *et al.* 2003). The observation that Cabernet Sauvignon may flower for a longer duration than Pinot noir is based on one season's data and should be treated as an observation only, though it is recognised that different cultivars vary in the timing and length of various phenological stages (McIntyre *et al.* 1982).

Flower size, as measured by ovary diameter, may influence the time when individual flowers undergo capfall. When examining the populations of tagged flowers, smaller flowers tend to undergo capfall after larger flowers (Figure 4.7). No relationship was found between the mean flowering date of individual inflorescences and ovary diameter (data not presented), suggesting that the relationship in Figure 4.7 is a result of differences within inflorescences and not between inflorescences. To a certain extent the vertical scatter on any one day is due

to small flowers opening on the early inflorescences at the same time as large flowers on late inflorescences.

A similar negative relationship between flower size and date of opening has been found in boysenberry (Trought 1983) and apple (Ferree *et al.* 2001; Westwood *et al.* 1967). However the arrangement of boysenberry and apple flowers shows less complexity than grape, which are arranged as a panicle with more or less cymose capfall. The influence of the complexity of the grapevine inflorescence on capfall is unknown, but could conceivably create a situation, particularly in regions with an extended flowering duration, where small (lateral) flowers on lower order branches could open before larger (terminal) flowers, on higher order branches.

Although the slopes of the regressions in Figure 4.7 have statistical significance, the fit of these is poor, suggesting that ovary diameter is not a strong predictor of capfall date. Analysis of the individual inflorescences over the three seasons of experimentation, found that the majority of inflorescences exhibit a negative relationship, but in only a small proportion of these is the relationship significant (Table 4.6), suggesting that other factors are more influential in determining capfall date of individual flowers.

It is of note that no flowers opened on 13 December 1999 and only three flowers opened on 16 December 2000 (Figure 4.7) during the peak of capfall. These dates coincided with rainfall events of 28mm and 1mm respectively. Decreases in air temperature are typically associated with rainfall events, and are evident on these occasions (Figure 4.9 & 4.12). This and part of the variation around the mean for each date is likely related to temperature or rainfall. Low temperatures and rainfall have been reported to disrupt (May 1986) and increase the duration (Staudt 1999) of capfall.

The effects of the treatments on the relationship between the date of capfall and ovary diameter were inconsistent with girdling altering the y intercept in Experiment Four (2000), but not in Experiment Three (1999). Shoots with 75% of their leaf area had a steeper slope than the control, but treatments with greater reductions in leaf area were no different. These inconsistencies and the failure of the treatments to alter flowering duration or progression suggest that these effects are probably not *bona fide*.

4.4.3 Temperature and flowering

Flowering may be advanced under warmer conditions and delayed under cooler conditions in many species such as kalanchoe (Englemann *et al.* 1974), *Cestium nocturnum* (Overland 1960), and almond (Degrandi-Hoffman *et al.* 1996). A similar relationship, based on observation, has been described for grapevines (Guillon 1905; May 1986; Randhawa & Negi 1965; Staudt 1999). An attempt has been made to confirm and define the effect of temperature conditions on the progression of capfall. Extrapolation from where the residual values are zero would suggest, at least in 1999 and 2001 (Figure 4.10 & 4.16) that temperature below about 15°C will delay flowering and above about 15°C will advance flowering in grapevines, though this is not evident for Pinot noir in 2000 (Figure 4.13). Temperatures above 15°C during flowering have been found to enhance other aspects of grapevine cropping. Average bunch weight increases suddenly when the average temperature during flowering is greater than 16°C (MacGregor 2000). Looking at the time during the day when flowers begin opening in South Africa, Perold (1927) found in fine weather flowers begin opening at 7am if the temperature has reached 15°C. Flowering is slow at 15°C, occurs at a normal rate at 17°C and rapidly at 20-25°C. In warm temperate zones, flowering often begins when the mean daily temperature reaches 20°C, but rather than being a temperature response may reflect the typical temperature environment when flowers have completed their development. Despite the above reports and the findings above (Figures 4.10, 4.13 &4.16) of 15°C being a turning point for the advancement of capfall, 15°C appears to be entirely arbitrary with no physiological basis.

It is of note that in 1999 the growing degree days (base 10°C) accumulated over December (i.e. capfall) were considerably less than the long term average and in 2001 the accumulated growing degree days for this period were slightly less than average, while in 2000 growing degree days were above average [Refer Appendix F, Figure 4]. Any temperature related advance or delay in capfall may be accentuated in cool to average seasons.

Care must be taken when interpreting the effect of temperature on the progression of capfall. To a certain extent the nature of this relationship (Figures 4.10, 4.13 & 4.16) may be a reflection of fitting a logistic curve to the data, however, fit of these curves to the whole population and to individual inflorescences (Appendix D) suggests it is accurate and appropriate. In addition, the experiments reported here were not specifically designed to elucidate such a relationship and more accurate interpretation of data may be gained from temperature controlled experiments.

Mean temperature over the preceding two days gave the greatest fit to the data in these experiments, but no biological basis for using this time frame can be identified. Staudt (1999) found that maximum temperature of the preceding day was a good predictor of capfall. The reasoning above and the inconsistent relationship in Pinot noir between the 1999 and 2000 seasons indicate further investigation is required regarding the precise effect of temperature on capfall.

Flowering is thought to be disrupted when rainfall events occur [accompanied with decreases in temperature] (Jackson 2000; May 1986; Perold 1927; Winkler *et al.* 1974); in light of this, data in Figures 4.10 and 4.13 on days with rainfall events has been excluded from the regression analysis. Doing so improves the fit of the regression to the data, providing further evidence of the negative impact that rainfall has on capfall. This is in agreement with Staudt (1999) who suggested that only adverse circumstances (i.e. very low temperature) would hinder developmental process, which result in capfall. No data associated with rainfall were excluded for Experiment Five (Cabernet Sauvignon 2001) (Figure 4.16), as rainfall occurred at the end of flowering and was likely to have had less of an impact on the progress of flowering as most flowers had undergone capfall.

4.4.4 Capfall and the inflorescence

The complexity of the grapevine inflorescence may be an important factor influencing capfall, potentially interacting with any temperature/flowering relationship. The grapevine inflorescence can be divided into a number of coflorescences, each with a reducing amount of branching, lending a conical shape. The individual flowers are typically arranged in dichasia that may be compressed into each other or reduced in flower numbers (especially as branching lessens). The nature of this complexity has been hard to classify with taxonomists yet to confirm either a monopodial (Barnard 1932; Bugnon 1953; May 1964; Winkler & Shemsettin 1937) or sympodial (Alleweldt & Balkema 1935; Troll 1969) organisation of the inflorescence. A number of researchers have tried to identify patterns of capfall across the inflorescence. In Pinot noir flowers of branches one and two of the main florescence (the tip of the inflorescence) open later than those of all other branches (May 1986). May (1986) also found that the terminal, central flowers of the primary and secondary branches are larger than lateral flowers and also undergo capfall before lateral flowers. Winkler *et al.* (1974) state that flowering progresses in an acropetal pattern across the inflorescence, but Castelli and Pisani

(1985) observed nine different patterns of capfall, depending on the cultivar being examined. It is hard to separate flower position effects from flower size effects as terminal flowers are larger than lateral flowers. More work is required on the influence of inflorescence complexity, above the level of the dichasium, on the progression of capfall, and possible effects on fruit set and berry development.

Chapter V

Grapevine fruit set

5.1 Introduction

At flowering yield potential is at its maximum; the extent to which this potential is realised is dependent on successful flowering, leading to fertilisation. Successful fertilisation occurs when the male and female gametes combine. However successful fertilisation is not a requirement for fruit set.

Fruit set occurs when a suitable stimulus, such as pollination, is received, preventing flower abscission. Typically fruit set represents a change over from the static condition of the flower to the rapidly growing conditions of the young fruit (Coombe 1962; Weaver 1976). The amount of fruit set is both genetically and climatically determined, with some cultivars setting most of their flowers, while others only a small percentage (Lavee & Nir 1986). Grapevines typically set between 5 to 35% of the flowers (Coombe 1973), depending on cultivar and season.

Nutrient deficiencies of boron (Gärtel 1974), molybdenum or zinc (Bindra 1989), cultural activities such as leaf removal at flowering (Candolfi-Vasconcelos & Koblet 1990), and environmental factors like low temperatures, overcast skies, or rain (Caspari *et al.* 1998) are often associated with reduced fruit set. These factors may reduce photosynthesis or interfere with carbohydrate availability. Caspari & Lang (1996) suggest that carbohydrate availability is a major determinant of fruit set in field grown grapevines.

Fruit set and fertilisation are not necessarily one and the same process. Once set, the extent to which a berry develops is determined by how successful the fertilisation was. Successful fertilisation will result in the formation of a seed; an arrest of seed development after fertilisation can result in a parthenocarpic berry, which is smaller than a seeded berry. Final berry size is closely linked to its total seed content (Coombe & Iland 2004; May 2000).

This chapter uses data from Experiments Three, Four, and Five, as described in Chapter Four. It examines the formation or setting of berries from individual flowers. Specifically, the

chapter will attempt to identify any effect of the progression of flowering on fruit set, examine any effect of temperature around flowering on fruit set and try to identify any role of leaf removal on fruit set.

5.2 Experimentation

5.2.1 Experimental aims

The aims of the three experiments were (For further details refer to Section 4.2):

Experiment Three (Pinot noir 1999): To examine the effect of shoot girdling on flowering, fruit set, and berry development. Two shoots per vine, of similar vigour were selected, and one of them girdled between nodes, two and three. Thirty flowers were tagged on the basal inflorescence of each shoot.

Experiment Four (Pinot noir 2000): To examine the effect of the antibiotic, spectinomycin, on flowering, fruit set, and berry development. Three shoots per vine, of similar vigour were selected, with one shoot girdled, another treated with the antibiotic Spectinomycin, and the other a control.

Experiment Five (Cabernet Sauvignon 2001): To examine how modifying carbohydrate supply influences flowering, fruit set, and berry development. Four shoots per vine, of similar vigour were selected and each one girdled. Portions of leaves were removed to reduce leaf area per shoot by approximately, 0, 25, 50, and 75%

These experiments followed the capfall of individual flowers to relate flower behaviour and environmental conditions to fruit set and berry development. In addition treatments were applied to alter the carbohydrate balance of shoots or modify seed development within berries, manipulating fruit set and berry development.

5.2.2 Data collection and statistical analysis

In addition to the date of capfall of individual flowers (Refer Section 4.2), data on the date of *véraison* and type of berries were collected.

Given the variation in the developmental stage of individual berries within bunches (Glynn & Boulton 2001), it is important to harvest berries at the same developmental stage to allow valid comparisons between berries. *Véraison* is a term used in a wide sense to embody a group of developmental changes in berries that occur as ripening begins (Coombe & Bishop 1980). These changes include: sugar accumulation, reduction in acidity, development of colour, loss of chlorophyll, increase in elasticity of the berry, and a renewed rapid expansion of berry volume. Given the numbers of berries that were being monitored berry colouration was used to define the point of *véraison*; this was defined as being when the first tinge of colour was visible on the berry (Figure 5.1) as per the original usage of the term in French.

At harvest berries were dissected to extract seeds, seed traces and ovule remnants, where present. The combined fresh weight of each berry's seed content was recorded. Berries were separated into three classes, based on the description of berries by Colin *et al.* (2002) and seed content:

1. Seeded berries – Large, coloured, soft berries containing at least one normal seed.
2. Seedless berries – Coloured, soft berries exhibiting some increase in diameter, but being considerably smaller than seeded berries. These berries contain seed traces.
3. Shot berries – Flowers/berries that show little to no growth and remaining on the bunch as small green berries, approximately two to three millimetres in diameter.

Statistical analysis of data by ANOVA and regression was completed using Genstat 5 Release 4.1 (Lawes Agricultural Trust, England). To examine the effect of time of capfall on berry development and fruit set, individual capfall events within inflorescences were expressed on a proportional basis and divided into five equal time periods; as the start date and duration of capfall for each inflorescence varied [Refer appendix D].

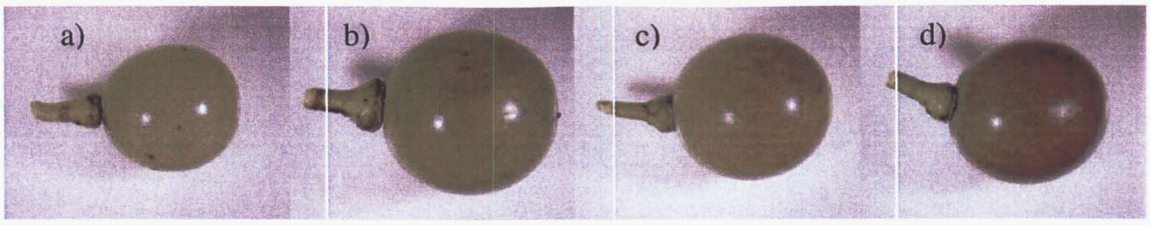


Figure 5.1 The development of colour in berries going through *véraison*: a) a green berry, b) first signs of colouration with a pink spot developing, c) enlargement of the pink spot to cover about one quarter of the berry, d) further spread and darkening of colour.

5.3 Results

5.3.1 Effect of girdling and spectinomycin on berry development and fruit set

Girdling shoots altered the development of berries and assessments of percentage fruit set (Table 5.1 & 5.2). In Experiment Three (Pinot noir 1999) girdling increased the proportion of seedless berries, decrease the proportion of shot berries and had no effect on the proportion of seeded berries or abscised flowers. A similar effect was evident in Experiment Four (Pinot noir 2000), however instead of decreasing the proportion of shot berries, the proportion of abscised flowers was decreased, with shot berries being unaffected (Table 5.2). The percent fruit set is increased with girdling, as calculated excluding shot berries (Table 5.1 & 5.2). However in Experiment Three (Pinot noir 1999) no difference in fruit set was evident when shot berries were included in the fruit set calculation (Table 5.1).

Spectinomycin had no effect on the development of berries or percentage fruit set (Table 5.2).

The progressive reduction in leaf area as applied in Experiment Five (Cabernet Sauvignon 2001) had a large effect on berry development and fruit set. Leaf area removal caused the proportion of seeded and shot berries to decrease and increased the proportion of seedless and abscised flowers (Table 5.3). However the treatment was not progressive in its effect, with changes in berry development only occurring when about 75% of a shoot's leaf area was removed (i.e. the 25% leaf area treatment). A covariate effect was also evident, with total shoot leaf area influencing the development of seedless berries. As shoot leaf area increased a greater proportion of seedless berries tended to set (Figure 5.2). Reducing leaf area did not alter fruit set when calculated excluding shot berries, but did so when including shot berries. Fruit set is dramatically reduced when about 75% of a shoots leaf area is removed (Table 5.3).

Table 5.1 Influence of girdling on the subsequent development and fruit set of tagged flowers for Experiment Three.

	Flower development (%)				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Girdled	14.6	34.4	4.4	46.7	49.1	53.4
Control	9.1	5.7	32.4	52.8	14.8	47.2
Significance	0.152	<0.001	0.001	0.402	<0.001	0.397
LSD (5%)	7.9	10.9	14.1	15.7	8.6	15.8

Table 5.2 Influence of girdling and spectinomycin treatment on the subsequent development and fruit set of tagged flowers for Experiment Four.

	Flower development (%)				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Spectinomycin	46.7	24.3	1.3	27.7	71.0	72.3
Girdled	43.2	36.9	1.8	22.0	76.2	78.0
Control	40.9	20.4	3.1	35.6	61.3	64.4
p value	0.700	0.018	0.595	0.044	0.027	0.044
LSD (5%)	14.48	11.37	3.67	10.49	10.63	10.49

Table 5.3 Influence of shoot leaf area on the subsequent development and fruit set of tagged flowers for Experiment Five.

	Flower development (%)				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Control (100% leaf area)	35.1	8.2	21.0	35.6	43.4	64.4
75% leaf area	27.5	8.4	37.3	26.9	35.8	73.1
50% leaf area	32.3	6.5	31.1	30.1	38.8	69.9
25% leaf area	0.0*	17.4	4.9	80.2	14.9	19.8
Significance	<0.001	0.423	0.017	<0.001	0.076	<0.001
LSD (5%)	12.2	19.6	30.8	23.4	23.44	26.4
Covariate	0.640	0.021	0.815	0.075	0.087	0.075

Note: Due to the abscission of most inflorescences with this treatment, ANOVA returned a value for seeded berries of -2.5.

5.3.2 Flower size and berry development

No relationship between flower size (ovary diameter) and berry development was observed, with small and large flowers being capable of forming seeded, seedless, and shot berries, or abscising. Likewise, small flowers did not form small berries, nor do large flowers form large berries (Figure 5.3 a, b, c). However flower size related to berry size in some experiments, though the relationship is not always consistent. In Experiments Three (Pinot noir 1999) and Five (Cabernet Sauvignon 2001) larger flowers resulted in larger shot berries; while seedless and seeded berries in Experiments Three (Pinot noir 1999) and in Five, respectively, also show the same relationship [Refer to Appendix F, Table 3 for regression equations and statistics].

5.3.3 Effect of time of capfall on berry development and fruit set

An examination of the effect of early, mid and late capfall on flower development and fruit set was attempted. In 1999 and 2001 (Experiments Three and Five, respectively) early, mid, or late flowering did not favour the development of any type of berry (Table 5.4 & 5.3). However, in 2000 (Experiment Four) flowers that opened early had a greater probability of developing into seeded berries, while flowers that opened mid to late capfall had a greater probability of developing into seedless berries (Table 5.5). The formation of shot berries or of flowers abscising were not influenced by the time of capfall of individual flowers.

5.3.4 Temperature and berry development

As the flowers monitored in Experiments Three to Five opened over an extended period of time, different flowers opened under a range of temperature conditions. This allowed an attempt to examine whether the probability of a particular berry type setting was related to temperature conditions immediately post capfall. A range of temperature indices were calculated and compared (i.e. Maximum, minimum, and mean temperature on the day of capfall, two days post capfall and three days post capfall). In Experiment Three (Pinot noir 1999) the mean air temperature for the three days post capfall was found to influence the formation of seedless berries, with warmer temperatures increasing the probability seedless berries forming (Figure 5.4). However in Experiment Four (Pinot noir 2000) cooler temperatures favoured the formation of seedless berries (Figure 5.5). In Cabernet Sauvignon (Experiment Five, 2001) no significant relationships were found, but the probability of a

seeded berry developing tended to be less with lower the mean minimum air temperature for the three days post capfall (Figure 5.6).

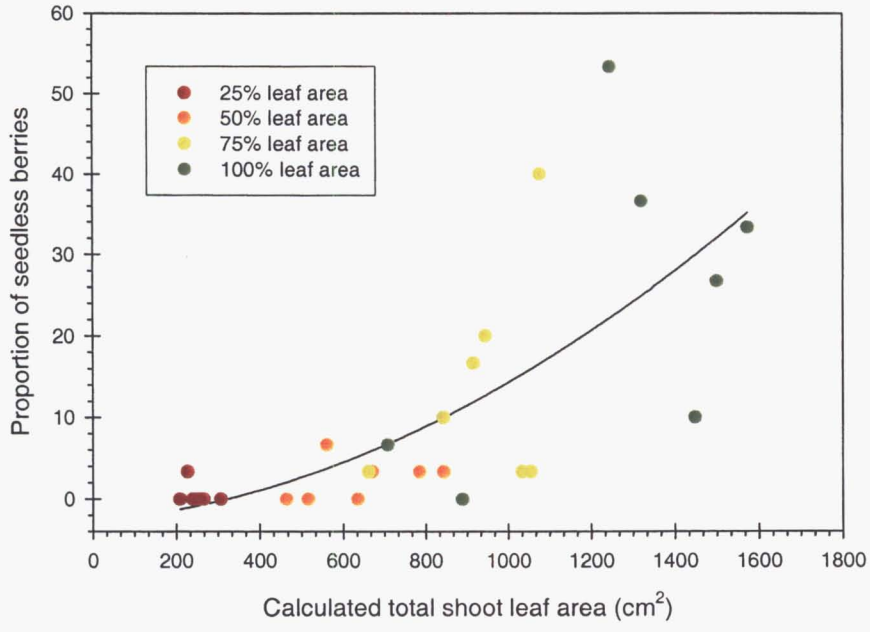


Figure 5.2 Relationship between shoot leaf area and the formation of seedless berries in Cabernet Sauvignon (Experiment Five 2001); $y = -24.9 + 19.6*1.00^2x$, $R^2 = 0.48$, *F* value = <0.001.

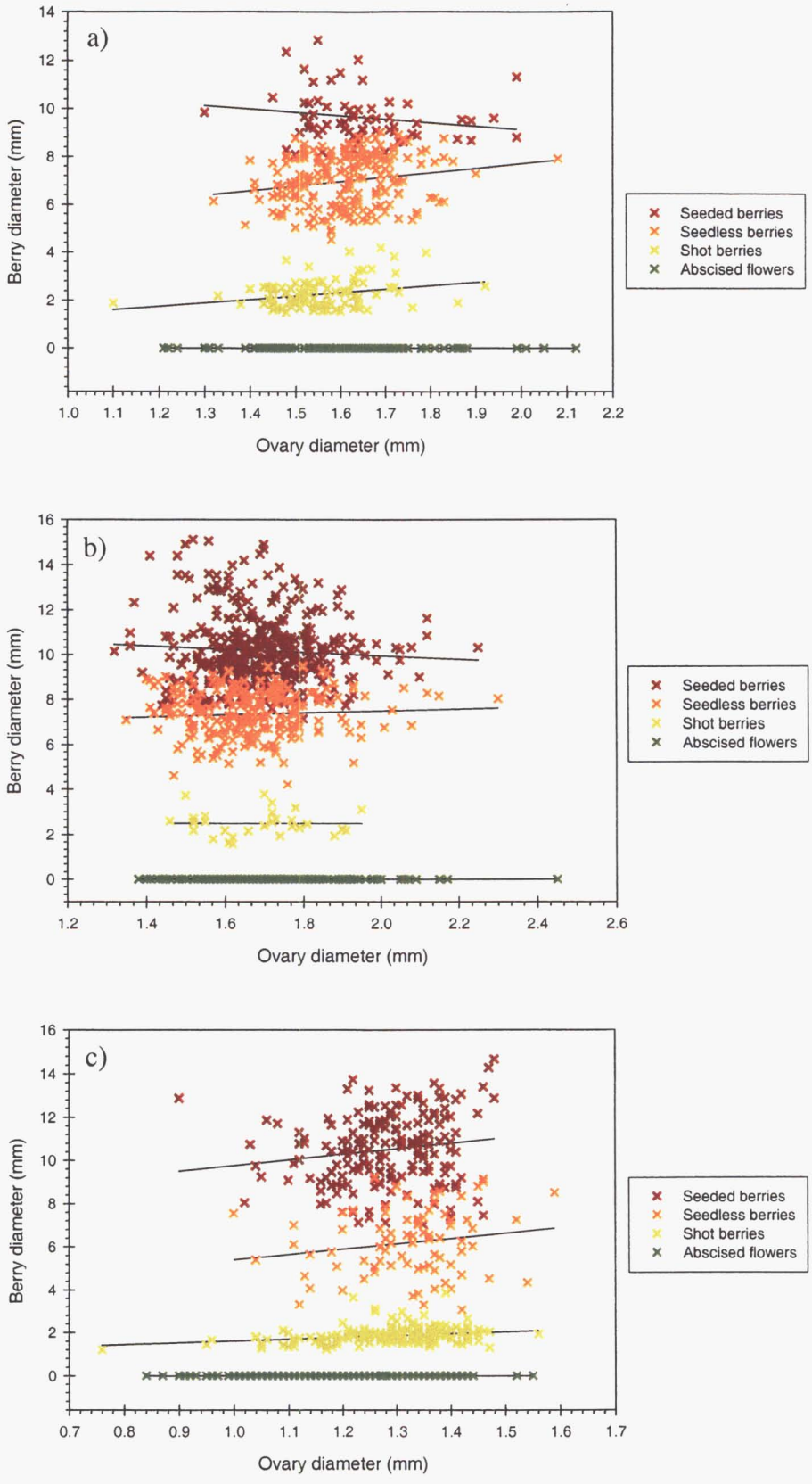


Figure 5.3 The relationship between flower size and berry development in a) Pinot noir from Experiment Three (1999); b) Pinot noir from Experiment Four (2000); c) Cabernet Sauvignon from Experiment Five (2001); Regression equations are presented in Appendix F Table 4.

Table 5.4 Effect of time of capfall on the percentage of flowers setting seeded, seedless, or shot berries or abscising for Pinot noir in Experiment Three (1999).

	Flower development				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Time period 1	0.086	0.149	0.146	0.619	23.5	38.1
Time period 2	0.106	0.167	0.102	0.526	27.2	37.4
Time period 3	0.074	0.284	0.162	0.431	35.7	51.9
Time period 4	0.095	0.225	0.137	0.494	31.9	45.6
Time period 5	0.157	0.144	0.302	0.397	30.1	60.3
P value	0.643	0.335	0.091	0.180	0.643	0.062
LSD	0.115	0.156	0.150	0.193	16.5	17.8

Table 5.5 Effect of time of capfall on the percentage of flowers setting seeded, seedless, or shot berries or abscising for Pinot noir in Experiment Four (2000).

	Flower development				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Time period 1	0.576	0.134	0.081	0.204	71.0	79.0
Time period 2	0.654	0.178	0.021	0.214	53.2	55.3
Time period 3	0.336	0.219	0.029	0.253	55.5	58.4
Time period 4	0.333	0.221	0.043	0.236	55.4	59.7
Time period 5	0.365	0.336	0.009	0.291	70.1	70.9
P value	0.007	0.018	0.125	0.724	0.111	0.035
LSD	0.150	0.120	0.057	0.134	17.64	17.0

Table 5.6 Effect of time of capfall on the percentage of flowers setting seeded, seedless, or shot berries or abscising for Cabernet Sauvignon in Experiment Five (2001).

	Flower development				Fruit set (%)	
	Seeded berries	Seedless berries	Shot berries	Abscised flowers	Excluding shot berries	Including shot berries
Time period 1	0.210	0.132	0.241	0.417	34.2	58.3
Time period 2	0.231	0.079	0.236	0.415	31.0	54.6
Time period 3	0.232	0.093	0.221	0.454	32.6	54.6
Time period 4	0.195	0.122	0.274	0.410	31.7	59.0
Time period 5	0.222	0.083	0.194	0.501	30.5	49.9
P value	0.957	0.765	0.815	0.548	0.984	0.633
LSD	0.111	0.099	0.131	0.123	13.0	12.7

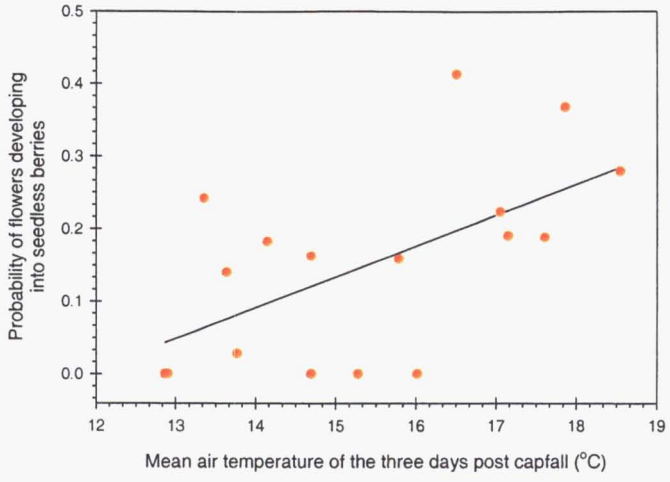


Figure 5.4 Relationship between the formation of seedless berries and air temperature in Experiment Three (Pinot noir 1999); y = -0.504 + 0.0425x, *F* value = 0.013, R^2 = 0.30.

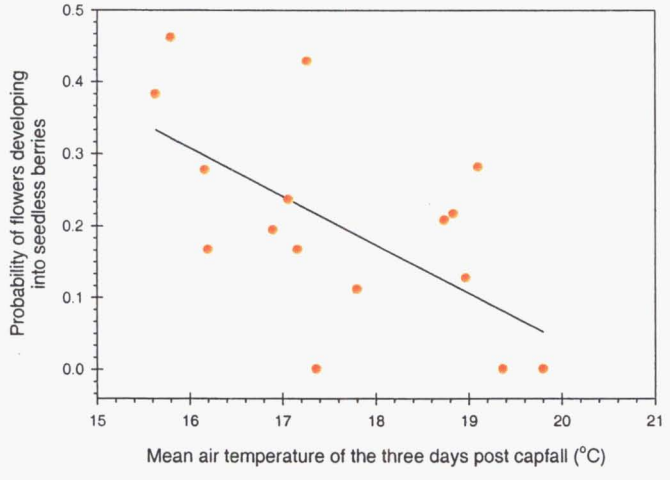


Figure 5.5 Relationship between the formation of seedless berries and air temperature in Experiment Four (Pinot noir 2000); y = 1.387 + -0.0674x, *F* value = 0.007, R^2 = 0.35.

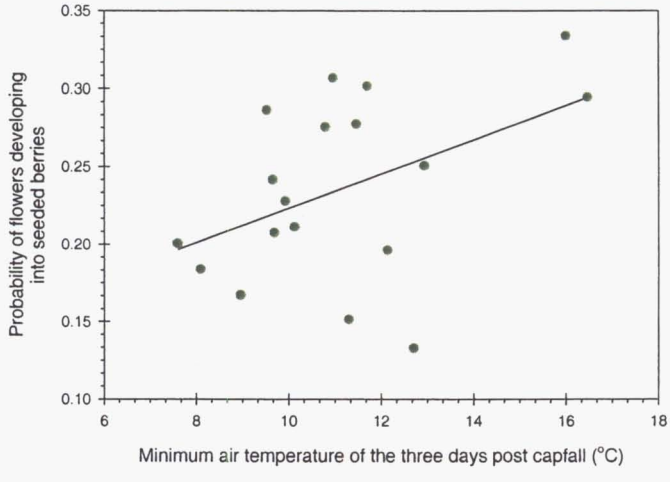


Figure 5.6 Relationship between the formation of seeded berries and air temperature in Experiment Five (Cabernet Sauvignon 2001); y = 0.113 + 0.011x, *F* value = 0.064, R^2 = 0.14.

5.4 Discussion

5.4.1 Effect of girdling and spectinomycin on berry development and fruit set

Girdling is known to increase fruit set in a range of perennial fruit crops (Noel 1968) including grapes (Brown *et al.* 1988; Caspari *et al.* 1998; Coombe 1959; Weaver *et al.* 1962). Typically girdling is used to enhance the productivity of seedless grapes (Winkler *et al.* 1974). Girdling without leaf removal increases fruit set on shoots (Caspari *et al.* 1998) and entire vines (Brown *et al.* 1988; Coombe 1959). Fruit set is increased as a greater number of seedless berries (Brown *et al.* 1988) or fewer-seeded berries (Coombe 1959) are retained, resulting in a decrease in average berry size; though Zhang *et al.* (2003) reported an increase in berry size when girdling Kyoho.

The findings from Experiments Three (1999) and Four (2000) in Pinot noir are in support of the literature, with fruit set (calculated excluding shot berries) being increased as a result of a greater number of seedless berries. Where these additional seedless berries originate from is of interest. In Experiment Three (1999) the shot berries appear to become seedless berries with girdling, while in Experiment Four (2000) girdling appears to prevent the abscission of abscised flowers, but also allow them to be converted into seedless berries. A possible explanation for the different origin of the increased number of seedless berries with girdling could be a reflection of the seasonal weather conditions. In Experiment Three (1999) the growing degree days accumulated over flowering (December) were considerably lower than the long term average, while in Experiment Four (2000) the growing degree days were higher than average [Appendix F, Figure 4]. Overall shot berries are much less prevalent in 2000 than in 1999. Shot berries are generally only prevalent in years of poor fruit set (Bindra 1989), which is often associated with cool and wet conditions during flowering (May 1987).

The absolute levels of fruit set differ between the warm 2000 experiment and the cool 1999 experiment. Percent fruit set is greater in 2000 (71% grand mean) than in 1999 (41% grand mean), while the proportions of shot berries and abscised flowers are lower. No statistics support these values as being different, but they are consistent with the observation that most commercially grown cultivars show reduced fruit set when weather conditions are unfavourable (May 1992).

The warmth of the 2000 flowering period appears to have hindered the formation of shot berries, with a higher proportion of seedless berries in the 2000 control, compared to the 1999 control, suggesting that under warm conditions flowers that would normally form shot berries develop into seedless berries. Under this situation girdling increases fruit set by allowing more flowers that would otherwise abscise to develop into seedless berries; this is reflected in the lower proportion of flowers that abscise. When flowering is cool (as in 1999), more flowers abscise and more shot berries form. In this situation, girdling appears to be insufficient a stimulus to encourage additional flowers from setting hence the lower percentage fruit set in 1999 (*c.f.* 2000), but will encourage the development of what would be shot berries into seedless berries.

At the time of fruit set, shot berries appear to form a pool from which seedless berries may develop. Shoot girdling provides a stimulus allowing a greater proportion of shot berries to develop into seedless berries. This may only occur in environments where temperatures over flowering are at the lower limit for fruit set. The modified carbohydrate (Weaver & McCune 1959) and plant growth hormone status (Weaver & Pool 1965) of girdled shoots may encourage further development of ovules, resulting in cell division and expansion of the mesocarp of what would otherwise be shot berries. It is expected that a cultivar's propensity to form shot berries will influence the effect of shoot girdling on the fate of flowers. These comments are reflected in the calculations of fruit set excluding and including shot berries in Tables 5.1 and 5.2. However these results are the result of only two seasons and require further confirmation. The lack of change in the proportion of seeded berries that form suggests girdling is not affecting the levels of fertilisation resulting in normal seeds.

Fruit set and flower abscission are thought to be under the control of carbohydrate supply (Aziz *et al.* 2001; Gomez-Cadenzas *et al.* 2000). Girdling halts the flow of phloem, preventing the export of carbohydrates to other sinks, increasing the availability above the girdle, leading to enhanced fruit set (Caspari *et al.* 1998). The increase in fruit set from girdling can be progressively reduced by leaf removal (Caspari & Lang 1996). Caspari *et al.* (1998) suggest that 38% percent of a girdled shoot's leaf area can be removed without affecting fruit set.

The effect of leaf area removal on girdled shoots in Experiment Five (Cabernet Sauvignon 2001) were similar to those presented by Caspari *et al.* (1998), with leaf area removal altering

fruit set, as well as berry development, however, rather than a progressive effect, only the 75% leaf area reduction treatment had an effect. With 75% shoot leaf area removal the proportion of seeded and shot berries were reduced and the number of abscised flowers increased dramatically. It was unexpected that fruit set and berry development were only altered with such a large decrease in leaf area, especially when the shoots are girdled. Examining the total shoot leaf area of individual shoots, it was noted that the leaf area of some shoots were closer to those of other treatments [Appendix F, Table 1]. The consequence of this may mean that some shoots of higher leaf area treatments could behave like treatments with lesser treatment leaf areas. These differences in total shoot leaf area are probably a reflection of variation in shoot vigour, with some shoots having different numbers of leaves of different sizes. The impact of these differences is that treatments were not consistent, confounding the data.

The decrease in the proportion of seeded berries with the 75% leaf area reduction suggests that seeded berry development is sensitive to carbohydrate supply, requiring some sort of base level to allow seeded berries to form. However once this demand is met, enhancing carbohydrate supply (i.e. girdling) above the demand will not increase the proportion of seeded berries that develop.

Identifying that total shoot leaf area differed within treatments, leaf area was used as a covariate in the analysis of leaf area removal and showed an effect on the proportion of seedless berries that develop (Table 5.3). As shoot leaf area increases a greater proportion of seedless berries form (Figure 5.3), although there is a greater amount of variation in the relationship at high leaf areas. The poor fit of this relationship may be a reflection of leaf area not being the dominant factor determining berry development, with pollination and fertilisation success also playing major roles, particularly when adequate leaf is present. Another possible explanation for the variation found was that greater leaf shading could be present with low levels of leaf removal. However, the slope of the regression suggests that shoot leaf area does influence the formation of seedless berries, and provides furthers support for carbohydrate supply being an important determinant of berry development.

Spectinomycin can be used to induce seedlessness in grapes by treating inflorescences at flowering (Widodo *et al.* 1999a), though the timing of application is an important determinant of it effectiveness (Widodo *et al.* 1999b). The spectinomycin treatment used in

experimentation in this study was applied at the rate of 200mg/L as per Widodo *et al.* (1999a) at the onset of flowering. However application at the peak of flowering for each inflorescence, as Widodo *et al.* (1999a) applied treatments may have enhanced the effectiveness of the treatment. Had the application of spectinomycin been at a more appropriate time it would be interesting to examine the effect on fruit set and berry development: would treatment disrupt seeded berry formation or enhance the development what would otherwise be shot berries, or increase fruit set and development of abscising flowers?

5.4.2 Flower size and berry development

May (1986) posed the question whether the variation in flower diameter influenced berry development. Small flowers could potentially have a greater chance of setting seed, due to the temperature dependent growth of pollen tubes (Staudt 1982). The shorter styles of small flowers mean pollen tubes have a shorter distance to grow to achieve pollination. Likewise larger flowers may have a competitive advantage, attracting more carbohydrate and increasing their chances of developing. However, Figure 5.3 does not suggest that flower size plays any major role in determining berry type. All berry types are capable of developing from the whole range of flower sizes, with small and large berries of each type forming from large or small flowers.

There may be a relationship between flower size (ovary diameter) and berry size in certain situations. Though some of the regressions in Figure 5.3 have significant slopes [Appendix F, Table 3], the fits are very poor and appear to be strongly influenced by a few values at the extremes in flower size. It was not expected to see a strong relationship between flower size and berry size in the seeded and seedless berries, as the extent to which fertilisation occurs is likely to play a far greater role on berry cellular composition and hence berry size. When examining the whole population, flowers of similar sizes open under different environmental conditions, which could potentially influence the success of fertilisation. To counter any such effect, the relationship was examined on a daily basis where sufficient flowers opened [Appendix F, Figures 1, 2 & 3]. Though some significant relationships exist, they are inconsistent and again are strongly influenced by single points.

If any relationship between flower size and berry size existed, it would most likely be evident with shot berries. Shot berries show no to little growth in size, presumably as a consequence

of the failure of ovules to develop (Kasseymeyer & Staudt 1982). Any growth in size that does occur is probably a true reflection of the cellular potential of the flower and an accurate reflection of the relationship between flower size and berry size. Again on a daily basis, though a number of relationships appear to exist [Appendix F, Figures 1, 2 & 3], they are not consistent on each day and the slopes are influenced by single points. The data suggest that any relationship between flower size and berry size is likely to be of little consequence.

5.4.3 Effect of time of capfall on berry development and fruit set

Grapevine flowers are small and have a relatively weak sink strength compared to other organs (Candolfi-Vasconcelos & Koblet 1990). This weak sink strength is likely to make grape flowers highly susceptible to competition for carbohydrates. At flowering, stored carbohydrate reserves are at their lowest levels (Bennett *et al.* 2005), while fruit set is closely linked to photoassimilate supply (Gomez-Cadenzas *et al.* 2000). The combination of poor competitive sink strength and limited carbohydrate supply could create a situation where the time of capfall might influence fruit set and berry development. Potentially flowers that open early during capfall have a greater access to carbohydrates increasing the percentage fruit set that occurs or favouring the formation of seeded, seedless or shot berries. Likewise, flowers that open later during capfall may have to compete for carbohydrates that are already allocated to stronger sinks (i.e. growing berries), resulting in a lower percent fruit set through greater flower abscission.

Only one trend in berry development with the time of capfall was evident. In Experiment Four (Pinot noir 2000), flowers that open at the end of capfall appear to have a greater chance of forming into seedless berries. This does not occur in Experiments Three or Five and does not support the hypothesis that due to carbohydrate competition, the time when flowers undergo capfall could influence their fruit set and berry development. Fruit set does not appear to be influenced by the time of capfall of flowers, with only Experiment Four (Pinot noir 2000) showing any significant results. Here fruit set (calculated including shot berries) was greater in the first period of flowering when compared to the middle periods of flowering. The data do not provide strong support of a relationship between the time of capfall and berry development or fruit set. This might be a reflection of how environmental conditions change with time rather than the consequence of using of reserves determining formation of different berry types.

5.4.4 Temperature and berry development

It is significant that no consistent or strong relationship with berry development and fruit set could be found, as it is generally recognised that cool temperature conditions during flowering result in poor fruit set. Comparing a number of seasons, MacGregor (2000) found that average bunch weights increased suddenly when the average temperature during capfall was 16°C or above. One consequence of the extended period of capfall in these experiments is that different flowers can set under quite different climatic conditions. Fruit set and potentially berry development could be determined by these conditions.

Comparisons of the relationship between the probability of different berry types setting and temperature suggest that temperature is a poor predictor of the probability of berry development. Perhaps temperature is not the ideal environmental factor to be using. Roubelakis and Kliewer (1976) found that a combination of low temperature and reduced light intensity caused a large reduction in percent fruit set. Additional experimentation incorporating solar radiation measurement with temperature data would confirm the relative importance of temperature and light and represents a point for further research.

Chapter VI

Berry growth

6.1 Introduction

Though most wine-grape cultivars are considered seeded (Coombe & Iland 2004), some berries possess imperfect seeds and are classified as seedless (May 2004). A strong relationship between seed content and berry size exists in grapevines (Cawthon & Morris 1982; Coombe 1960; Müller-Thurgau 1898; Olmo 1946; Petrie *et al.* 2000; Scienza *et al.* 1978; Winkler & Williams 1936), and it is tempting to hypothesise that seed development, mediated through plant growth regulators, drives initial berry development and hence final berry size.

Considerable variation in berry size exists within grape bunches (Ebadi *et al.* 1996a; Glynn & Boulton 2001; Milne *et al.* 2003; Trought & Tannock 1996). At fruit maturity, the diameter of normal Merlot berries can range between 8 and 15 mm. However, sometimes berry development is abnormal, resulting in ripe berries with a harvest diameter of 4 to 7 mm, and some berries are very small, being 1 to 3 mm in diameter and green in colour (Colin *et al.* 2002).

The final shape and size of a berry is strongly influenced by the number, shape and size of cells in the flesh and skin (Coombe & Iland 2004). Berry growth is a result of cell division (mitosis) and cell expansion. At anthesis the pericarp of Sultana already has approximately 200,000 cells (Harris *et al.* 1968), and will undergo one to two further divisions to achieve 600,000 cells at harvest (Coombe 1976). Cell division in the pericarp is most active during the first 14 days after anthesis, especially during the first week (Ojeda *et al.* 1999). Cells enlarge in two main episodes – from one to five weeks after anthesis, then during phase III. Limitation of either cell division or expansion during phase I berry development could lead to the differences in berry size found in bunches.

Normal growth of the seeded grape berry is characterised by a double sigmoid curve and is typically divided into three phases (Coombe 1976). During phase I, increase in the size of a

berry is due initially to pericarp cell division, with subsequent growth due to cell enlargement (Harris *et al.* 1968). Little to no berry growth occurs in phase II (lag phase), however the seed continues to mature. Phase II is an artificial division physiologically, representing the often indistinct boundary between phase I and III (Coombe & Iland 2004). Phase III is a second sigmoid growth period, where increase in berry volume is due entirely to cell expansion in the exocarp. The onset of the second growth period is referred to as *véraison* and signifies the beginning of sugar accumulation, colour development and ultimately, ripening (Colin *et al.* 2002). A fourth phase may also be considered, where berries enter senescence and shrivel. In contrast to seeded 'normally' developing berries, the growth curves of seedless and shot berries are not as well defined.

6.2 Experimentation

6.2.1 Experiment aim

This experiment was run to describe the growth curves and cellular makeup of seeded, seedless and shot berries and to relate their development and cellular characteristics to berry seed content.

6.2.2 Site, experimental design and data collection

The vines used for data collection were growing in the Lincoln University experimental vineyard, located in the province of Canterbury, New Zealand (43°39' S, 172°28' E) during the 2001/2002 growing season.

A single vine each of Pinot noir and Cabernet Sauvignon were used for data collection; the Pinot noir (clone AM10/5) vine was five-years-old and grafted to Riparia Gloire rootstock, while the Cabernet Sauvignon vine was 26-years-old, grown on its own roots. Three basal inflorescences, each on a separate shoot of typical vigour, were selected; ten flowers on each inflorescence were randomly tagged as per previous experimentation (Refer Chapter 4.0). Tagging was completed and monitoring of flower/berry diameter began on 13 December 2001.

Diameters were measured on a near-daily basis until 9 May 2002, using a pair of digital vernier callipers (Sylvac, Crissier, Switzerland; ± 0.01mm). Measurements were taken during

the early afternoon (NZST). Monitoring of berries was halted when shrivelling was evident (whether a result of damage or reaching physiological ripeness [phase IV]).

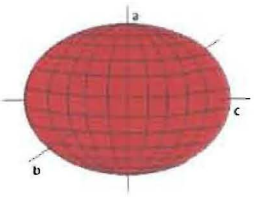
At the completion of monitoring, berries were harvested and dissected to extract seeds, seed traces and ovule remnants, where present. The combined fresh and dry weight of each berry's seed content was recorded. Berries were also separated into three classes, based on the berry description of berries by Colin *et al.* (2002) and seed content:

1. Seeded berries – Large, coloured, soft berries containing at least one normal seed.
2. Seedless berries – Coloured, soft berries exhibiting some increase in diameter, but being considerably smaller than seeded berries. These berries contain seed traces.
3. Shot berries – Flowers/berries that show little to no growth and remaining on the bunch as small green berries, approximately two to three millimetres in diameter.

Six of the Cabernet Sauvignon flowers abscised early during capfall, and were replaced with other berries, as indicated in Table 6.1 and in Figures 6.4 to 6.6. By 6 March 2002 it was obvious that very few seedless Cabernet Sauvignon berries were being monitored. In the interests of attaining a balanced data set, an additional 10 seedless berries were tagged on a fourth Cabernet Sauvignon bunch. The growth curves for these berries have not been graphed as the days after capfall cannot be accurately calculated. However, their final berry diameter and seed content have been incorporated into the regression in Figure 6.8.

The cellular makeup of ten randomly selected seeded, seedless and shot Cabernet Sauvignon berries was assessed at *véraison*. Berries were considered to have been at *véraison* when the first signs of colouration were visible (Figure 5.1). An estimate of the number of cells within each berry, and the average pericarp cell volume (size) was collected from stained berry cross-sections (equatorial cut), cut by hand with a razor blade. Cells were stained using tannic acid and ammonium iron (III) sulphate, as described by Goffinet *et al.* (1995) and the cross-sections micro-photographed through a stereo microscope (SZ60, Olympus. Tokyo, Japan). Photographs were printed to paper allowing assessments of cell volume and number to be made. The width and length of ten randomly selected adjacent cells, within the inner mesocarp, were measured to calculate cell volume (adjusting for magnification). Cell volume was calculated by assuming that the cells were prolate ovoid (ellipsoid) in shape, similar to the method of Harris *et al.* (1968) using the equation:

Cell volume = 4/3 π (abc/2)



Where:

a, b, c are measures of diameter

axis a = b.

An estimate of the number of cells per berry was made by calculating the number of cells present in the cross-section of each berry. A quadrat, representing 20% of the cross-sectional area, was randomly placed within the inner mesocarp of each berry, and the numbers of cells contained within were counted. Cells were counted if at least 50% of their area was within the quadrat. Cell counts were then multiplied by a factor of five to estimate the number of cells within the cross-section. The assessments of cell volume and number were used to describe their relationship with berry volume. Berry volume was calculated from measurements of berry diameter, assuming the berries to be a sphere, using the equation:

$$\text{Berry volume} = 4/3\ \pi\ (\text{diameter}/2)^3$$

6.2.3 Statistical analyses

The data in figures 6.1 to 6.6 have been graphed ignoring any missing data to enhance the clarity of the curves, allowing description of individual growth curves.

In order to identify the point where seeded berries began Stage I growth and when seedless and shot berries take an alternative path of development to seeded berries, the growth curves of each individual berry were 1/log(x) transformed, as per Ott (1993), to provide a curve with two linear sections. Linear regressions were then fitted to the linear portions of each part of the curve, and the point of interception calculated from the regression equations [See appendix H]. The points of interception were used to calculate the days after capfall when berries began their characteristic growth patterns. The resulting data were analysed via analysis of variance (ANOVA) using Genstat 5 (Release 4.1; Lawes Agricultural Trust. Rothamsted, England).

Regressions of seed content, cell volume and cell numbers were calculated using Genstat 5 and graphed using SigmaPlot 9.0 (Systat Software. Richmond, CA, USA).

6.3 Results

6.3.1 Berry growth

The tagging of flowers to monitor berry growth was undertaken pre-anthesis, which meant that ensuring all berry classes were represented was a random event. Over all, the three berry classes were reasonably well represented, with only seedless Cabernet Sauvignon berries being few in number (Table 6.1). The limited number of seedless Cabernet Sauvignon berries may be a reflection of the low proportion of seedless berries normally present in a given population.

Pinot noir berries (mean diameter 9.86; SD 2.97) in general were larger with a greater range in size than Cabernet Sauvignon berries (mean diameter 8.76; SD 1.60). The growth curves of individual berries, regardless of cultivar, showed varied patterns that could be classified according to berry type (Figures 6.1-6.6). Berries classified as being 'seeded' showed a double sigmoid growth curve. The growth of some berries diverged mid way through Stage I growth and failed to achieve the same extent of growth in Stage III; these berries were classified as being 'seedless' as they possessed a total seed content of about less than 1 mg. The growth of the remaining 'seedless' berries tracked that of seeded berries with an initial sigmoidal growth curve of lesser extent; however, no further growth was evident post Stage II. Shot berries showed no growth after an initial expansion of approximately 0.6mm post capfall. Similar trends can be noted for both Pinot noir (Figures 6.1-6.3) and Cabernet Sauvignon (Figures 6.4-6.6) berries. The diameter of several berries decreases during Stage III growth (shrivelling); this may either relate to the berry attaining physiological ripeness and entering a senescence phase [similar to the post-*véraison* shrivelling seen in Shiraz (Coombe & McCarthy 2000)], or due to damage through continued handling and measurement.

Considerable variation (as a percentage) in berry diameter is noted between measurements, which may be due to either, daily fluctuations in berry turgor or inaccuracies in measurement and placement of the callipers across the widest point on the berry equator. Repeated measures of Sultana berries (Appendix H) suggest that placement of the callipers may be the greatest source of error.

Table 6.1 Classifications of berries in Figure 6.1 to 6.6; numbers in red indicate flowers that were replaced during capfall as the original flower abscised.

Classification	Bunch one	Bunch two	Bunch three
Pinot noir			
Seeded	2, 3, 5, 7, 9, 10	17, 18, 19, 20	21, 22, 23, 25, 26, 29, 30
Seedless	1, 4, 6, 8	16	28
Shot	-	11, 12, 13, 14, 15	24, 27
Cabernet Sauvignon			
Seeded	1, 3, 4, 6, 8, 10	11, 13, 17, 20	23
Seedless	2	12, 14	30
Shot	5, 7, 9	15, 16, 18, 19	21, 22, 24, 25, 26, 27, 28, 29

Immediately post-capfall, berries show little to no increase in diameter for approximately three days. After this period growth begins, increasing dramatically, resulting in Stage I berry development. The main burst of Stage I berry growth in seeded berries begins 19 days after flowering (Table 6.2). Pinot noir seedless and shot berries follow the growth of seeded berries immediately post capfall, but diverge 17 days and 13-14 days after capfall, respectively. These trends were not evident in Cabernet Sauvignon berries.

In general, the lag phase between Stage I and III berry growth of seeded berries was not distinct, with growth slowing before the next period of development. Although the phases of berry growth can be identified, at no time does growth cease; it appears that the slope of the lag phase reflects final berry size (Figures 6.1-6.6). Seedless berries with a double sigmoid growth curve demonstrated a stronger lag phase, however neither Stage I or III berry growth occurred to the same extent as for that of seeded berries. After a negligible increase in diameter, shot berries showed no growth throughout the remainder of the growing season, but did show significant variations in turgor, particularly after rainfall events later in the season (observation).

Table 6.2 Days after flowering when growth curves of seedless and shot berries deviate from those of seeded berries.

	Berry type			p value	Least sig. difference
	Shot	Seedless	Seeded		
Pinot noir	13.5	17.2	19.1	<0.001	1.08
Cabernet Sauvignon	10.7	9.4	15.4	0.313	9.12

6.3.2 The seed and berry size

Seed dry weight was closely related to berry diameter in both Pinot noir (Figure 6.7) and Cabernet Sauvignon (Figure 6.8), with the regression for Pinot noir being a stronger predictor than that of Cabernet Sauvignon. The greater the seed content, the larger a berry's diameter. No seed or seed traces could be found in berries less than 4 mm in diameter for either Cabernet Sauvignon or Pinot noir. The Cabernet Sauvignon data show a continuum over all berries greater than 6 mm diameter; however Pinot noir berries have two populations, berries between 5 to 8 mm in diameter and berries greater than about 10 mm diameter.

6.3.3 Berry cellular composition

The response of Cabernet Sauvignon berry volume to the size and number of cells is dependent on berry type. The volume of seeded and seedless berries increase as a result of greater cell volume (Figure 6.9), while the volume of shot berries is a function of the number of cells (Figure 6.10). Seedless and shot berries have a similar range in cell number despite seedless berries being consistently larger in volume. Considerable variation exists within these populations.

The differences in cellular composition between seeded, seedless, and shot berries are reiterated in Table 6.3. The differences in the size of the various classes of berries are a consequence of cell expansion with seedless berries having larger cells than shot berries and seeded berries having larger cells than seedless berries. The same pattern is not evident with regard to cell number. Seedless and shot berries possess similar numbers of cells within the berry cross-section, but the formation of a seed results in greater numbers of cells, contributing to the larger size of seeded berries.

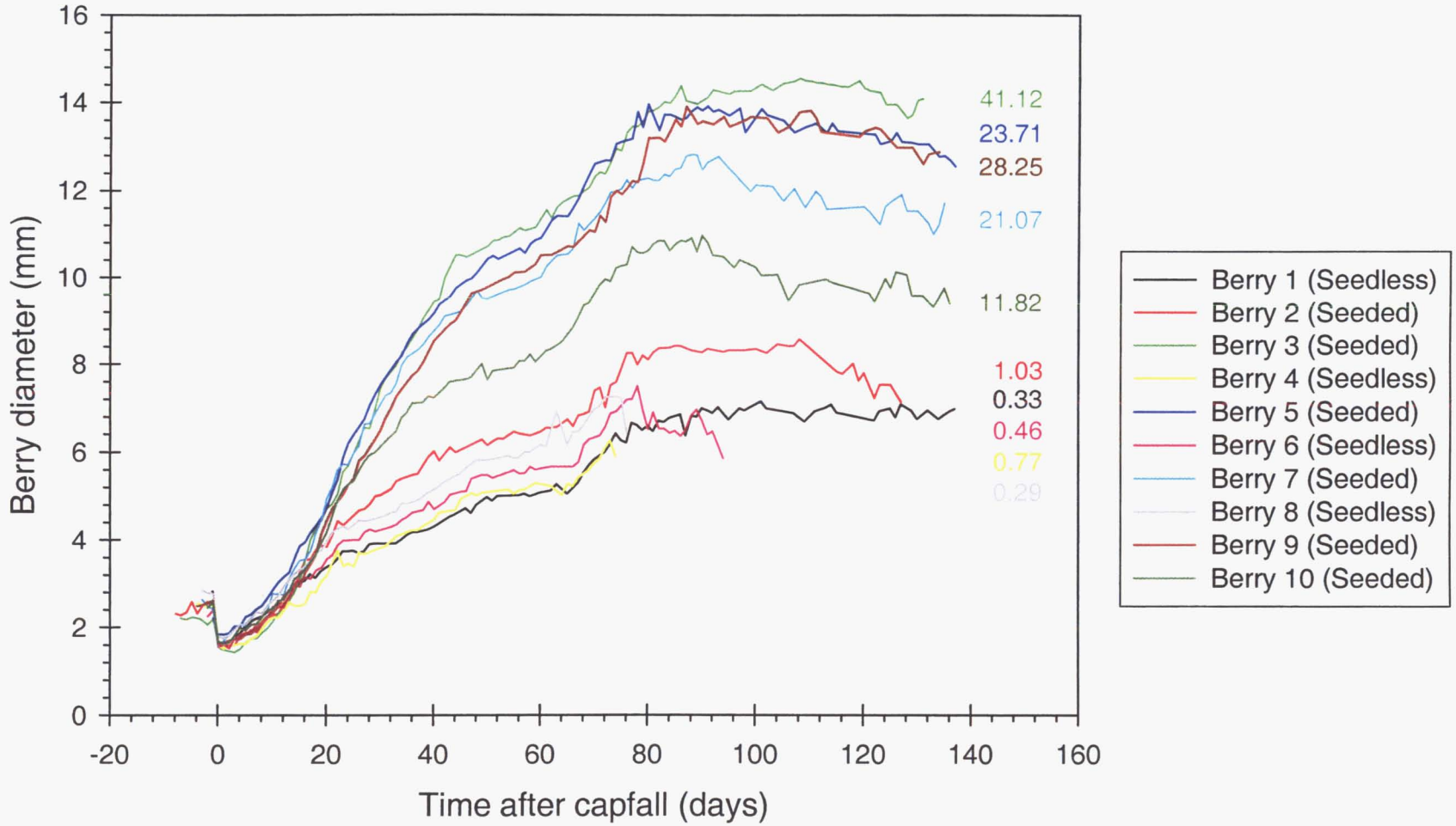


Figure 6.1 Growth of Pinot noir berries from bunch one. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

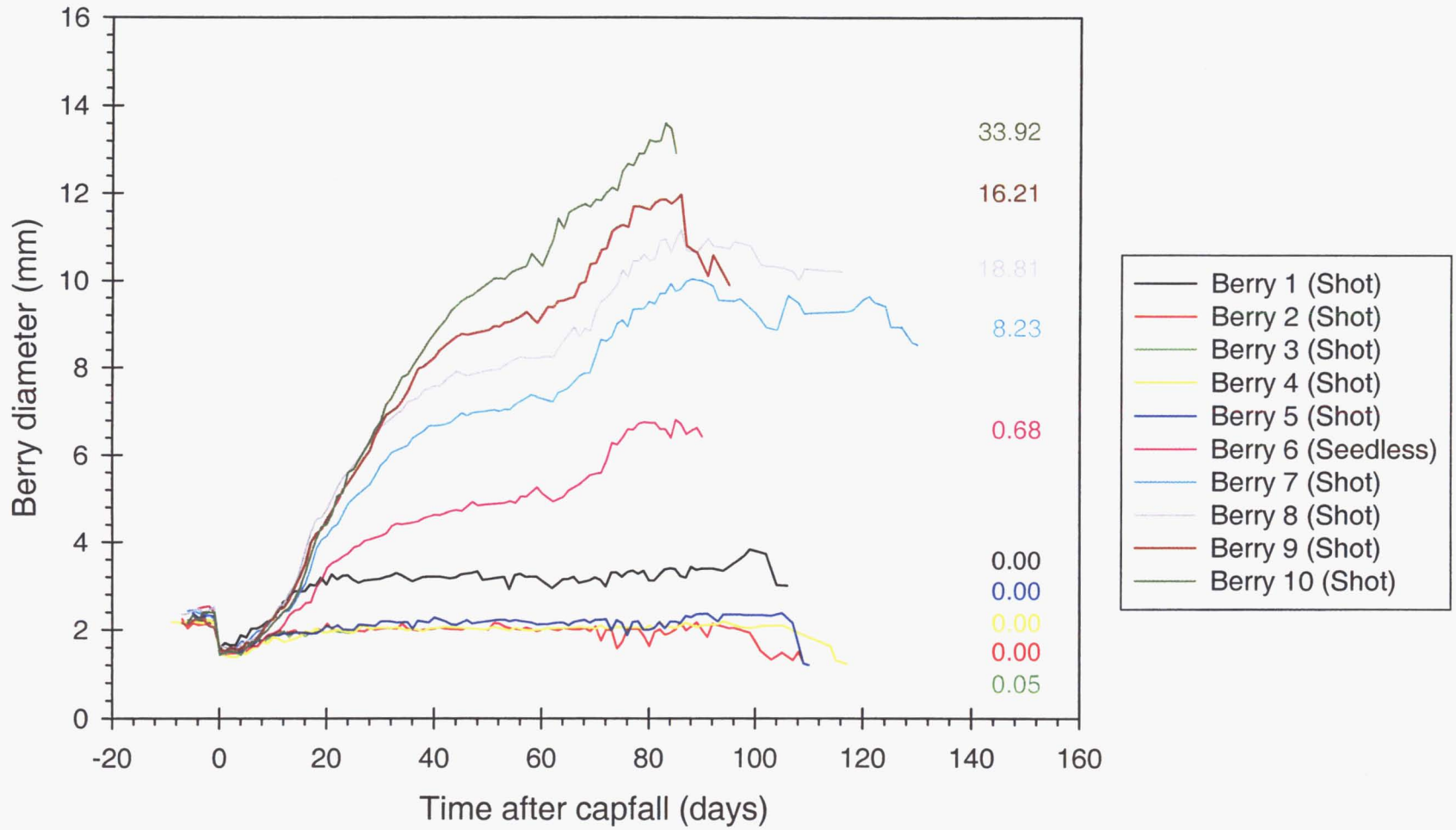


Figure 6.2 Growth of Pinot noir berries from bunch two. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

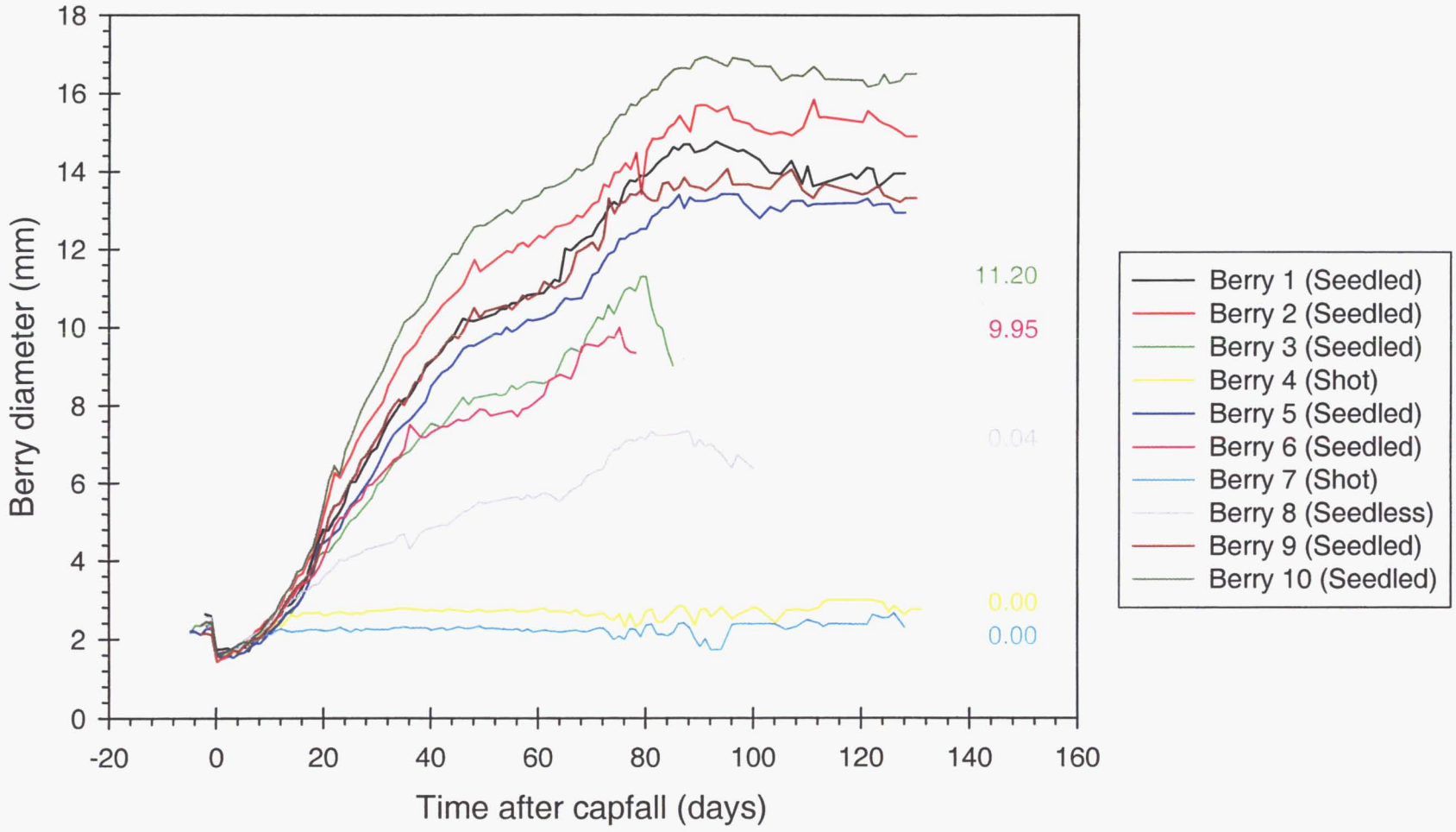


Figure 6.3 Growth of Pinot noir berries from bunch three. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

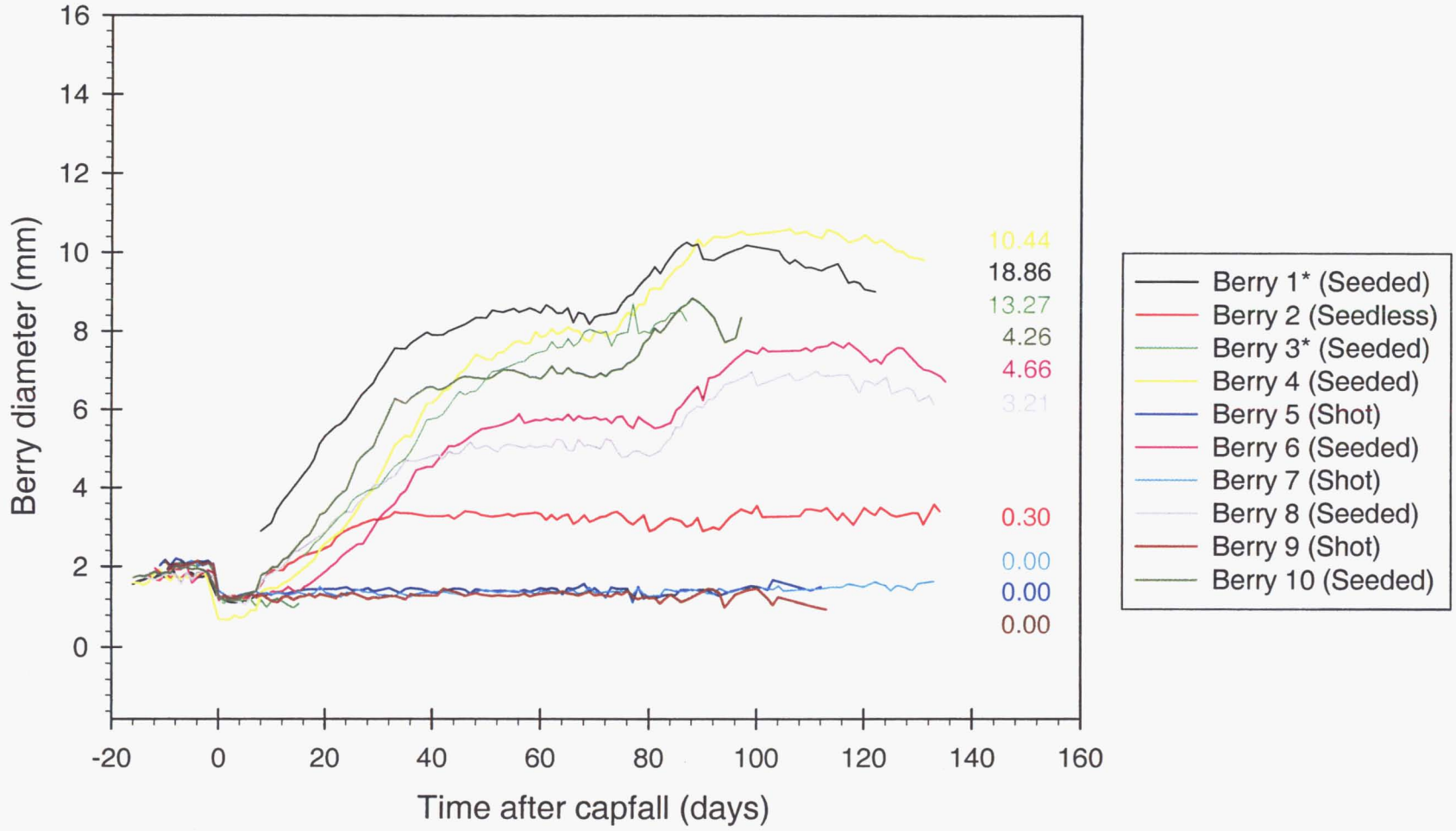


Figure 6.4 Growth of Cabernet Sauvignon berries from bunch one; asterisk indicate replacement berries, for those that abscised. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

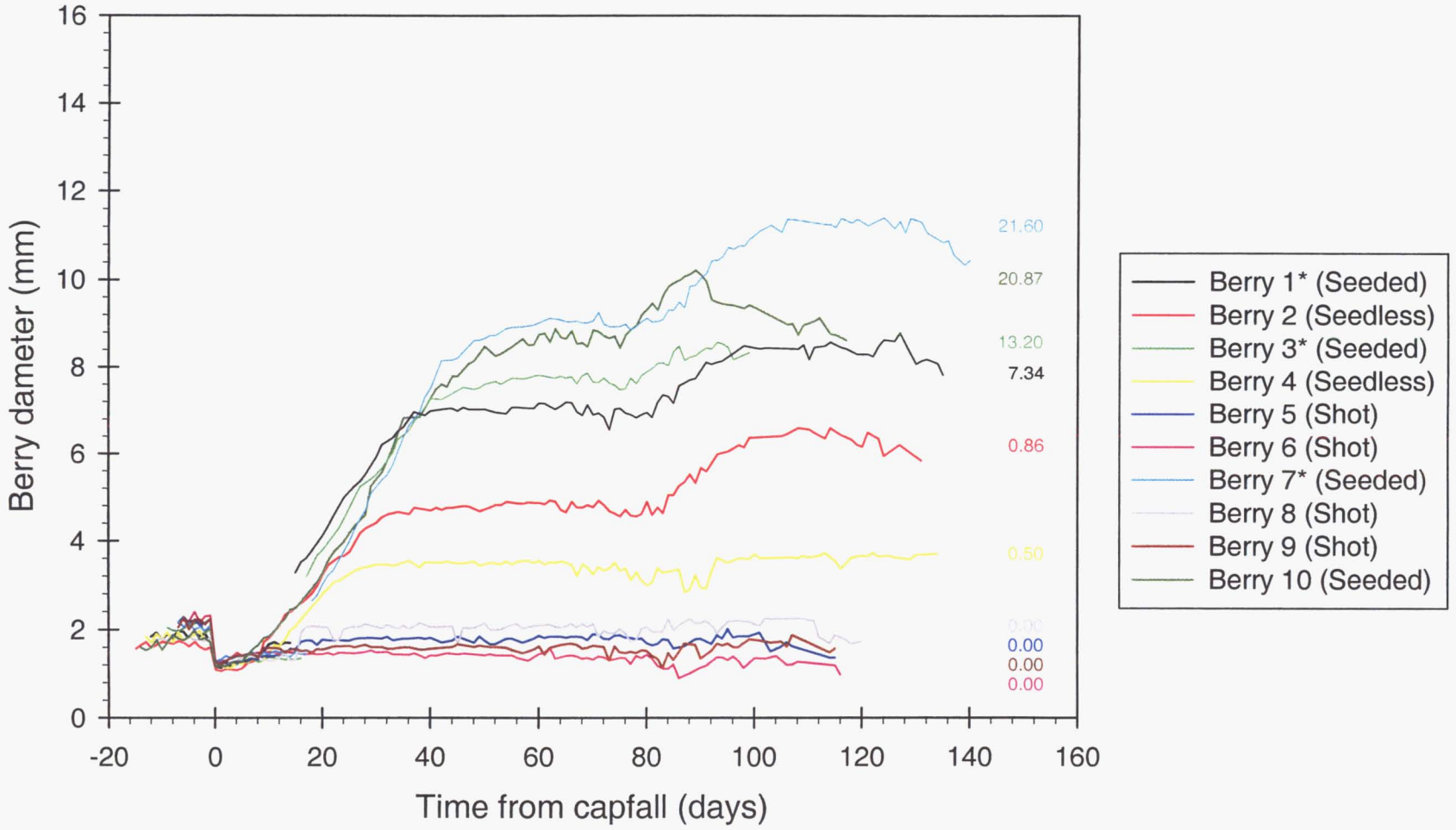


Figure 6.5 Growth of Cabernet Sauvignon berries from bunch two; asterisk indicate replacement berries, for those that abscised. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

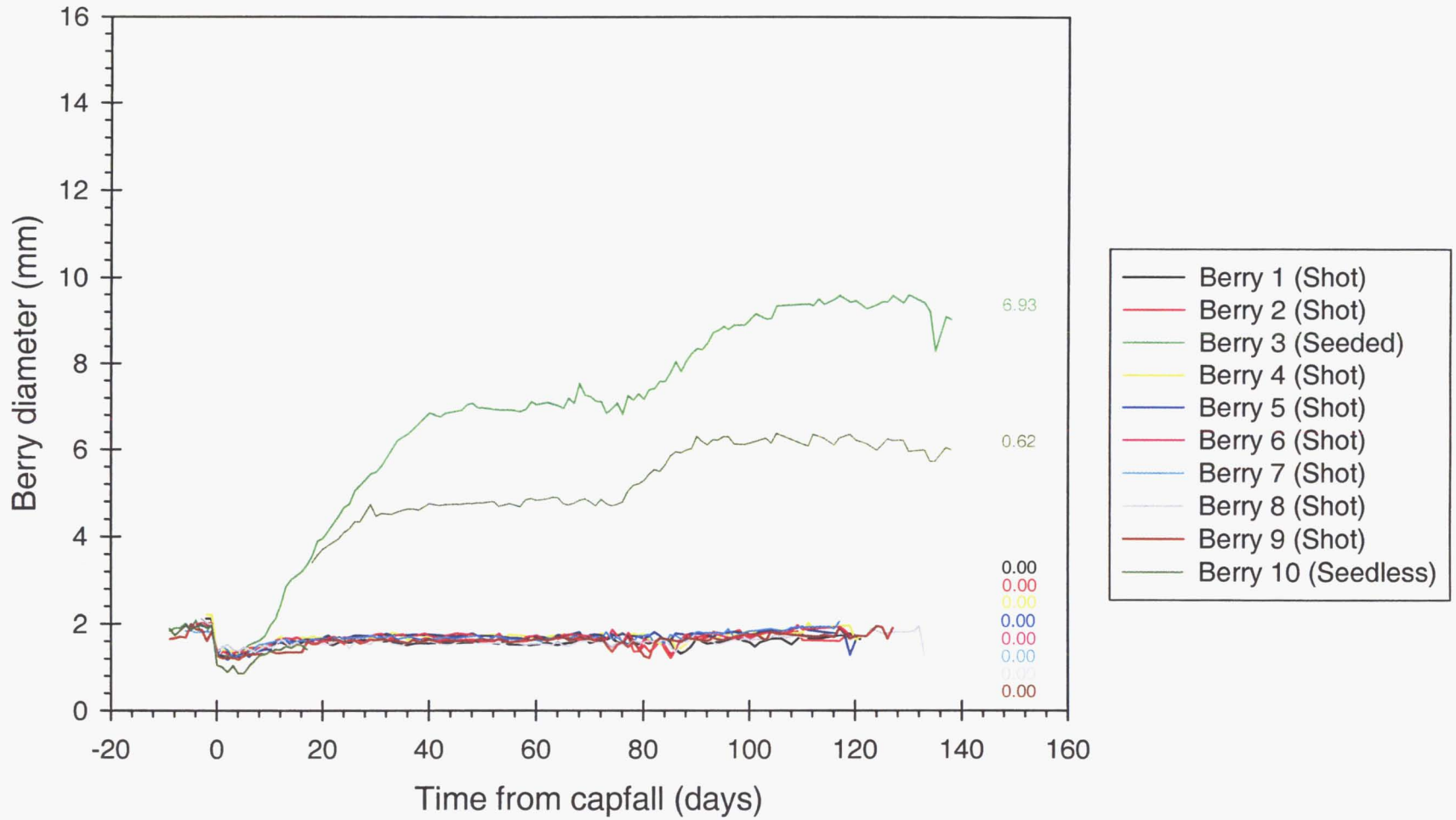


Figure 6.6 Growth of Cabernet Sauvignon berries from bunch three; asterisk indicates replacement berry, for one that abscised. The combined weight (mg) of each berry's seed content is indicated for each growth curve.

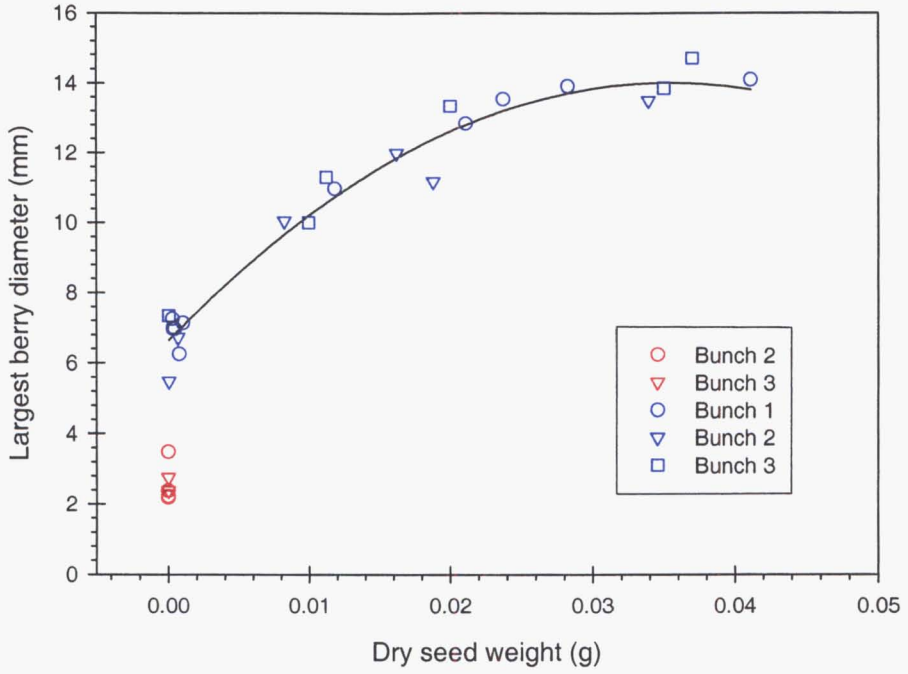


Figure 6.7 The relationship between berry size and seed content of Pinot noir berries. Red symbols indicate berries where no seed or seed traces could be found; Regression line is calculated using the blue symbols only; $y=14.792+-8.120*0.62E-27^2x$, $R^2=0.97$, $P=< 0.001$.

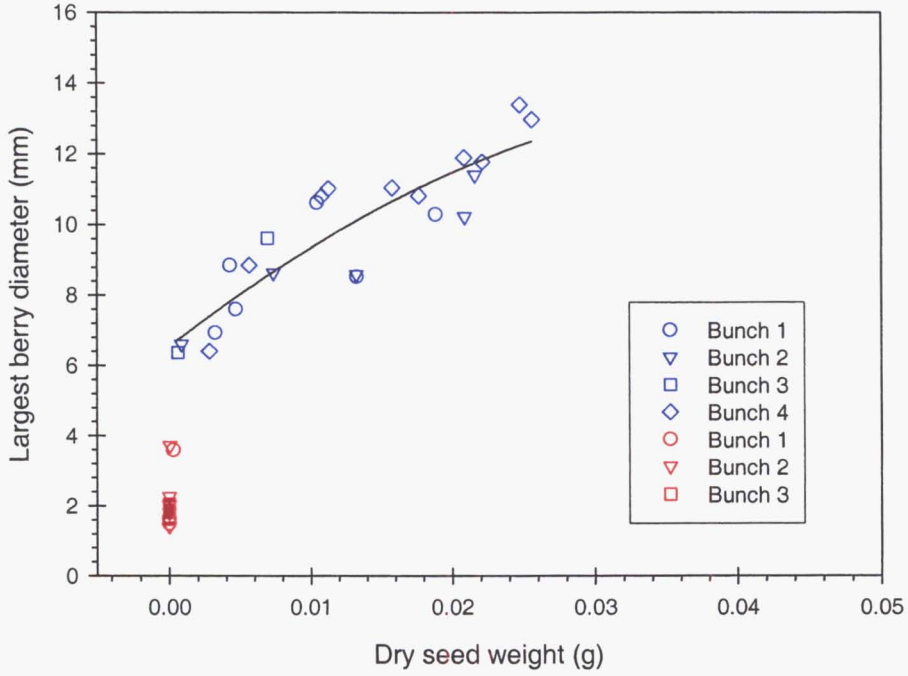


Figure 6.8 The relationship between berry size and seed content of Cabernet Sauvignon berries. Red symbols indicate berries where no seed or seed traces could be found; Regression line is calculated using the blue symbols only; $y=14.91+-8.61*0.17E-19^2x$, $R^2=0.78$, $P = < 0.001$.

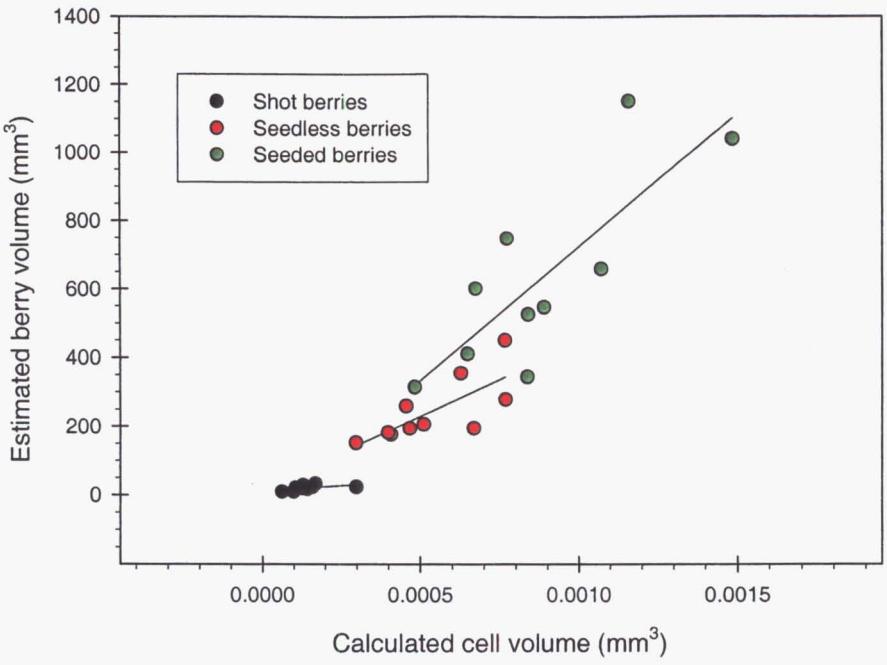


Figure 6.9 The relationship between berry size and mesocarp cell volume for Cabernet Sauvignon. Regression for seeded berries: $y = -56 + 778519x$, $R^2 = 0.60$, $P = 0.005$, seedless berries: $y = 16 + 426799x$, $R^2 = 0.49$, $P = 0.015$, and shot berries: $y = 13 + 58026x$, $R^2 = 0.16$, $P = 0.138$.

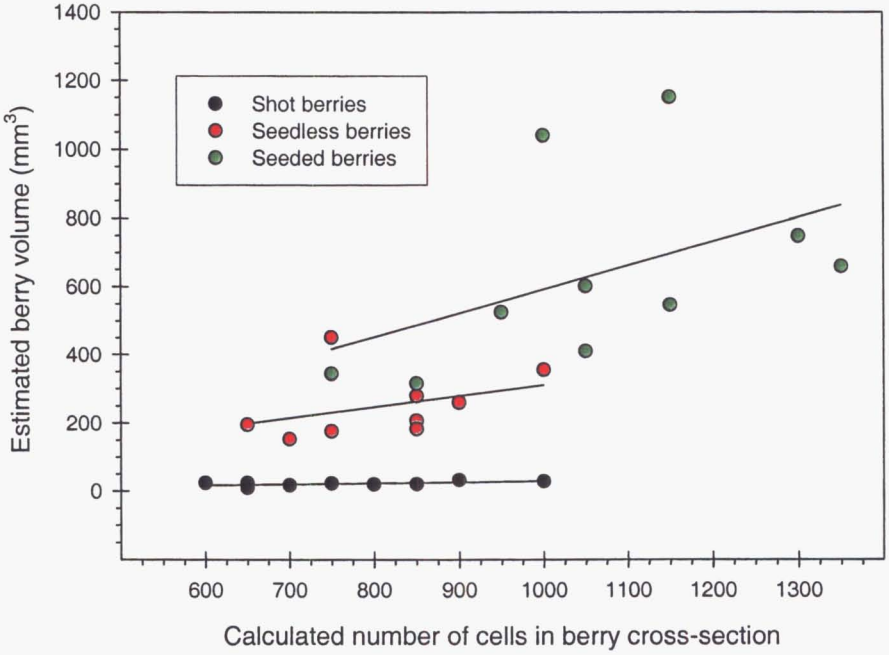


Figure 6.10 The relationship between berry size and the number of mesocarp cells within a berry cross-section for Cabernet Sauvignon. Regression for seeded berries: $y = -109 + 0.700x$, $R^2 = 0.12$, $P = 0.175$, seedless berries: $y = -9 + 0.320x$, $R^2 = 0.05$, $P = 0.266$, and shot berries: $y = -5.1 + 0.0347x$, $R^2 = 0.32$, $P = 0.053$.

Table 6.3 Differences in mean cellular composition of seeded, seedless and shot Cabernet Sauvignon berries

Berry class	Seeded	Seedless	Shot	*P* value	L.S.D.
Berry diameter (mm)	10.46	7.66	3.38	<0.001	0.981
Cell volume (μl)	0.886	0.537	0.143	<0.001	0.196
Cell number in berry cross-section	1060	795	755	<0.001	146

6.4 Discussion

6.4.1 Berry growth

The growth curves and total growth of berries varied as a function of their seed content, with a greater seed content per berry resulting in a larger berry diameter. Seeded berries showed a double sigmoid growth curve with characteristic phases of growth, as reported in the literature (Cawthon & Morris 1982; Coombe 1976, 1989; Jackson 2000; Mullins *et al.* 1992; Nitsch *et al.* 1960; Staudt *et al.* 1986; Winkler *et al.* 1974). The growth curves of seedless and shot berries differ to those of seeded berries. Seedless berries had variable patterns of growth with most berries exhibiting a double sigmoid growth curve (e.g. Pinot noir berries 4, 6, 8, 16 & 28; Cabernet Sauvignon berries 12 & 30), while a few berries only showed Phase I growth with no additional increase in diameter post-*véraison* (e.g. Pinot noir berry 1; Cabernet Sauvignon berries 2 & 14).

The differences in the growth curves of seedless berries may relate to their seed content or the failure of seed to complete normal development. Seedless berries demonstrating a double sigmoid growth curve contained between 1 to 0.5 mg of seed, while those lacking Phase III enlargement had a seed content of less than 0.5mg (Figures 6.1-6.6). It would appear that these berries require a minimum level of seed development to undergo a double sigmoid growth pattern. Examining a range of *V. vinifera* cultivars, Staudt & Kassemeyer (1984) identified that a minimum seed content was required for normal berry development, akin to the requirement of Concord berries to posses a minimum of one normal seed, in order for the berries to progress through *véraison*, colour and sugar accumulation (Cawthon & Morris 1982).

After a brief period of initial growth post-capfall, shot berries show no growth for the remainder of the season, while a proportion of tagged flowers do not develop into fruit and abscise (i.e. 20% of tagged Cabernet Sauvignon flowers). It is difficult to make inferences as to the role of seed on the development of shot berries, as no microscopic assessment of seed traces was made on abscised flowers or shot berries. It might be assumed that abscised flowers were not pollinated when the pistil was receptive to pollen; hence their abscission. Shot berries may have formed in response to pollination that failed to result in an aborted or successful fertilization (i.e. seedless or seeded berry, respectively). Ovules of shot berries lack an embryo sac but possess a normal nucellus (Kassemeyer and Staudt 1982), suggesting that no further development can occur. Pollen from other crop plants has been found to contain ethylene inhibitors and auxin (Taylor and Hepler 1997), compounds that encourage fruit set. Friend *et al.* (2003) hypothesise that shot berries are formed by a hormone stimulus from pollination. This stimulus induces a pollen-regulated development response sufficient to prevent abscission, but insufficient to ensure seed and as a consequence berry development. Similar development is found in the parthenocarpic cultivar Zante Currant, where pollination alone is the trigger for fruit development (Mullins *et al.* 1992).

By extrapolating the linear portions of transformed berry growth curves during the initial stage of phase I berry growth, the point where seeded berries begin their rapid increase in berry diameter can be identified. This occurred 19 days after capfall for Pinot noir, but could not be identified for Cabernet Sauvignon (Table 6.2). Harris *et al.* (1968) found that cell division is the primary means of berry enlargement for up to 21 days, relating to the initial quadratic stage of Phase I berry development, while cell expansion is the primary means of berry enlargement after this period, relating to the linear portion of Phase I berry growth. The point of rapid Phase I berry growth in Pinot noir (19 days), is a similar timeframe to the 21 days identified by Harris *et al.* (1968) in Sultana. During this period of time not only is the pericarp of the fruit undergoing significant cellular development, but the seeds are also forming. Around 14-16 days after capfall the zygote of a seed will have formed and be about to begin division to form an embryo; the endosperm will be at the free-nuclear stage and about to become cellularised (Ebadi *et al.* 1996b; Nitsch *et al.* 1960; Pratt 1971). However in flowers that fail to develop into 'normal' seeded berries, the zygote and/or the free-nuclear endosperm can begin to degenerate from this time onwards (Ebadi *et al.* 1996b). Failure of seed to undergo normal development may limit the extent to which seedless berries undergo rapid Phase I growth and explain the growth of berries around this time in Pinot noir. Such

development can be observed in the stenospermocarpic cultivar Sultana, where ovule development may be normal or near normal (Mullins *et al.* 1992). Studying seedlessness in a range of cultivars and progenies from crosses, Stout (1936), described the development of seeds and seed traces as having a continuous nature. Comparing progenies from parthenocarpic and stenospermocarpic crosses, Striem *et al.* (1992), found that despite of the continuous nature of the fresh weight of seed, significant morphological differences could be observed in the appearance of the different groups of size of the seeds.

In Pinot noir, seedless berries follow the growth curves of seeded berries for about 17 days after capfall, at which point their growth slows. A portion of these seedless berries maintain a double sigmoid growth curve, but fail to attain the diameter of seeded berries, while the remainder appear to halt Phase I berry growth early, and remain as small berries. The growth of shot berries, in Pinot noir, halts about 13 days after flowering. These patterns of the timing of growth in seedless and shot berries are not evident in Cabernet Sauvignon (Table 6.2); with some berries seemingly experiencing a phase shift in time when development occurs (i.e. post capfall flowers show no enlargement for several days after most flowers have begun development (Figures 6.4 to 6.6)).

The cause of the shift in time, when Cabernet Sauvignon berries begin phase I growth, is unknown and may be a consequence of inadequate or self-pollination, which may result in shot berry formation or abscission of the flower. Although self-pollination is thought to occur readily in grapevines, perhaps not all cultivars have an equal ability to form seedless or seeded berries from self-pollination. Possibly the available carbohydrate status of the vine improves during the time when the flower shows no growth, allowing development of a berry to occur. No evidence is presented to support this proposition, which represents a possible angle of further research.

The occurrence of this shift may be significant, as competitively, flowers that are delayed may be unable to acquire the carbohydrate resources required for initial seed and berry development. Adequate carbohydrate nutrition is a controlling influence in fruit set and abscission of grapes (Caspari *et al.* 1998) and citrus (Gomez-Cardenzas *et al.* 2000). Given the number of berries followed in this experiment it is difficult to say that phase shift is a common occurrence. The presence of a phase shift and the likely competitive disadvantage

these flowers are likely to be under may explain why seedless berries tend to be rare in Cabernet Sauvignon (based on casual observation).

6.4.2 The seed and berry size

The difference between Pinot noir and Cabernet Sauvignon berries may be a function of the relationship between seed content and berry size and season. Both cultivars show a strong quadratic relationship (Figures 6.7 & 6.8) consistent with reports in the literature (Coombe & Iland 2004; May 2000). Only a small amount of seed development is required to result in a berry about 6 mm in diameter, but while Cabernet Sauvignon berries show a continuum within the seed:berry relationship, Pinot noir berries show two distinct populations: berries 6 to 8 mm in diameter with very little seed and berries greater than 10 mm with at least 10 mg of seed. The occurrence of these two berry populations provides further support for the requirement of a minimum extent of seed development to ensure a normal seeded berry. Given the growth of seedless berries deviates from those of seeded berries at a time close the formation of the embryo and endosperm of the seed, and assuming there are no differences between seasons, this could be considered the test of a successful fertilisation, resulting in either partial or normal seed formation. That Pinot noir and Cabernet Sauvignon show different seed:berry relationships, suggests that the causes for failure of normal seed and hence berry development have different origins. This deserves further investigation.

5.4.3 Berry cellular composition

The difference in size between seeded, seedless and shot Cabernet Sauvignon berries is a consequence of their cellular development. Larger berries have larger mesocarp cells, regardless of berry classification. Seeded berries show growth, due to cell expansion and division (Table 6.3), but it is the greater range in the number of cells within seeded berries (compared to seedless and shot berries) (Figure 6.10) that demonstrates the importance of seed development as a stimulus of cell division within the mesocarp. This finding is supported by the literature, where it is assumed that berry size is determined by the number of cells in the mesocarp [as small parthenocarpic berries show no cell division after flowering compared to the considerable cell division found in seeded berries] (Coombe & Iland 2004), and with Ojeda *et al.* (1999) suggesting that seed growth has a positive effect on cell mitosis.

It is apparent that shot berries and some seedless berries undergo a distinctly different path of development to the remaining seeded berries and those that abscise. The lack of at least one fully formed seed after fertilisation severely limits berry size by hindering both cell division and expansion in the developing fruit (Table 6.3). Failure of a seed to develop normally after fertilisation, resulting in an incomplete seed, stimulates cells expansion, but this is insufficient to stimulate cell division, which results in the smaller size of seedless berries. The presumed failure of any seed development in shot berries prevents both cell expansion and cell division.

It is possible that the complete lack of seeds in shot berries means that the initial expansion of these berries, immediately post anthesis is a consequence of the stimulus that prevents their abscission. The growth of shot berries deviates from that of seeded berries, in Pinot noir, about 13 days after capfall (Table 6.2). Friend *et al.* (2003) hypothesise that pollination prevents abscission of shot berries, but does not result in fertilisation noting that ovules of shot berries lack an embryo sac but possess a normal nucellus (Kassemeyer & Staudt 1982). The lack of an embryo sac will prevent normal seed development from occurring as the nucellus degenerates; this has been observed to occur in Chardonnay anytime between 4 to 28 days after capfall (Ebadi *et al.* 1996b).

Chapter VII

Final Summary and Conclusions

7.1 Conclusions

A series of experiments were run to study the set and development of individual flowers into berries. Berries are the basic unit of yield (May 2000), but surprising little information is available on their development from flowers. Different aspects of berry development were studied, examining changes in yield components, the behaviour of individual flowers and the growth and cellular characteristics of various types of berries.

The variation in flower size described by Ezzili (1992) and May (1986) early in flower development was still present at capfall. Flower size (ovary diameter) was found to influence the timing of capfall, with smaller flowers tending to undergo capfall after larger flowers (Chapter Four). May (1986) pondered whether flower size influenced the persistence of flowers at anthesis and the further development of the berries. Although flowers do differ in their size and the date when they undergo capfall, these characteristics appear to have little effect on whether a flower will set and the type of berry that results (Chapter V). The lack of a strong relationship would suggest that other factors, perhaps resource availability (Caspari *et al.* 1998), pollination or fertilisation (Milne *et al.* 2003), may have a greater impact on berry development than flower characteristics.

The progression of capfall was influenced by environmental conditions. Results presented in Chapter Four demonstrated how rainfall and associated cool temperatures disrupt the progression of capfall. Air temperatures above 15°C advanced the progression of capfall in two out of three seasons, partially in support of Millardet (quoted in Perold 1927, from Guillon 1905), who found that Chasselas flowers opened slowly at 15°C, normally at 17°C and rapidly at 20-25°C. However, flowers that opened early during capfall had no greater chance of developing into berries, than flowers opening later (Chapter Five).

Most studies of fruit set in grapevines only consider the total number of berries set. However, the ability of grapevines to form shot berries, parthenocarpic or stenospermocarpic berries,

and seeded berries means that valuable information regarding berry formation can be gained from identifying the types of berries that set.

The development of individual flowers can be divided into four types:

1. Flowers that abscise.
2. Flowers that develop into seeded berries; showing normal development of the mesocarp with sugar accumulation and development of anthocyanins in the skin. At least one fully developed seed is present.
3. Flowers that develop into seedless berries; showing less development of the mesocarp than seeded berries, with sugar accumulation and development of anthocyanins in the skin. Either ovule traces or at least one aborted seed is present.
4. Flowers that form shot berries. These do not develop nor abscise; they remain within the cluster as small green berries (1 to 3 mm diameter), though sometimes they do shrivel on the bunch.

The timing of phenological growth stages may have an important role in determining berry development (Chapter Three). Delaying winter spur pruning, delayed the timing of bud break, and resulted in an increase in bunch weight, similar to that reported by Barnes (1958), Coombe (1964), and Whittle (1986). Bunch weight increased due to a larger average berry weight. The increase in average berry weight resulted from changes in the berry population, with the proportion of large seeded berries increasing within bunches, associated with a possible reduction in the proportion of smaller seedless berries. Treatments that delayed bud break also delayed flowering date, perhaps to a time when weather conditions were more favourable for berry development. Temperature is considered an important factor affecting fruit set and berry development (Galet 2000; Jackson 2000). A weak relationship was evident between the warmth of the bud break period and yield, as well as bunch weight; this may be an indirect relationship, as current yield is being determined at flowering not bud break, and does not provide evidence of the role of temperature on fruit set and berry development.

Carbohydrate availability plays an important role in berry set and development. Increasing carbohydrate supply does not affect fertilisation success, as girdling did not alter the proportion of seeded berries that formed, however a minimum supply of carbohydrate is required for seeded berry formation, as removing 75% of shoot leaf area reduces the proportion of seeded berries (Chapter Five). As expected, the proportion of seedless berries

that form was enhanced with girdling and reduced by leaf area removal, in agreement with the findings of Coombe (1959) and Caspari *et al.* (1998), respectively. The extreme level of leaf area removal (75%) required to illicit a change in berry development was a surprise, and quite different to the findings of Caspari *et al.* (1998), who suggest a leaf area removal greater than 38% was required to reduce fruit set. Also of note, seedless berry formation was not influenced by leaf area removal. Total shoot leaf area was found to vary considerably within treatments, and when used as a covariate, was correlated to seedless berry formation. A weak positive relationship between total shoot leaf area and the development of seedless berries was found, reinforcing the role that carbohydrate supply plays in the development of seedless berries.

The changes in seedless berry formation associated with carbohydrate supply had an impact on the development of shot berries and flowers that would otherwise abscise. The data from yield components (Chapter Three) and of individual flowers (Chapter Five) suggest that the population of berries are fluid in nature. It is proposed that shot berries and flowers that abscise, form a pool from which seedless berries can develop; carbohydrate availability appears to determine the extent to which this occurs. The warmth of the flowering period may also influence whether it is the shot berries or flowers that abscise that instead develop into seedless berries. Seasons with cool flowering periods could limit the formation of seeded and seedless berries (i.e. reducing overall fruit set as found in 1999), resulting in greater shot berry formation. Shot berries are more prevalent in seasons of poor fruit set and are associated with cool weather conditions (Bindra 1989). Such a situation would explain why seedless berries appear to form from what would be shot berries in 1999 and from flowers that would otherwise abscise in 2000.

MacGregor (2000) found seasonal Chardonnay bunch weights increased about two-fold between an average air temperature of 13.3 and 19.4°C during flowering (presumably due to differences in either berry number or weight). In contrast no strong relationship between temperature at flowering and fruit set or berry development within seasons could be found (Chapter Five). This implies that the relationship between vine yield and warmth at bud break in Chapter Three is indirect and the role of temperature may not be as important as suggested in the literature. It was noted that mean overall fruit set differed between the 1999 (41%) season with a cool flowering period, and the 2000 (71%) season with a warm flowering period, suggesting that temperature does play some role in fruit set. It is difficult to comment

further without greater understanding of the role of climate and periods of high and low temperature on the physiology of the grapevine; particularly if vines respond differently to climate type and general warmth or cold of a season. Other indices such as light intensity, sunlight hours or cloud cover may be more appropriate environmental measures, relating to photosynthesis and photoassimilate availability. Stored carbohydrates are at a minimum during flowering (Bennett 2005) and hence fruit set may be solely dependent on current photoassimilate supply. Application of girdling enhances berry development, supporting such a notion.

In agreement with Coombe and Iland (2004) a strong quadratic relationship was found between the diameter of seeded and seedless berries and their seed and seed trace content (Chapter Six). Berries of Cabernet Sauvignon showed a range of berry sizes in this relationship, but Pinot noir berries had two populations, berries 5-8mm and berries greater than 10mm. Only a very small amount of seed development (>0.5mg) is required to stimulate moderate (>5mm diameter) berry development in both Pinot noir and Cabernet Sauvignon.

The final size of a berry is determined by its growth curve and relates to the berry's seed content. Seeded berries required at least one normal seed to undergo the characteristic double sigmoid growth curve (Cawthon & Morris 1982; Staudt & Kassemeyer 1984). Most seedless berries also grow via a double sigmoid growth curve; however this curve diverges from that of seeded berries during phase I berry growth. Seedless berries contained less than 1mg fresh seed weight. Some seedless berries lacked Phase III growth; these berries possessed less than 0.5mg fresh weight of seed. The growth of non-seeded Pinot noir berries deviated away from that of seeded berries at defined times. It was proposed that the time when the growth of seedless Pinot noir berries deviates from seeded berries was similar to when the zygote and free nuclear stage endosperm begin degeneration in seedless table grape cultivars (Pratt 1971).

No consistent time when growth of non-seeded Cabernet Sauvignon berries deviated from seeded berries could be found. This may relate to the range of berry sizes found in Cabernet Sauvignon, reflecting the ability of seed development to fail at any time. Stout (1936) found a continuum of seed trace development existing when examining the progeny of seeded and seedless grape crosses. The reasons for failure of seed development were not examined.

Apart from a short initial period of growth immediately post capfall, shot berries do not grow. The size differences between seeded, seedless and shot berries are due to cell division and expansion. Seedless berries and shot berries possess a similar number of cells, but seedless berries are larger than shot berries because they have larger cells. Seeded berries have more cells and larger cells than seedless or shot berries. It would appear that incomplete fertilisation will allow cell expansion to occur, but complete seed development is necessary for cell division.

A model explaining berry development has been proposed (Figure 7.1) and differs to that described in Figure 1.3. In this model, flowers reach maturity and undergo capfall, allowing pollen release and pollination. Pollination is the first requirement for berry development. Failure of a flower to be pollinated will result in abscission of that flower. Successful pollination provides a stimulus allowing fruit set to occur, providing a pollen-regulated development response. In this model fruit set is considered the alternative to flower abscission. Once a flower has set, further growth is determined by fertilisation, and the extent that fertilisation allows seed development. If no fertilisation occurs a shot berry will form. The lack of an embryo sac in the ovules of shot berries (Kassemeyer & Staudt 1982) would prevent seed formation, limiting growth of the berry. The extent to which fertilisation allows seeds to develop will determine the size of the seeds at maturity, whether the development of seeds is normal and as a consequence whether the berry is seeded or seedless.

Failure of at least one seed to reach maturity in a berry results in a seedless berry. The continuous range in seed and ovule traces, as described by Stout (1936) studying seedless cultivars, would suggest that development of the seed can fail for many reasons at any stage during seed development. The strong relationship between seed content and berry size, as described in Chapter Six and the literature (Cawthon & Morris 1982; Coombe 1960; Müller-Thurgau 1898; Olmo 1946; Petrie *et al.* 2000; Scienza *et al.* 1978; Winkler & Williams 1936), would suggest that the extent to which seeded and seedless berries grow is governed by development of the seed. In turn this is related to a berry's growth curve, which is determined by cell division and expansion (Coombe & Iland 2004).

The model in Figure 7.1 identifies five processes (purple) that lead to the formation of various berry types (yellow). Of note, this model considers abscission and fruit set as contrary processes; it also regards shot berries as the initial phase of berry formation that only result

when a failure in the process of normal berry growth and development occurs. Fruit set occurs before fertilisation, and fertilisation determines the extent to which seeds will develop.

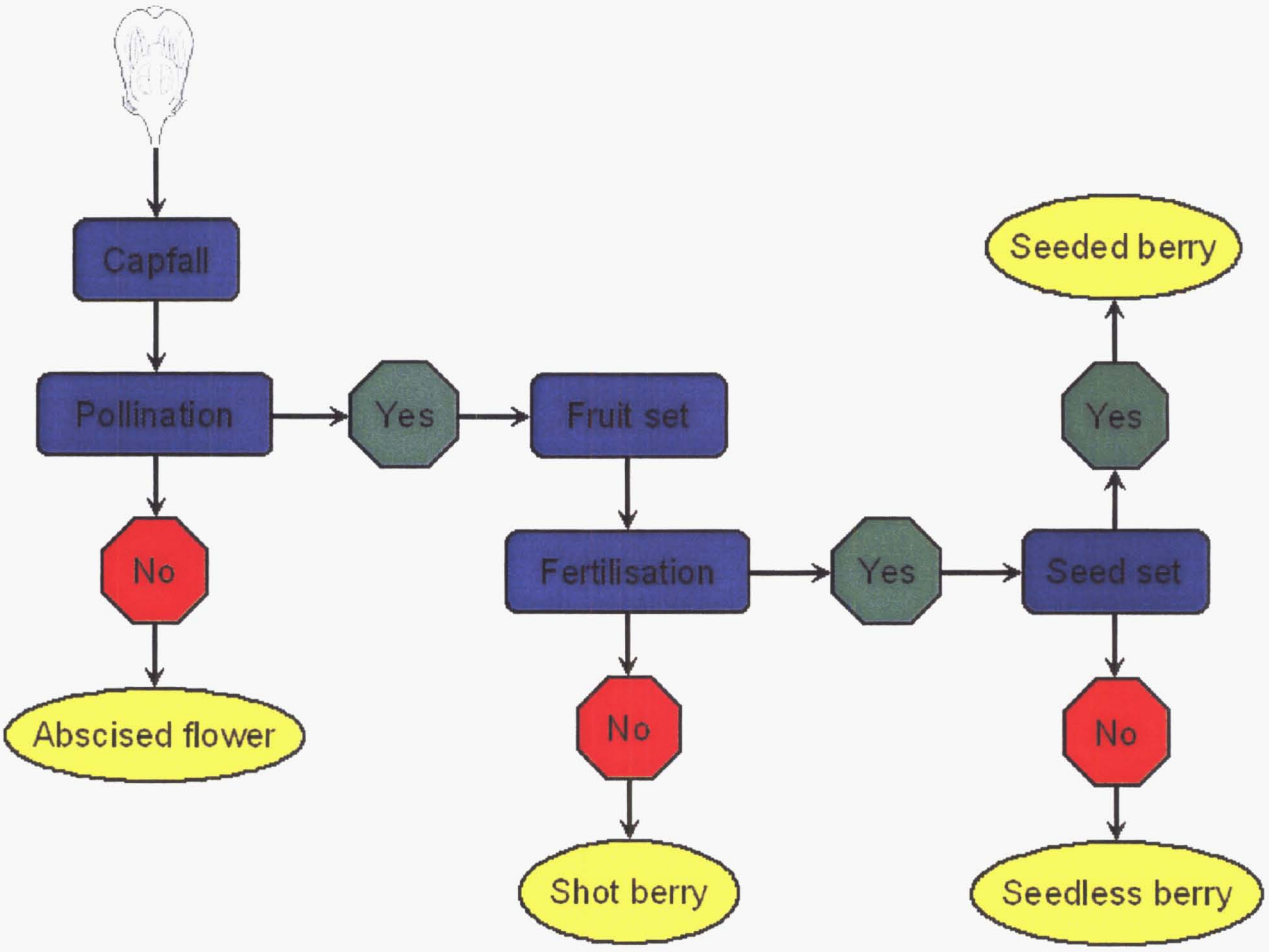


Figure 7.1 Proposed model of berry development

Future studies investigating the physiological basis of berry development should include an assessment of berry type, particularly when manipulating berry number per bunch (fruit set) and average berry weight. Inclusion of an assessment of berry type would allow identification of the stage of berry formation that treatments are influencing.

Many questions have arisen from this work. Confirming the role of pollination in preventing abscission and the relative importance of self- versus cross-pollination, deserves further attention. Microscopic examination of the cellular composition of flowers at capfall and of

developing seeds in shot, seedless and seeded berries would identify the importance of pollination and fertilisation in determining berry type and final size; linking berry development to pollination, fertilisation and the seed. Further research into the causal factors leading to the formation of shot, seedless and seeded berries is required to confirm the findings discussed in previous chapters.

When starting this research project, the strong bias of the literature, towards the role of temperature in determining fruit set and berry development, lead to the idea that temperature would be a dominating factor importance. However, the difficultly experienced trying to elucidate strong relationships between temperature and flower behaviour, fruit set, and berry development would suggest that other factors are, just as, if not more important. The use of a phytotron, to control environmental conditions, would allow the relative importance of temperature in the development of yield to be described in detail; focusing on heat accumulation over time, heat at key phenological stages, and the rate of heat accumulation could provide results of interest. However, temperature must not be looked at in isolation, as in phytotron studies, one could risk over emphasising its role. The influence of girdling and shoot leaf area manipulation on berry development, suggests that carbohydrate supply may play an important role, particularly in flower abscission and seedless berry formation. Environmental conditions that would affect carbohydrate availability, such as light intensity, should also be included in any future work focusing on temperature and berry development.

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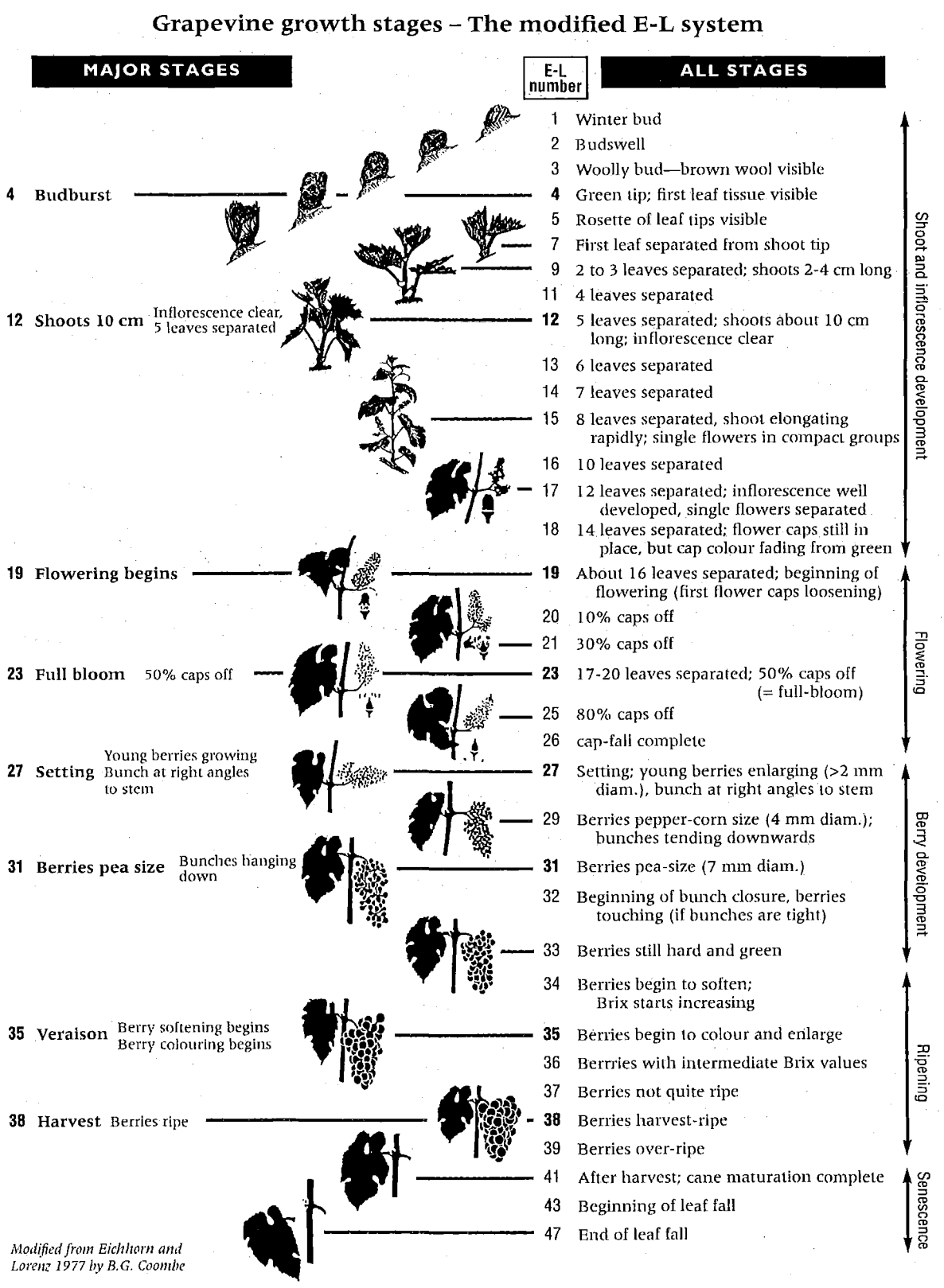
APPENDIX A



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APPENDIX B - ANOVA input matrix used in Experiment Two analysis of bud development, testing for linear and quadratic relationships within treatments, including a covariate (frost incidence).

```
"General Analysis of Variance."
 BLOCK Rep/Pruning/Gell_trt/Spur/Node
 TREATMENTS pol(Pruning;3)*reg(Gell_trt;3;m1)*Node
 COVARIATE Frost
 ANOVA [PRINT=aovtable,information,mean,covariate; FACT=3;
FPROB=yes; PSE=diff,lsd, means] Phenology 26 Sept

***** Analysis of variance (adjusted for covariate) *****

Variate: Penology26Sept
Covariate: Frost
```

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Rep stratum						
Covariate	1	7.5297	7.5297	12.68		0.009
Residual	7	4.1555	0.5936	1.05	2.46	
Rep.Pruning stratum						
Pruning	2	14.0154	7.0077	12.42	0.48	<.001
Lin	1	13.8662	13.8662	24.57	0.32	<.001
Quad	1	0.1491	0.1491	0.26	0.96	0.615
Covariate	1	2.0154	2.0154	3.57		0.078
Residual	15	8.4661	0.5644	1.04	1.16	
Rep.Pruning.Gell_trt stratum						
Gell_trt	3	9.2524	3.0841	5.69	0.70	0.001
nil vs rest	1	2.9302	2.9302	5.41	0.67	0.023
twice vs once	1	5.6498	5.6498	10.43	0.56	0.002
early vs late	1	0.6724	0.6724	1.24	0.99	0.269
Pruning.Gell_trt	6	12.2784	2.0464	3.78	0.96	0.003
Lin.nil vs rest	1	7.9342	7.9342	14.65	1.00	<.001
Quad.nil vs rest	1	0.0401	0.0401	0.07	1.00	0.786
Lin.twice vs once	1	2.0092	2.0092	3.71	0.83	0.058
Quad.twice vs once	1	1.3227	1.3227	2.44	0.99	0.123
Lin.early vs late	1	0.4535	0.4535	0.84	1.00	0.363
Deviations	1	0.5187	0.5187	0.96	0.99	0.331
Covariate	1	0.9794	0.9794	1.81		0.183
Residual	71	38.4651	0.5418	1.02	1.01	
Rep.Pruning.Gell_trt.Spur stratum						
Covariate	1	4.3134	4.3134	8.14		0.005
Residual	107	56.6866	0.5298	1.06	1.07	
Rep.Pruning.Gell_trt.Spur.Node stratum						
Node	1	21.9097	21.9097	43.82	0.87	<.001
Pruning.Node	2	0.3260	0.1630	0.33	1.00	0.722
Lin.Node	1	0.1314	0.1314	0.26	1.00	0.609
Quad.Node	1	0.1946	0.1946	0.39	1.00	0.533
Gell_trt.Node	3	0.2574	0.0858	0.17	0.97	0.915
nil vs rest.Node	1	0.0001	0.0001	0.00	0.96	0.992
twice vs once.Node	1	0.1192	0.1192	0.24	0.98	0.626
early vs late.Node	1	0.1381	0.1381	0.28	0.96	0.600
Pruning.Gell_trt.Node	6	2.8167	0.4695	0.94	1.00	0.468
Lin.nil vs rest.Node	1	0.0000	0.0000	0.00	1.00	1.000
Quad.nil vs rest.Node	1	0.1897	0.1897	0.38	1.00	0.539
Lin.twice vs once.Node	1	0.4600	0.4600	0.92	1.00	0.339
Deviations	3	2.1631	0.7210	1.44	0.99	0.232
Covariate	1	2.2133	2.2133	4.43		0.037
Residual	203	101.5090	0.5000		1.02	
Total	431	375.1852				

```
* MESSAGE: the following units have large residuals.
Rep 5.00 Pruning 3.00                                  0.318   s.e. 0.140
Rep 3.00 Pruning 2.00 Gell_trt 3.00                    0.839   s.e. 0.298
Rep 6.00 Pruning 3.00 Gell_trt 2.00                   -0.840   s.e. 0.298
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 1.00 Node 1.00
                                                      -1.822   s.e. 0.485
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 1.00 Node 2.00
                                                       1.822   s.e. 0.485
Rep 2.00 Pruning 1.00 Gell_trt 4.00 Spur 2.00 Node 1.00
                                                      -1.738   s.e. 0.485
Rep 2.00 Pruning 1.00 Gell_trt 4.00 Spur 2.00 Node 2.00
                                                       1.738   s.e. 0.485

***** Covariate regressions *****

Variate: P26Sept
```

Covariate	coefficient	s.e.
Rep stratum		
Frost	1.35	0.379
Rep.Pruning stratum		
Frost	0.84	0.446
Rep.Pruning.Gell_trt stratum		
Frost	0.28	0.209
Rep.Pruning.Gell_trt.Spur stratum		
Frost	0.51	0.178
Rep.Pruning.Gell_trt.Spur.Node stratum		
Frost	0.29	0.137
Combined estimates		
Frost	0.407	0.0922

```
***** Tables of means (adjusted for covariate) *****

Variate: P26Sept
Covariate: Frost
Grand mean  2.815
```

Pruning	1.00	2.00	3.00	
	3.193	2.842	2.410	
Gell_trt	1.00	2.00	3.00	4.00
	2.523	2.908	2.793	3.035
Node	1.00	2.00		
	3.056	2.574		

Pruning	Gell_trt	1.00	2.00	3.00	4.00
1.00		3.204	3.374	3.076	3.116
2.00		2.413	2.923	2.947	3.083
3.00		1.951	2.428	2.357	2.906

Pruning	Node	1.00	2.00
1.00		3.397	2.988
2.00		3.113	2.570
3.00		2.658	2.163

Gell_trt	Node	1.00	2.00
1.00		2.736	2.310
2.00		3.187	2.629
3.00		3.023	2.564
4.00		3.278	2.792

Pruning	Gell_trt	Node	1.00	2.00
1.00	1.00		3.295	3.113
	2.00		3.695	3.052
	3.00		3.242	2.910
	4.00		3.354	2.878
2.00	1.00		2.691	2.135
	2.00		3.328	2.519
	3.00		3.130	2.765
	4.00		3.305	2.861
3.00	1.00		2.221	1.681
	2.00		2.539	2.317
	3.00		2.698	2.016
	4.00		3.175	2.636

```
*** Least significant differences of means (at 95%) ***
```

Table	Pruning	Gell_trt	Node	Pruning Gell_trt
rep.	144	108	216	36
l.s.d.	0.2733	0.2384	0.1436	0.4010
d.f.	15	71	203	82.04
Except when comparing means with the same level(s) of				
Pruning				0.3999
d.f.				71

Table	Pruning Node	Gell_trt Node	Pruning Gell_trt Node
rep.	72	54	18
l.s.d.	0.2960	0.3023	0.5149
d.f.	50.43	202.28	224.63
Except when comparing means with the same level(s) of			
Pruning	0.2817		0.5139
d.f.	203		202.28
Gell_trt		0.2961	
d.f.		203	
Pruning.Gell_trt			0.5034
d.f.			203
Pruning.Node			0.5139
d.f.			202.28

```
"General Analysis of Variance."
 BLOCK Rep/Pruning/Gell_trt/Spur/Node
 TREATMENTS pol(Pruning;3)*reg(Gell_trt;3;m1)*Node
 COVARIATE Frost
 ANOVA [PRINT=aovtable,information,mean,covariate; FACT=3;
FPROB=yes; PSE=diff,lsd, means] Phenology 5 Oct

***** Analysis of variance (adjusted for covariate) *****

Variate: P5Oct
Covariate: Frost
```

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Rep stratum						
Covariate	1	0.672	0.672	0.33		0.584
Residual	7	14.263	2.038	0.88	0.92	
Rep.Pruning stratum						
Pruning	2	75.552	37.776	16.24	0.48	<.001
Lin	1	64.733	64.733	27.82	0.32	<.001
Quad	1	10.819	10.819	4.65	0.96	0.048
Covariate	1	17.249	17.249	7.41		0.016
Residual	15	34.899	2.327	1.12	1.40	

```
Rep.Pruning.Gell_trt stratum
Gell_trt                3   3.710   1.237   0.60  0.70  0.618
  nil vs rest           1   0.629   0.629   0.30  0.67  0.583
  twice vs once         1   2.336   2.336   1.13  0.56  0.292
  early vs late         1   0.745   0.745   0.36  0.99  0.550
Pruning.Gell_trt        6  49.842   8.307   4.02  0.96  0.002
  Lin.nil vs rest       1  30.279  30.279  14.64  1.00  <.001
  Quad.nil vs rest      1   1.935   1.935   0.94  1.00  0.337
  Lin.twice vs once     1   9.261   9.261   4.48  0.83  0.038
  Quad.twice vs once    1   4.924   4.924   2.38  0.99  0.127
  Lin.early vs late     1   1.013   1.013   0.49  1.00  0.486
  Deviations            1   2.430   2.430   1.17  0.99  0.282
Covariate               1  53.798  53.798  26.01        <.001
Residual               71 146.841   2.068   1.19  1.35

Rep.Pruning.Gell_trt.Spur stratum
Covariate               1  30.899  30.899  17.84        <.001
Residual              107 185.351   1.732   1.27  1.16

Rep.Pruning.Gell_trt.Spur.Node stratum
Node                    1  83.815  83.815  61.66  0.87  <.001
Pruning.Node            2   1.522   0.761   0.56  1.00  0.572
  Lin.Node              1   1.415   1.415   1.04  1.00  0.309
  Quad.Node             1   0.108   0.108   0.08  1.00  0.779
Gell_trt.Node           3   5.595   1.865   1.37  0.97  0.252
  nil vs rest.Node      1   3.747   3.747   2.76  0.96  0.098
  twice vs once.Node    1   1.847   1.847   1.36  0.98  0.245
  early vs late.Node    1   0.000   0.000   0.00  0.96  0.993
Pruning.Gell_trt.Node   6  11.028   1.838   1.35  1.00  0.236
  Lin.nil vs rest.Node  1   0.091   0.091   0.07  1.00  0.796
  Quad.nil vs rest.Node 1   0.532   0.532   0.39  1.00  0.532
  Lin.twice vs once.Node 1  0.165   0.165   0.12  1.00  0.728
  Deviations            3  10.241   3.414   2.51  0.99  0.060
Covariate               1  90.471  90.471  66.55        <.001
Residual              203 275.946   1.359        1.32

Total                 431  1137.831

* MESSAGE: the following units have large residuals.
Rep 1.00 Pruning 3.00                                    -0.601 s.e. 0.284
Rep 5.00 Pruning 3.00                                     0.617 s.e. 0.284
Rep 3.00 Pruning 2.00 Gell_trt 3.00                       2.062 s.e. 0.583
Rep 7.00 Pruning 1.00 Gell_trt 1.00                       1.629 s.e. 0.583
Rep 1.00 Pruning 1.00 Gell_trt 3.00 Spur 1.00             2.410 s.e. 0.655
Rep 1.00 Pruning 1.00 Gell_trt 3.00 Spur 2.00 -2.410 s.e. 0.655
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 1.00 -2.250 s.e. 0.655
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 2.00  2.250 s.e. 0.655
Rep 4.00 Pruning 1.00 Gell_trt 1.00 Spur 1.00 -2.250 s.e. 0.655
Rep 4.00 Pruning 1.00 Gell_trt 1.00 Spur 2.00  2.250 s.e. 0.655
Rep 9.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00  1.750 s.e. 0.655
Rep 9.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 -1.750 s.e. 0.655
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 1.00
                                                         -3.333 s.e. 0.799
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 2.00
                                                          3.333 s.e. 0.799

***** Covariate regressions *****

Variate: P50ct
Covariate                coefficient        s.e.
Rep stratum
 Frost                          0.40       0.701
Rep.Pruning stratum
 Frost                         -2.46       0.905
Rep.Pruning.Gell_trt stratum
 Frost                         -2.08       0.407
Rep.Pruning.Gell_trt.Spur stratum
 Frost                         -1.36       0.322
Rep.Pruning.Gell_trt.Spur.Node stratum
 Frost                         -1.84       0.225
Combined estimates
 Frost                         -1.71       0.163

***** Tables of means (adjusted for covariate) *****

Variate: P50ct
Covariate: Frost
Grand mean  4.086
   Pruning     1.00     2.00     3.00
             4.859    4.314    3.084
 Gell_trt     1.00     2.00     3.00     4.00
             3.952    4.174    4.048    4.169
      Node     1.00     2.00
             4.540    3.631
   Pruning Gell_trt     1.00     2.00     3.00     4.00
      1.00             5.310    5.050    4.604    4.469
      2.00             3.988    4.433    4.608    4.228
      3.00             2.559    3.038    2.931    3.810
   Pruning      Node     1.00     2.00
      1.00             5.376    4.341
      2.00             4.788    3.840
      3.00             3.457    2.712
 Gell_trt      Node     1.00     2.00
      1.00             4.573    3.332
      2.00             4.632    3.715
      3.00             4.502    3.593
      4.00             4.454    3.884
   Pruning Gell_trt      Node     1.00     2.00
      1.00     1.00              5.926    4.695
               2.00              5.754    4.347
               3.00              4.934    4.274
               4.00              4.891    4.047
      2.00     1.00              4.821    3.154
               2.00              4.938    3.927
               3.00              4.947    4.269
               4.00              4.446    4.009
      3.00     1.00              2.971    2.147
               2.00              3.205    2.871
               3.00              3.627    2.236
               4.00              4.024    3.595

*** Least significant differences of means (at 95%) ***

Table                Pruning    Gell_trt        Node     Pruning
                                                         Gell_trt
rep.                     144         108         216          36
l.s.d.                0.5549      0.4657      0.2367      0.7918
d.f.                      15          71         203       80.59
Except when comparing means with the same level(s) of
 Pruning                                                  0.7813
 d.f.                                                        71

Table                Pruning    Gell_trt     Pruning
                        Node        Node    Gell_trt
                                             Node
rep.                     72          54          18
l.s.d.                0.5560      0.5489      0.9413
d.f.                  36.72       169.41      186.06
Except when comparing means with the same level(s) of
 Pruning              0.4645                  0.9331
 d.f.                    203                  169.41
 Gell_trt                        0.4883
 d.f.                              203
 Pruning.Gell_trt                        0.8301
 d.f.                                      203
 Pruning.Node                            0.9331
 d.f.                                    169.41
```

```
"General Analysis of Variance."
 BLOCK Rep/Pruning/Gell_trt/Spur/Node
 TREATMENTS pol(Pruning;3)*reg(Gell_trt;3;m1)*Node
 COVARIATE Frost
 ANOVA [PRINT=aovtable,information,mean,covariate; FACT=3;
FPROB=yes; PSE=diff,lsd, means] P20Oct

***** Analysis of variance (adjusted for covariate) *****

Variate: P20Oct
Covariate: Frost

Source of variation d.f.(m.v.) s.s.    m.s.  v.r. cov.ef. F pr.

Rep stratum
Covariate               1   12.575  12.575  2.73        0.142
Residual                7   32.242   4.606  1.06  1.22

Rep.Pruning stratum
Pruning                 2  155.983  77.991 18.03  0.49  <.001
  Lin                   1  104.738 104.738 24.22  0.32  <.001
  Quad                  1   51.245  51.245 11.85  0.98  0.004
Covariate               1  135.326 135.326 31.29        <.001
Residual               15   64.878   4.325  1.23  2.89

Rep.Pruning.Gell_trt stratum
Gell_trt                3   7.974   2.658  0.75  0.69  0.524
  nil vs rest           1   0.232   0.232  0.07  0.67  0.798
  twice vs once         1   3.659   3.659  1.04  0.55  0.312
  early vs late         1   4.084   4.084  1.16  0.98  0.285
Pruning.Gell_trt        6  95.118  15.853  4.50  0.96  <.001
  Lin.nil vs rest       1  47.355  47.355 13.44  1.00  <.001
  Quad.nil vs rest      1   1.367   1.367  0.39  1.00  0.535
  Lin.twice vs once     1  27.181  27.181  7.71  0.82  0.007
  Quad.twice vs once    1  14.670  14.670  4.16  1.00  0.045
  Lin.early vs late     1   0.252   0.252  0.07  1.00  0.790
  Deviations            1   4.293   4.293  1.22  1.00  0.273
Covariate               1 292.935 292.935 83.12        <.001
Residual               71 250.229   3.524  0.94  2.14

Rep.Pruning.Gell_trt.Spur stratum
Covariate               1 354.024 354.024 94.73        <.001
Residual              107 399.890   3.737  1.34  1.87

Rep.Pruning.Gell_trt.Spur.Node stratum
Node                    1 112.578 112.578 40.38  0.88  <.001
Pruning.Node            2   3.246   1.623  0.58  0.99  0.560
  Lin.Node              1   2.524   2.524  0.91  0.99  0.343
  Quad.Node             1   0.723   0.723  0.26  0.99  0.611
Gell_trt.Node           3  17.959   5.986  2.15  0.97  0.096
  nil vs rest.Node      1  10.020  10.020  3.59  0.96  0.059
  twice vs once.Node    1   6.817   6.817  2.44  0.98  0.120
  early vs late.Node    1   1.122   1.122  0.40  0.96  0.527
Pruning.Gell_trt.Node   6  16.317   2.719  0.98  1.00  0.443
  Lin.nil vs rest.Node  1   2.358   2.358  0.85  1.00  0.359
  Quad.nil vs rest.Node 1   0.393   0.393  0.14  1.00  0.708
  Lin.twice vs once.Node 1  0.010   0.010  0.00  1.00  0.952
  Deviations            3  13.556   4.519  1.62  0.99  0.186
Covariate               1 647.084 647.084 232.08        <.001
Residual          198(5) 552.070   2.788        2.16

Total                426(5)     3520.656

* MESSAGE: the following units have large residuals.
Rep 1.00 Pruning 2.00                                     0.890 s.e. 0.388
Rep 1.00 Pruning 3.00                                    -1.002 s.e. 0.388
Rep 6.00 Pruning 3.00 Gell_trt 3.00                       2.473 s.e. 0.761
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 1.00 -3.250 s.e. 0.962
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 2.00  3.250 s.e. 0.962
```

```
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 1.00 -3.164 s.e. 0.962
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 2.00  3.164 s.e. 0.962
Rep 4.00 Pruning 3.00 Gell_trt 3.00 Spur 1.00  3.000 s.e. 0.962
Rep 4.00 Pruning 3.00 Gell_trt 3.00 Spur 2.00 -3.000 s.e. 0.962
Rep 5.00 Pruning 2.00 Gell_trt 2.00 Spur 1.00  2.914 s.e. 0.962
Rep 5.00 Pruning 2.00 Gell_trt 2.00 Spur 2.00 -2.914 s.e. 0.962
Rep 1.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 1.00
                                                3.611 s.e. 1.130
Rep 1.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 2.00
                                               -3.611 s.e. 1.130
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 1.00
                                               -4.889 s.e. 1.130
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 2.00
                                                4.889 s.e. 1.130
Rep 3.00 Pruning 3.00 Gell_trt 1.00 Spur 2.00 Node 1.00
                                                3.556 s.e. 1.130
Rep 3.00 Pruning 3.00 Gell_trt 1.00 Spur 2.00 Node 2.00
                                               -3.556 s.e. 1.130

***** Covariate regressions *****

Variate: P20Oct
Covariate                coefficient        s.e.
Rep stratum
 Frost                        -1.8         1.08
Rep.Pruning stratum
 Frost                        -6.5         1.16
Rep.Pruning.Gell_trt stratum
 Frost                       -4.84        0.531
Rep.Pruning.Gell_trt.Spur stratum
 Frost                       -4.65        0.478
Rep.Pruning.Gell_trt.Spur.Node stratum
 Frost                       -4.99        0.328
Combined estimates
 Frost                       -4.86        0.233

***** Tables of means (adjusted for covariate) *****

Variate: P20Oct
Covariate: Frost

Grand mean  6.846
   Pruning     1.00     2.00     3.00
             7.739    7.338    5.462
 Gell_trt     1.00     2.00     3.00     4.00
             6.780    7.031    6.725    6.848
     Node     1.00     2.00
             7.378    6.314
   Pruning Gell_trt     1.00     2.00     3.00     4.00
      1.00              8.628    7.814    7.391    7.122
      2.00              6.893    7.587    7.680    7.191
      3.00              4.817    5.693    5.104    6.232
   Pruning      Node     1.00     2.00
      1.00              8.211    7.267
      2.00              7.809    6.866
      3.00              6.115    4.808
 Gell_trt      Node     1.00     2.00
      1.00              7.604    5.956
      2.00              7.475    6.587
      3.00              7.322    6.128
      4.00              7.112    6.584
   Pruning Gell_trt      Node     1.00     2.00
      1.00      1.00              9.267    7.989
                2.00              8.176    7.453
                3.00              7.956    6.826
                4.00              7.445    6.799
      2.00      1.00              7.782    6.005
                2.00              8.169    7.004
                3.00              7.818    7.542
                4.00              7.467    6.914
      3.00      1.00              5.761    3.873
                2.00              6.082    5.304
                3.00              6.191    4.017
                4.00              6.426    6.039

*** Least significant differences of means (at 95%) ***

Table                Pruning    Gell_trt        Node     Pruning
                                                       Gell_trt
rep.                     144         108         216          36
l.s.d.                0.7499      0.6114      0.3383      1.0460
d.f.                      15          71         198       78.66
Except when comparing means with the same level(s) of
 Pruning                                                  1.0192
 d.f.                                                        71

Table                Pruning    Gell_trt     Pruning
                       Node        Node    Gell_trt
                                              Node
rep.                     72          54          18
l.s.d.                0.7667      0.7461      1.2843
d.f.                   39.33      186.03      196.72
Except when comparing means with the same level(s) of
 Pruning               0.6620                  1.2649
 d.f.                     198                  186.03
 Gell_trt                          0.7010
 d.f.                                198
 Pruning.Gell_trt                             1.1884
 d.f.                                         198
 Pruning.Node                                 1.2649
 d.f.                                         186.03

(Not adjusted for missing values)
```

```
"General Analysis of Variance."
 BLOCK Rep/Pruning/Gell_trt/Spur/Node
 TREATMENTS pol(Pruning;3)*reg(Gell_trt;3;m1)*Node
 COVARIATE Frost
 ANOVA [PRINT=aovtable,information,mean,covariate; FACT=3;
FPROB=yes; PSE=diff,lsd, means] P1Nov

***** Analysis of variance (adjusted for covariate) *****

Variate: P1Nov
Covariate: Frost

Source of variation d.f.(m.v.) s.s.   m.s.   v.r. cov.ef. F pr.

Rep stratum
Covariate               1   29.126  29.126  22.78       0.002
Residual                7    8.949   1.278   0.32  3.72
Rep.Pruning stratum
Pruning                 2   46.611  23.306   5.88  0.53 0.013
  Lin                   1   42.346  42.346  10.69  0.36 0.005
  Quad                  1    4.266   4.266   1.08  0.99 0.316
Covariate               1  239.146 239.146  60.38       <.001
Residual               15   59.408   3.961   1.36  4.71
Rep.Pruning.Gell_trt stratum
Gell_trt                3    6.640   2.213   0.76  0.74 0.520
  nil vs rest           1    3.955   3.955   1.36  0.72 0.248
  twice vs once         1    0.096   0.096   0.03  0.61 0.856
  early vs late         1    2.589   2.589   0.89  0.98 0.349
Pruning.Gell_trt        6   93.785  15.631   5.37  0.97 <.001
  Lin.nil vs rest       1   44.907  44.907  15.43  1.00 <.001
  Quad.nil vs rest      1    4.317   4.317   1.48  1.00 0.227
  Lin.twice vs once     1   10.074  10.074   3.46  0.85 0.067
  Quad.twice vs once    1   11.081  11.081   3.81  0.99 0.055
  Lin.early vs late     1   19.279  19.279   6.63  1.00 0.012
  Deviations            1    4.128   4.128   1.42  1.00 0.238
Covariate               1  349.072 349.072 119.97       <.001
Residual               71  206.585   2.910   0.70  2.65
Rep.Pruning.Gell_trt.Spur stratum
Covariate               1  502.950 502.950 120.20       <.001
Residual              107  447.701   4.184   1.19  2.10
Rep.Pruning.Gell_trt.Spur.Node stratum
Node                    1  124.743 124.743  35.44  0.85 <.001
Pruning.Node            2   15.125   7.562   2.15  1.00 0.120
  Lin.Node              1    6.443   6.443   1.83  1.00 0.178
  Quad.Node             1    8.682   8.682   2.47  0.99 0.118
Gell_trt.Node           3   11.663   3.888   1.10  0.96 0.349
  nil vs rest.Node      1    2.796   2.796   0.79  0.95 0.374
  twice vs once.Node    1    7.179   7.179   2.04  0.97 0.155
  early vs late.Node    1    1.688   1.688   0.48  0.95 0.490
Pruning.Gell_trt.Node   6   30.771   5.128   1.46  1.00 0.195
  Lin.nil vs rest.Node  1    0.703   0.703   0.20  0.99 0.655
  Quad.nil vs rest.Node 1    1.668   1.668   0.47  1.00 0.492
  Lin.twice vs once.Node 1   0.081   0.081   0.02  1.00 0.880
  Deviations            3   28.319   9.440   2.68  1.00 0.048
Covariate               1  727.103 727.103 206.58       <.001
Residual          178(25)  626.503   3.520         2.15

Total             406(25) 3824.654

* MESSAGE: the following units have large residuals.
Rep 8.00 Pruning 3.00        -0.887   s.e. 0.371
Rep 9.00 Pruning 2.00 Gell_trt 4.00        -1.957   s.e. 0.692
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 1.00 -2.750 s.e. 1.018
Rep 2.00 Pruning 1.00 Gell_trt 1.00 Spur 2.00  2.750 s.e. 1.018
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 1.00 -3.574 s.e. 1.018
Rep 2.00 Pruning 1.00 Gell_trt 2.00 Spur 2.00  3.574 s.e. 1.018
Rep 4.00 Pruning 1.00 Gell_trt 3.00 Spur 1.00  3.074 s.e. 1.018
Rep 4.00 Pruning 1.00 Gell_trt 3.00 Spur 2.00 -3.074 s.e. 1.018
Rep 9.00 Pruning 2.00 Gell_trt 4.00 Spur 1.00  3.130 s.e. 1.018
Rep 9.00 Pruning 2.00 Gell_trt 4.00 Spur 2.00 -3.130 s.e. 1.018
Rep 1.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 1.00
                                                4.056 s.e. 1.204
Rep 1.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 2.00
                                               -4.056 s.e. 1.204
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 1.00
                                               -3.444 s.e. 1.204
Rep 3.00 Pruning 2.00 Gell_trt 1.00 Spur 1.00 Node 2.00
                                                3.444 s.e. 1.204
Rep 7.00 Pruning 1.00 Gell_trt 3.00 Spur 2.00 Node 1.00
                                               -3.643 s.e. 1.204
Rep 7.00 Pruning 1.00 Gell_trt 3.00 Spur 2.00 Node 2.00
                                                3.643 s.e. 1.204
Rep 8.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 1.00
                                               -4.444 s.e. 1.204
Rep 8.00 Pruning 2.00 Gell_trt 1.00 Spur 2.00 Node 2.00
                                                4.444 s.e. 1.204
Rep 9.00 Pruning 3.00 Gell_trt 4.00 Spur 1.00 Node 1.00
                                               -4.212 s.e. 1.204
Rep 9.00 Pruning 3.00 Gell_trt 4.00 Spur 1.00 Node 2.00
                                                4.212 s.e. 1.204

***** Covariate regressions *****

Variate: P1Nov
Covariate                coefficient        s.e.
Rep stratum
 Frost                       -2.58        0.541
Rep.Pruning stratum
 Frost                        -8.4         1.09
```

```
Rep.Pruning.Gell_trt stratum                                    (Not adjusted for missing values)
 Frost                          -5.04       0.460
Rep.Pruning.Gell_trt.Spur stratum
 Frost                          -5.29       0.483
Rep.Pruning.Gell_trt.Spur.Node stratum
 Frost                          -5.75       0.400
Combined estimates
 Frost                          -5.37       0.245

***** Tables of means (adjusted for covariate) *****

Variate: P1Nov
Covariate: Frost
Grand mean  10.722
  Pruning      1.00     2.00     3.00
             11.306   10.863    9.997
 Gell_trt      1.00     2.00     3.00     4.00
             10.869   10.659   10.863   10.497
     Node      1.00     2.00
             11.287   10.157
  Pruning Gell_trt      1.00     2.00     3.00     4.00
     1.00              12.149   10.709   11.841   10.525
     2.00              10.723   11.263   11.076   10.392
     3.00               9.736   10.004    9.673   10.575
  Pruning      Node      1.00     2.00
     1.00              12.123   10.488
     2.00              11.226   10.501
     3.00              10.512    9.482
 Gell_trt      Node      1.00     2.00
     1.00              11.272   10.466
     2.00              11.467    9.851
     3.00              11.489   10.238
     4.00              10.921   10.073
  Pruning Gell_trt      Node      1.00     2.00
     1.00      1.00              12.627   11.671
               2.00              12.172    9.246
               3.00              12.484   11.198
               4.00              11.211    9.838
     2.00      1.00              11.167   10.278
               2.00              11.780   10.747
               3.00              11.192   10.959
               4.00              10.766   10.018
     3.00      1.00              10.023    9.449
               2.00              10.449    9.560
               3.00              10.790    8.556
               4.00              10.787   10.362

*** Least significant differences of means (at 95%) ***

Table              Pruning    Gell_trt        Node     Pruning
                                                      Gell_trt
rep.                   144         108         216          36
l.s.d.              0.6886      0.5382      0.3862      0.9445
d.f.                    15          71         178          76
Except when comparing means with the same level(s) of
 Pruning                                                0.9056
 d.f.                                                      71

Table              Pruning    Gell_trt     Pruning
                      Node        Node    Gell_trt
                                             Node
rep.                    72          54          18
l.s.d.              0.7648      0.7437      1.2868
d.f.                 50.17      218.90      221.67
Except when comparing means with the same level(s) of
 Pruning            0.7290                  1.2614
 d.f.                  178                  218.90
 Gell_trt                       0.7791
 d.f.                              178
 Pruning.Gell_trt                        1.3216
 d.f.                                       178
 Pruning.Node                            1.2614
 d.f.                                    218.90
```

APPENDIX C - The effect of the frost on shoot development in Experiment Two (2000)

The spring frost on the 26 September 2000 caused death of developing primary buds (Figure 3.4a). The incidence of primary bud death across the tagged shoots was 33%; however bud death was not distributed evenly across all treatments. Treatments that exhibited a delay in bud development (Table 1) experienced a reduced incidence of primary bud death (Table 1); suggesting that both treatments were effective in maintaining bud ecodormancy. Treatments showed an interaction (P = 0.018), with the early gel treatment showing less damage when combined with the late pruning treatment. Delayed winter pruning showed a quadratic reduction in primary bud death (P = < 0.001). Application of the alginate gel reduced the incidence of primary bud death (P = < 0.001), with a multiple application being more effective than a single (P = 0.003) and there being no difference between the early and mid application (P = 0.416).

Table 1 Percentage incidence of primary bud death as rated on 5 October 2000 and subsequent secondary shoot development as rated on 1 November 2000.

	Death of primary buds			
Sodium alginate gel treatment	Control	Early	Mid	Repeat
Time of pruning				
Early (August)	63.9	61.1	58.3	2.8
Mid (September)	58.3	36.1	47.2	0.0
Late (October)	38.9	11.1	16.7	2.8
			LSD (at 95%) = 19.56	
Except when comparing means at the same level of pruning = 19.55				
	Development of secondary shoots			
Sodium alginate gel treatment	Control	Early	Mid	Repeat
Time of pruning				
Early (August)	60.3	59.1	60.7	5.9
Mid (September)	50.3	36.3	45.5	11.1
Late (October)	32.0	16.7	23.6	2.6
			LSD (at 95%) = 23.39	
Except when comparing means at the same level of pruning = 20.24				

Associated with the frost event was the development of secondary shoots. The incidence of secondary shoot development across the tagged shoots was 31%, and showed an uneven distribution across treatments. Treatments that exhibited a delay in bud development (Table

1), tended to have a lower incidence of secondary shoot formation (Table 1). No interaction between the treatments occurred. Delayed winter pruning showed a quadratic reduction in the incidence of secondary shoot formation ($P = 0.004$). Application of alginate gel also reduced the incidence of secondary shoot development ($P = < 0.001$), with there being no effect between the timing of application ($P = 0.157$), or the number of applications ($P = 0.318$). Regression analysis of the incidence of bud death against secondary shoot development revealed a strong linear relationship (Figure 1), suggesting that secondary shoot formation was a consequence of primary bud death.

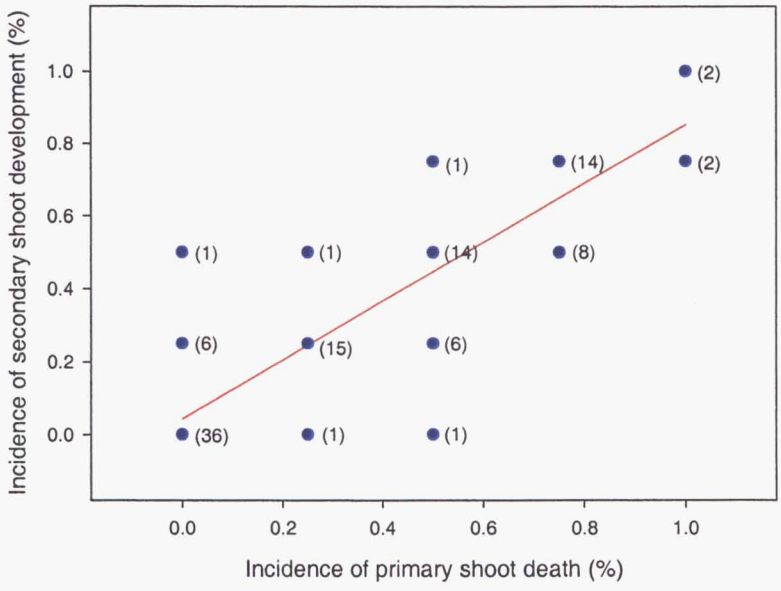


Figure 1 The relationship between the incidence of primary bud death from frost damage and the development of secondary shoots; numbers in brackets indicate the number of values behind each point; y=4.267+0.808x, R^2=0.82, $P = < 0.001$ (• Vine treatment means).

The impact of the frost in experiment two was significant, with a greater incidence of primary shoot death (Table 1) mirrored by a more advanced bud developmental stage (Table 3.2). Associated with the death of primary shoots from the September frost, was the development of secondary shoots (Figure 1). The incidence of secondary shoots is likely to have a profound effect on yield, as secondary shoots are less fruitful than primary shoots, with reduced bunch numbers per shoot and smaller bunches (Hu *et al.* 1999; Wallace 1973).

APPENDIX D - Progression of capfall for individual inflorescences in Experiments Three, Four and Five (Refer Chapter 4.0, Section Table 4.6).

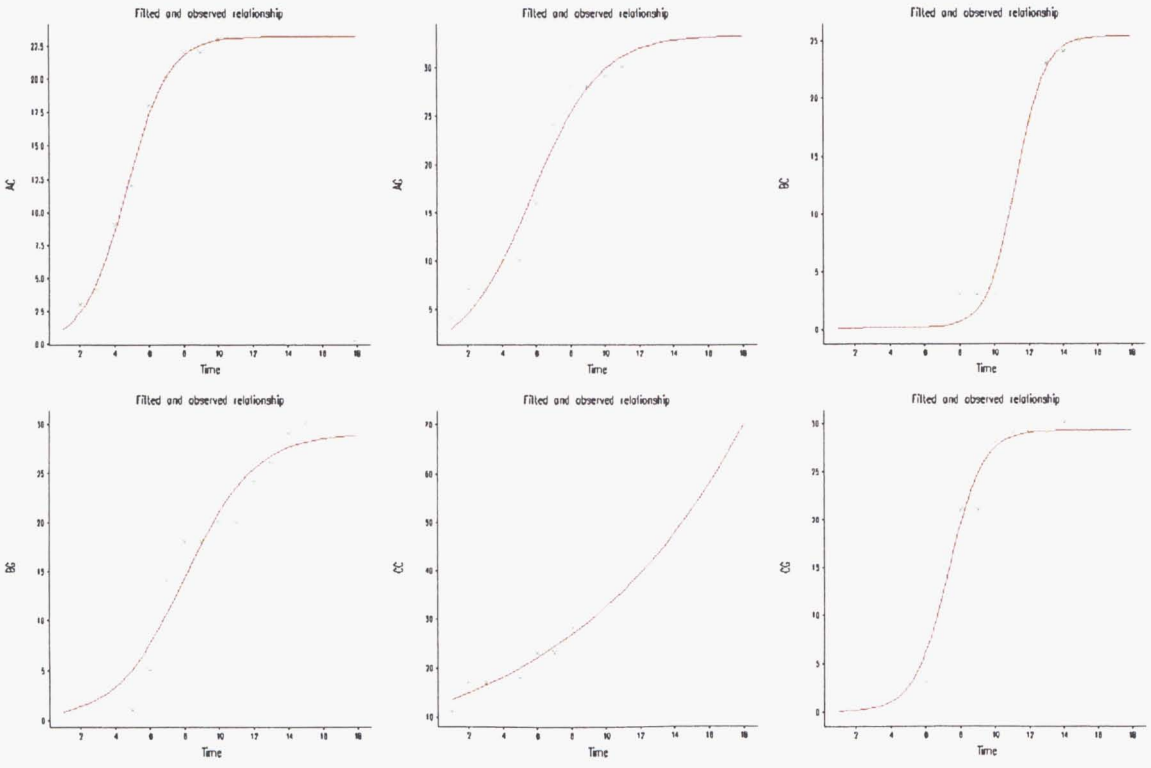
Plt	Trt	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	Control	7	3	1	5	3	6	2	2	0	1								
A	Girdled	4	3	0	3	0	6	8	4	0	1	1							
B	Control								3	0	2	2	8	7	5	1	1		
B	Girdled					1	4	9	4	0	2	0	4	2	3	1			
C	Control	11	6	0	1	0	5	0	5										
C	Girdled						3	12	6	0	7	1	0	0	1				
D	Control	8	7	0	2	1	0	7											
D	Girdled	5	0	1	2	3	4	4	6	0	1	2	0	0	0	0	1		
E	Control	1	2	0	0	2	2	13	2	0	6	0	0	0	0	0	1		
E	Girdled						1	6	9	0	9	0	1	0	2	1	1		
F	Control						1	4	3	0	9	3	1	0	5	1	1		
F	Girdled								2	0	9	3	6	2	3	1	2		
G	Control	3	7	0	1	0	5	7	3	0	1	1							
G	Girdled	4	6	3	0	0	8	5	3										
H	Control										3	0	2	2	9	5	3	1	1
H	Girdled						3	8	6	0	7	0	4	1	1				
I	Control		1	0	3	6	5	6	2	0	2	1							
I	Girdled								6	0	8	1	2	2	7	1	1	0	1
J	Control					2	4	5	8	0	5	1	0	1	1	0	1		
J	Girdled		1	0	0	0	3	9	9	0	0	0	2	1	1				

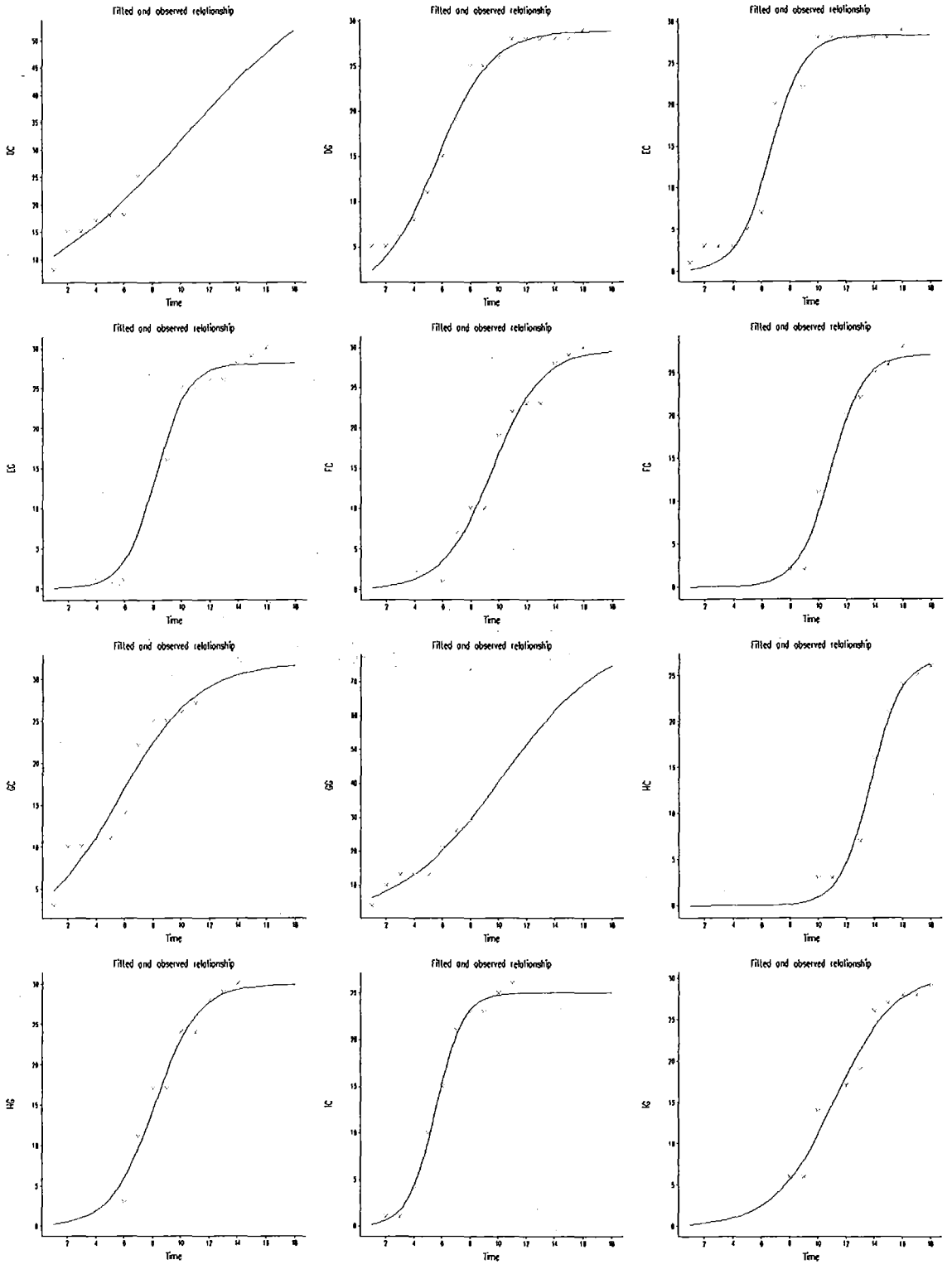
Plt	Trt	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Control								1	1	3	9	2	2	1	2	3	2				
A	Girdled					1	0	1	2	2	6	6	4	0	5	1	1					
A	Antibiotic									4	8	10	3	0	1	0	0	1				
B	Control	1	0	0	0	0	0	3	4	4	6	9	2	0	1							
B	Girdled					1	0	0	1	7	7	11	3									
B	Antibiotic					1	0	0	5	3	7	6	5	0	0	1	0	1				
C	Control									2	4	5	10	2	2	2	3					
C	Girdled								1	2	3	4	6	0	8	0	2	3				
C	Antibiotic											3	10	0	8	0	4	2	1			
D	Control											4	8	0	9	0	4	0	2	1		
D	Girdled										4	7	10	0	3	1	0	3	2			
D	Antibiotic											2	4	0	11	3	6	3	0	0	0	1
E	Control										2	8	15	0	2	0	2					
E	Girdled					1	1	1	1	5	7	5	3	0	2	0	1					
E	Antibiotic								3	2	3	12	6									
F	Control						1	0	2	4	2	8	10	0	0	0	1	1				
F	Girdled	1	0	0	0	0	0	0	0	0	0	12	5	0	4	0	1	3	1			
F	Antibiotic									1	4	10	6	0	3	1						
G	Control								1	1	4	16	6	0	2							
G	Girdled						1	0	1	0	3	10	6	0	3	2	0	1				
G	Antibiotic					2	3	0	3	7	7	3	4									
H	Control										7	6	13	0	2	1						
H	Girdled								2	2	3	7	10	0	1	4						
H	Antibiotic												6	1	5	4	4	6	3			
I	Control							1	1	4	7	6	3	0	1	1	3	2				
I	Girdled											2	7	0	6	2	5	3	2			
I	Antibiotic												6	0	6	1	13	1				
J	Control									1	1	8	15	0	2	0	1					
J	Girdled											2	10	0	8	1	4	2	1			
J	Antibiotic											9	7	1	10							

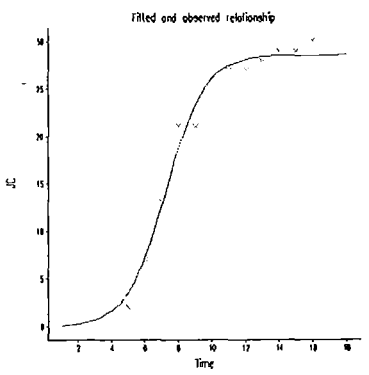
Plt	Trt	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2
A	100			4	3	2	4	4	3	1	1	4	1	2	1						
A	75		1	4	3	2	1	4	1	7	2	0	0	4	1						
A	50					1	4	2	4	1	1	2	3	3	5	3	1				
A	25		2	4	8	1	4	3	2	4	1	0	0	1							
B	100		5	8	6	1	5	2	1	0	1	1									
B	75		3	2	5	3	1	4	5	4	0	1	1	1							
B	50	1	1	2	1	3	2	2	5	3	2	5	1	2							
B	25		6	5	4	1	2	0	2	4	1	2	1	1	1						
C	100		1	5	2	2	3	3	7	3	1	1	0	1	1						
C	75			2	6	5	1	2	1	5	3	3	1	1							
C	50		2	3	7	4	1	0	3	4	3	1									
C	25		1	2	2	2	7	2	1	3	4	1	0	2	1	1					
D	100											1	1	3	6	3	2	2	6		
D	75													5	11	4	4	2	1		
D	50				1	0	0	0	0	0	0	0	0	3	10	1	4	0	10	0	1
D	25			4	9	2	4	2	0	2	2	2	0	1	2						
E	100		2	5	10	1	0	1	2	3	3	2	0	1							
E	75			1	1	1	0	2	6	1	4	3	5	3	1	0	1	0	0	1	
E	50			7	4	1	4	3	2	3	1	1	0	1	3						
E	25							3	3	5	5	0	2	7	1	2	1				
F	100		7	5	10	2	3	1	0	1	0	0	0	1							
F	75			1	0	0	0	2	3	1	2	3	3	7	7	0	1				
F	50		5	4	9	3	3	0	1	0	2	2	0	1							
F	25		4	10	10	1	1	2	0	1	0	1									
G	100			3	4	1	2	3	0	4	2	5	0	2	2	2					
G	75			1	5	0	0	2	5	3	1	6	3	3	1						
G	50				2	2	0	3	3	4	3	3	3	2	5						
G	25					2	3	4	5	1	1	3	4	2	1	4					

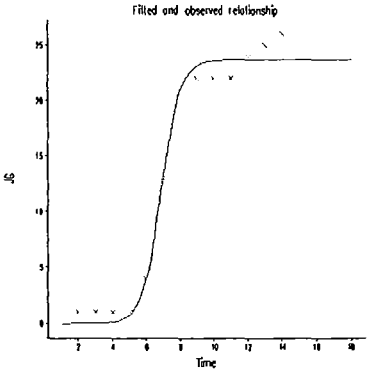
Relationship between the date of capfall and ovary diameter for individual inflorescences (Refer Chapter 4.0, Table 4.6)

Experiment Three - Pinot noir 1999.




Fitted and observed relationship
DC
Time
Fitted and observed relationship
DG
Time
Fitted and observed relationship
EC
Time
Fitted and observed relationship
EG
Time
Fitted and observed relationship
FC
Time
Fitted and observed relationship
FG
Time
Fitted and observed relationship
GC
Time
Fitted and observed relationship
GG
Time
Fitted and observed relationship
HC
Time
Fitted and observed relationship
HG
Time
Fitted and observed relationship
IC
Time
Fitted and observed relationship
IG
Time

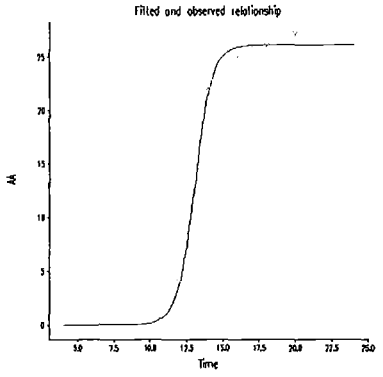


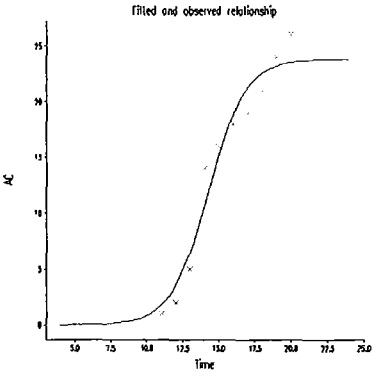


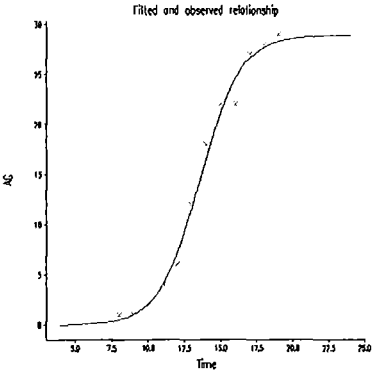
Rep/Trt	A	B	M	C	R^2
A/C	0.0	0.816	4.699	23.18	99.1
A/G	0.0	0.096	0.542	33.24	95.5
B/C	0.0	1.189	11.258	25.34	97.7
B/G	0.0	0.502	8.100	28.95	89.5
C/C	0.0	0.098	55.920	2979	85.7
C/G	0.0	1.022	7.301	29.18	93.0
D/C	0.0	0.173	10.400	66.00	74.2
D/G	0.0	0.516	5.606	28.97	98.4
E/C	0.0	0.855	6.638	28.31	97.3
E/G	0.0	0.886	8.208	28.10	95.0
F/C	0.0	0.566	9.524	29.74	95.5
F/G	0.0	0.851	10.883	27.12	97.4
G/C	0.0	0.368	5.660	31.88	91.6
G/G	0.0	0.268	10.300	84.00	91.6
H/C	0.0	0.915	13.702	26.67	98.0
H/G	0.0	0.655	8.137	29.94	94.5
I/C	0.0	1.021	5.528	25.06	99.3
I/G	0.0	0.465	11.180	30.56	95.9
J/C	0.0	0.868	7.257	28.54	97.8
J/G	0.0	1.825	6.867	23.71	98.5

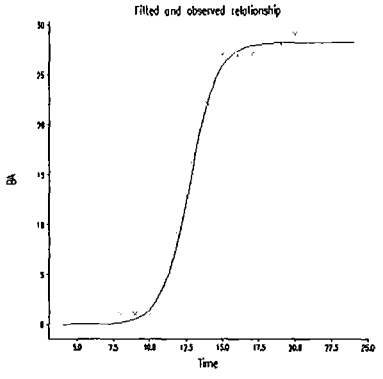
Where fitted logistic curve: $y = A + C / (1 + EXP(-B * (X - M)))$

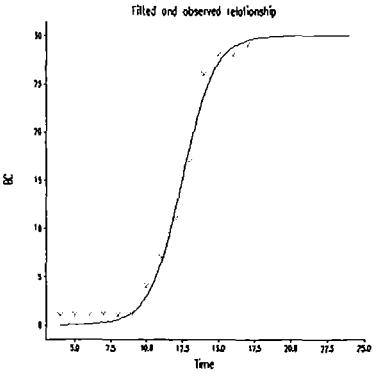
Experiment Four - Pinot noir 2000.

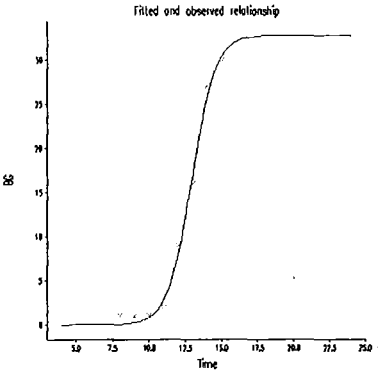


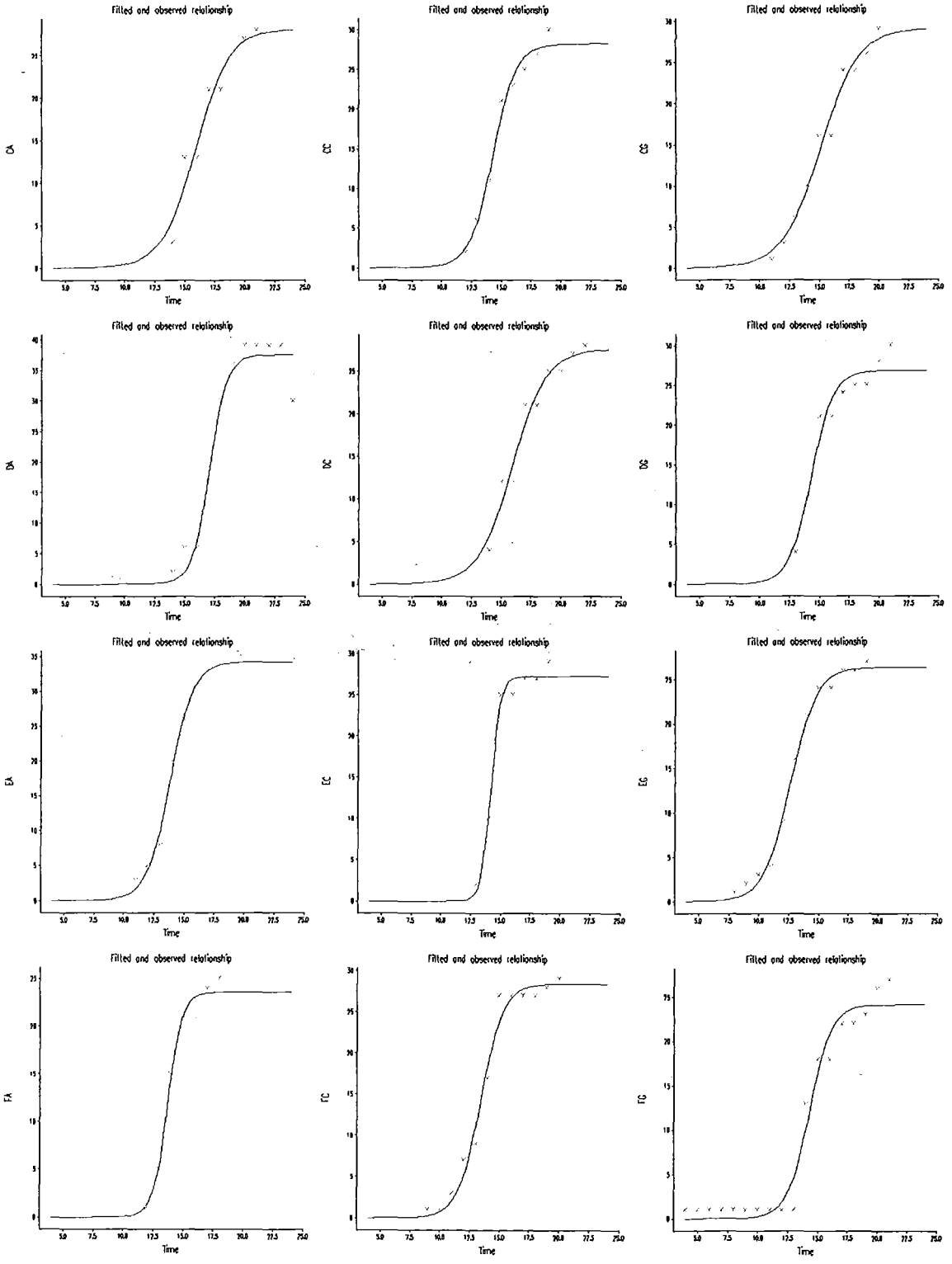


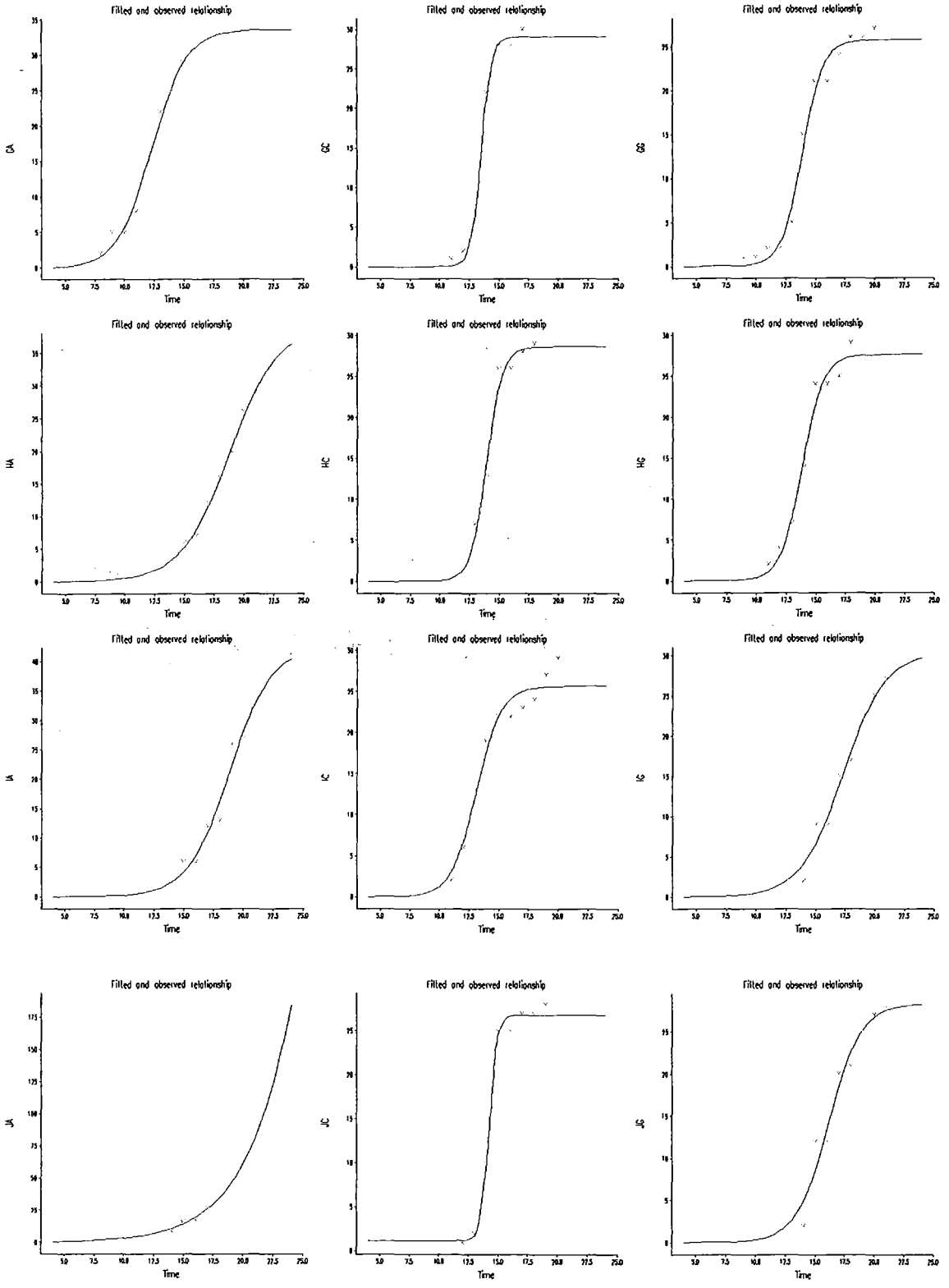








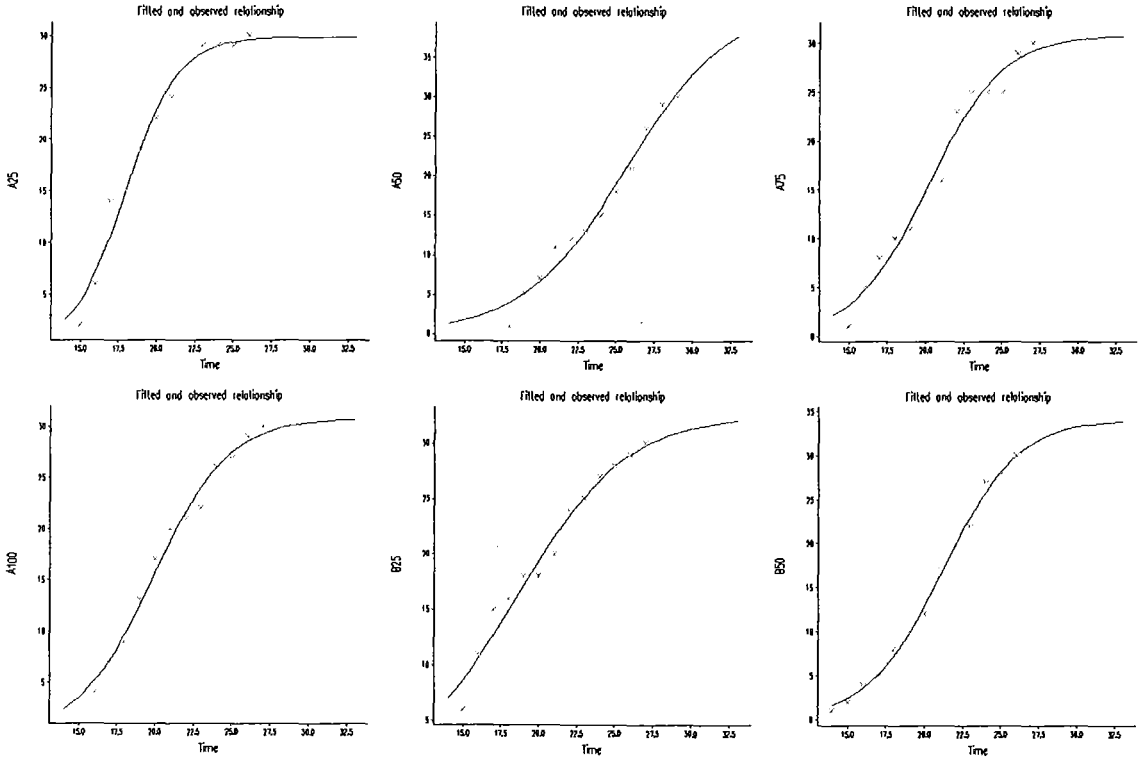

Fitted and observed relationship
CA
CC
CG
DA
DC
DG
EA
EC
EG
FA
FC
FG
Time

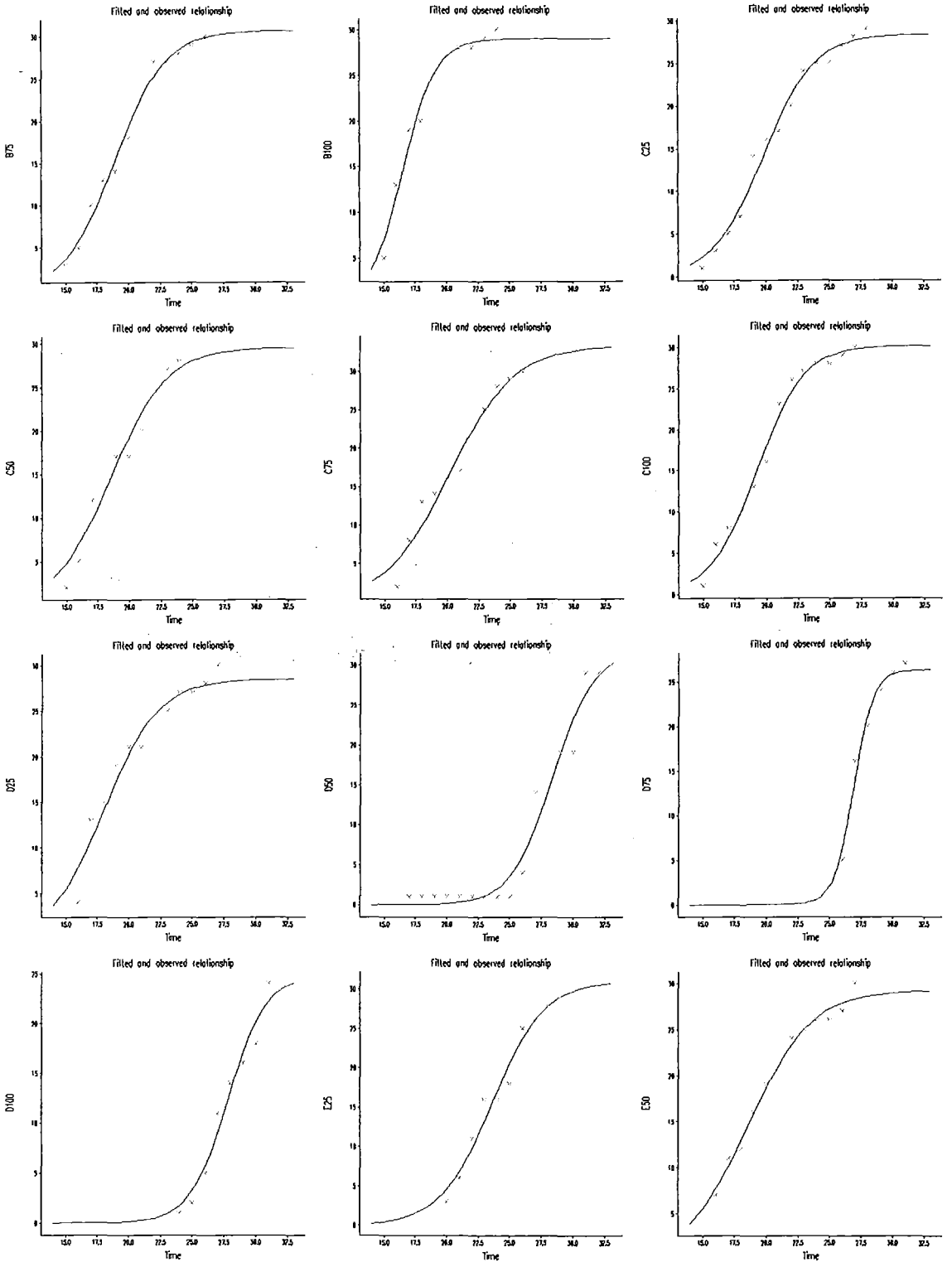

Fitted and observed relationship
CA
Time
Fitted and observed relationship
QC
Time
Fitted and observed relationship
QG
Time
Fitted and observed relationship
HA
Time
Fitted and observed relationship
HC
Time
Fitted and observed relationship
HG
Time
Fitted and observed relationship
IA
Time
Fitted and observed relationship
IC
Time
Fitted and observed relationship
IG
Time
Fitted and observed relationship
JA
Time
Fitted and observed relationship
JC
Time
Fitted and observed relationship
JG
Time

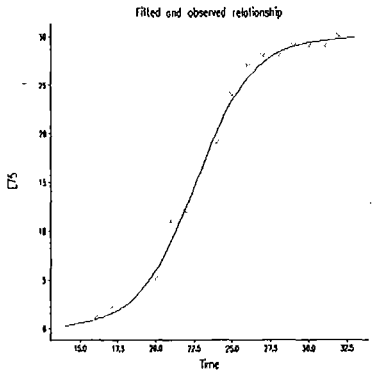
Rep/Trt	A	B	M	C	R^2
A/C	0.00	0.775	14.252	23.84	94.4
A/G	0.00	0.715	13.548	28.91	99.0
A/A	0.00	1.680	13.065	26.07	99.5
B/C	0.00	0.893	12.475	30.07	99.1
B/G	0.00	1.233	12.927	32.84	99.2
B/A	0.00	1.069	12.733	28.19	99.7
C/C	0.00	1.027	14.305	28.24	97.6
C/G	0.00	0.634	15.107	29.08	97.5
C/A	0.00	0.710	15.907	28.13	92.3
D/C	0.00	0.702	15.917	27.56	94.5
D/G	0.00	1.074	14.303	26.80	92.3
D/A	0.00	1.391	17.059	37.51	95.1
E/C	0.00	2.604	14.187	27.20	98.0
E/G	0.00	0.919	12.611	26.37	99.4
E/A	0.00	1.055	13.836	34.20	95.7
F/C	0.00	1.064	13.428	28.42	98.1
F/G	0.00	1.098	14.299	24.16	96.7
F/A	0.00	1.680	13.727	23.60	98.0
G/C	0.00	2.336	13.353	29.06	99.4
G/G	0.00	1.151	13.909	25.71	98.3
G/A	0.00	0.694	12.325	33.60	98.3
H/C	0.00	1.480	13.943	28.64	94.9
H/G	0.00	1.110	13.846	27.60	97.1
H/A	0.00	0.490	18.781	39.17	99.2
I/C	0.00	0.955	13.109	25.69	96.6
I/G	0.00	0.559	17.291	30.32	96.4
I/A	0.00	0.574	18.810	42.50	88.3
J/C	0.00	2.665	14.175	26.95	99.1
J/G	0.00	0.732	16.126	28.31	93.3
J/A	0.00	0.296	31.180	1735	78.0

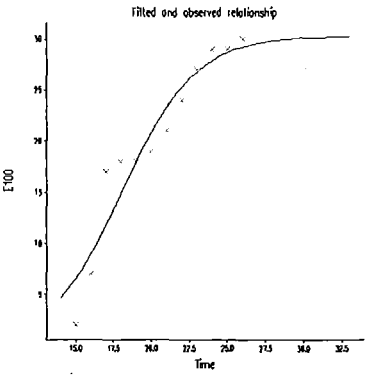
Where fitted logistic curve: $y = A + C / (1 + EXP(-B * (X - M)))$

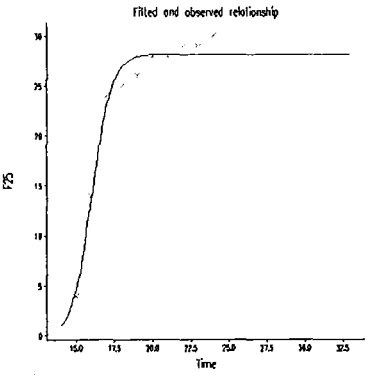
Experiment Five - Cabernet Sauvignon 2001.

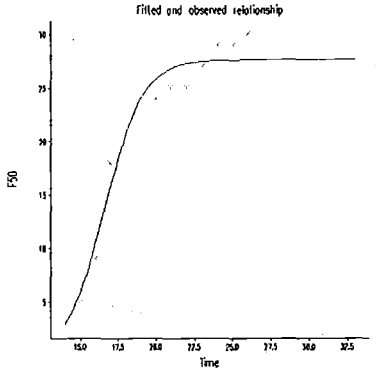


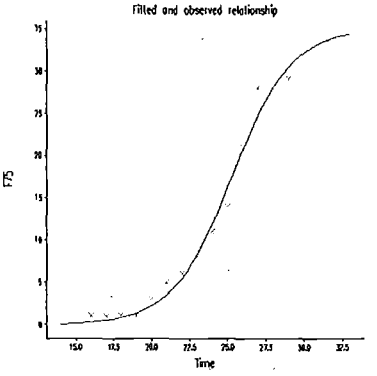

Fitted and observed relationship
B75
Time
Fitted and observed relationship
B100
Time
Fitted and observed relationship
C25
Time
Fitted and observed relationship
C50
Time
Fitted and observed relationship
C75
Time
Fitted and observed relationship
C100
Time
Fitted and observed relationship
D25
Time
Fitted and observed relationship
D50
Time
Fitted and observed relationship
D75
Time
Fitted and observed relationship
D100
Time
Fitted and observed relationship
E25
Time
Fitted and observed relationship
E50
Time

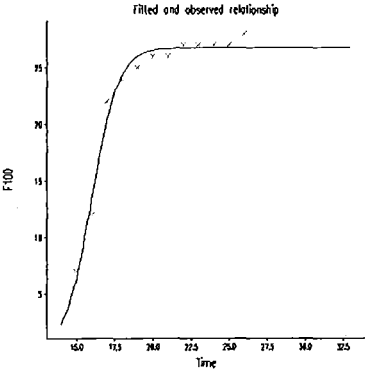

Fitted and observed relationship
E75
Time

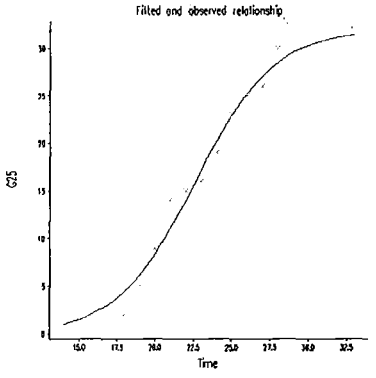

Fitted and observed relationship
E100
Time

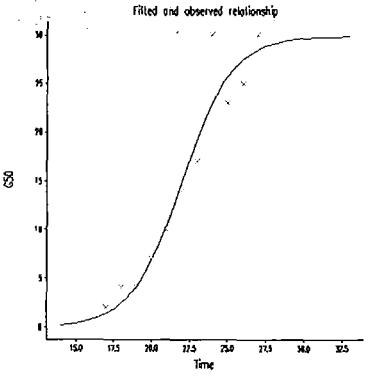

Fitted and observed relationship
F25
Time

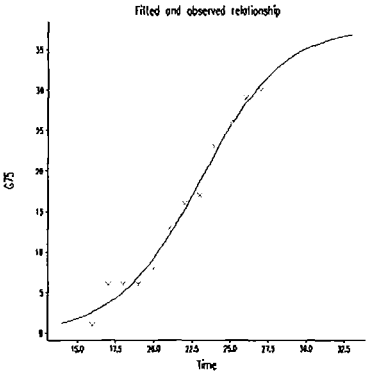

Fitted and observed relationship
F50
Time

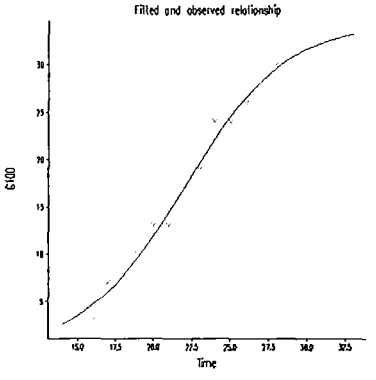

Fitted and observed relationship
F75
Time


Fitted and observed relationship
F100
Time


Fitted and observed relationship
G25
Time


Fitted and observed relationship
G50
Time


Fitted and observed relationship
G75
Time

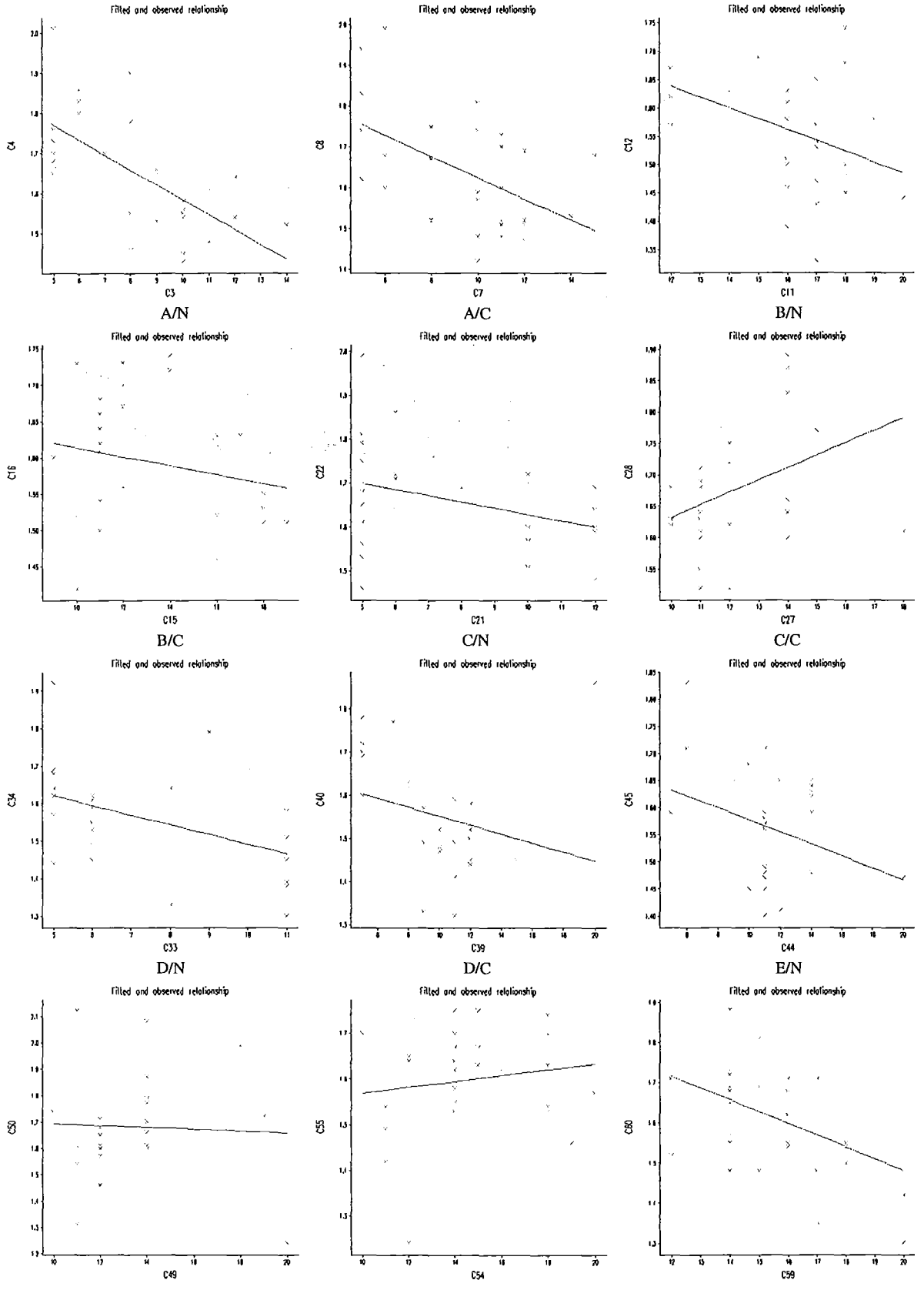

Fitted and observed relationship
G100
Time

Rep/Trt	A	B	M	C	R^2
A/25	0.00	0.5882	17.994	29.83	97.3
A/50	0.00	0.2943	25.670	42.08	97.2
A/75	0.00	0.4153	20.152	30.89	97.2
A/100	0.00	0.5470	19.270	30.15	98.5
B/25	0.00	0.2794	18.617	32.65	96.4
B/50	0.00	0.4114	21.208	34.19	99.5
B/75	0.00	0.5012	18.955	30.75	98.5
B/100	0.00	0.7450	16.517	29.05	96.9
C/25	0.00	0.5005	19.833	28.32	98.2
C/50	0.00	0.4540	18.602	29.54	92.3
C/75	0.00	0.3865	20.198	33.39	95.7
C/100	0.00	0.5470	19.270	30.15	98.4
D/25	0.00	0.4671	18.056	28.49	93.1
D/50	0.00	0.6140	28.350	31.78	96.7
D/75	0.00	1.2860	26.890	26.33	96.0
D/100	0.00	0.6750	27.822	24.77	95.2
E/25	0.00	0.4858	23.604	31.07	96.3
E/50	0.00	0.4042	18.599	29.24	98.4
E/75	0.00	0.5354	22.611	29.96	99.3
E/100	0.00	0.4150	18.102	30.34	89.2
F/25	0.00	1.5690	16.032	28.20	97.2
F/50	0.00	0.7990	16.651	27.63	94.8
F/75	0.00	0.5137	25.275	34.97	98.2
F/100	0.00	1.1570	16.006	26.82	97.9
G/25	0.00	0.3939	22.633	31.94	96.3
G/50	0.00	0.6100	21.990	29.87	91.8
G/75	0.00	0.3711	23.013	37.74	98.0
G/100	0.00	0.3078	22.037	34.34	98.5

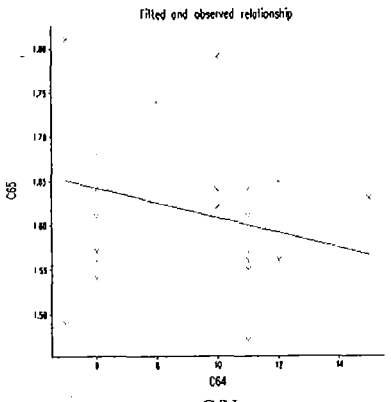
Where fitted logistic curve: $y = A + C / (1 + EXP (-B * (X - M)))$

APPENDIX E - Relationship between the date of capfall and ovary diameter for individual inflorescences (Refer Chapter 4.0, Table 4.6)

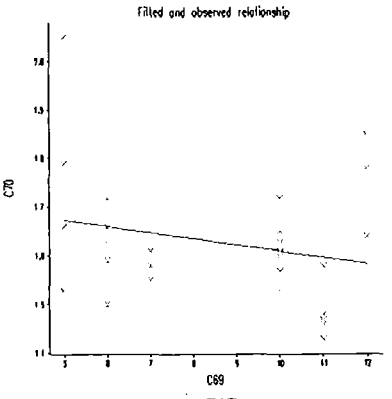
Experiment Three Pinot noir 1999



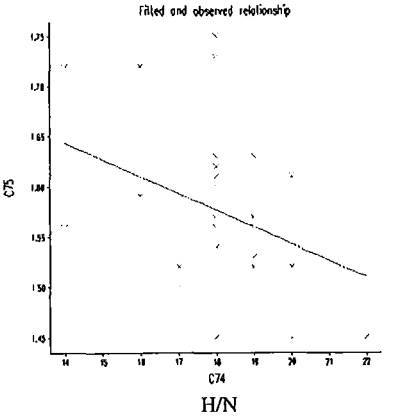
E/C



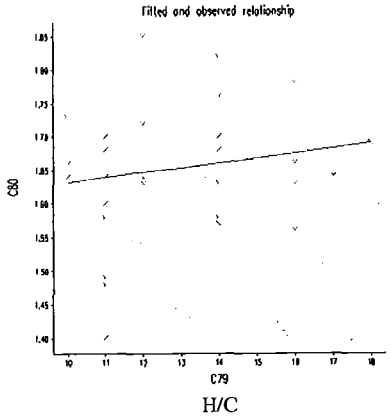
F/N



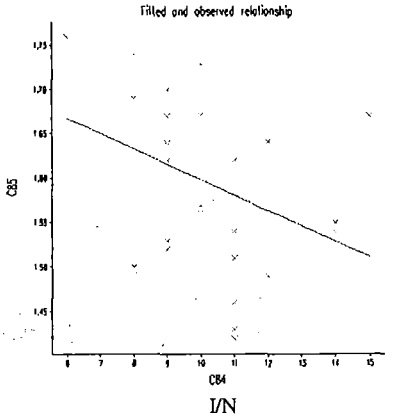
F/C



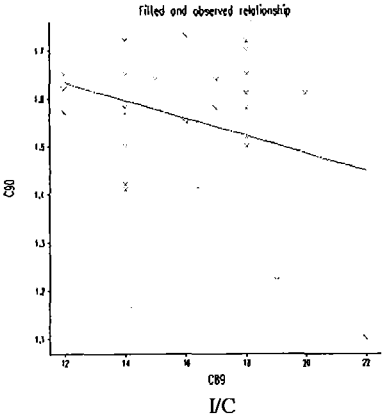
G/N



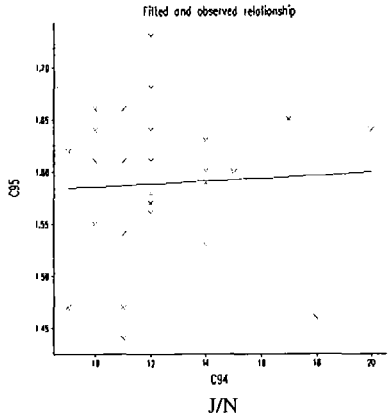
G/C



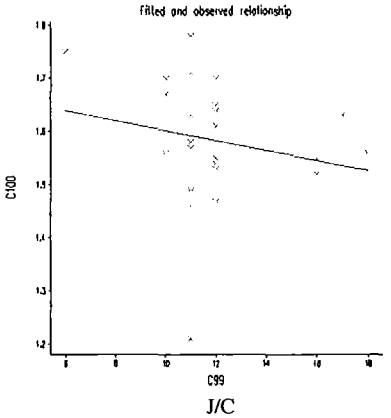
H/N



H/C



I/N



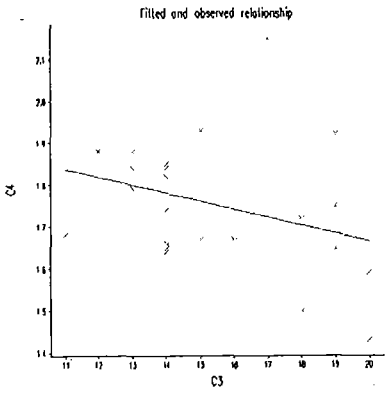
I/C

J/N

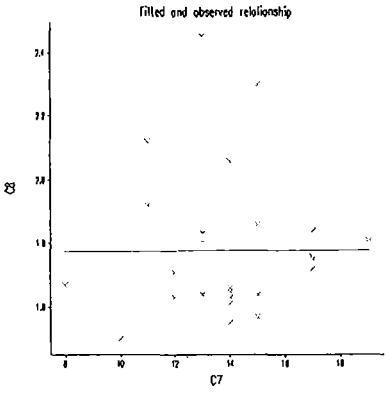
J/C

Rep/Trt	y axis	x axis	Regression line	*P* value	R^2
A/N	C4	C3	y = -0.037 x + 1.955	<0.001	40.9
A/C	C8	C7	y = -0.026 x + 1.885	0.005	22.7
B/N	C12	C11	y = -0.019 x + 1.868	0.050	10.3
B/C	C16	C15	y = -0.006 x + 1.676	0.259	1.1
C/N	C22	C21	y = -0.014 x + 1.771	0.076	8.2
C/C	C28	C27	y = 0.020 x + 1.435	0.048	10.2
D/N	C34	C33	y = -0.026 x + 1.750	0.019	18.2
D/C	C40	C39	y = -0.010 x + 1.656	0.147	4.2
E/N	C45	C44	y = -0.011 x + 1.689	0.102	6.2
E/C	C50	C49	y = -0.004 x + 1.730	0.789	
F/N	C55	C54	y = 0.006 x + 1.505	0.148	
F/C	C60	C59	y = -0.029 x + 2.069	0.007	22.0
G/N	C65	C64	y = -0.008 x + 1.692	0.201	3.0
G/C	C70	C69	y = -0.013 x + 1.738	0.190	2.8
H/N	C75	C74	y = -0.017 x + 1.877	0.044	12.3
H/C	C80	C79	y = 0.007 x + 1.558	0.367	
I/N	C85	C84	y = -0.017 x + 1.772	0.070	9.4
I/C	C90	C89	y = -0.019 x + 1.855	0.049	10.4
J/N	C95	C94	y = 0.001 x + 1.573	0.803	
J/C	C100	C99	y = -0.009 x + 1.695	0.321	0.1

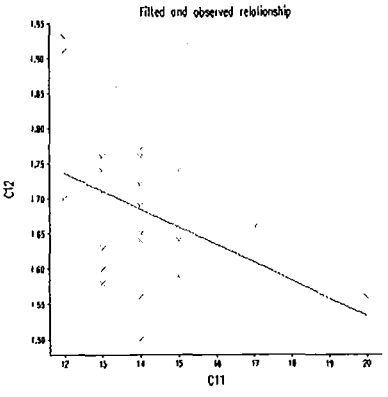
Flower size vs. capfall date on each inflorescence – Experiment Four Pinot noir 2000



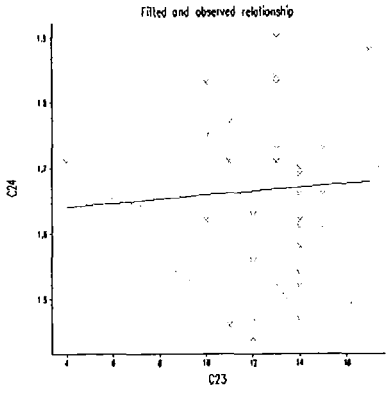
A/C



A/G



A/A



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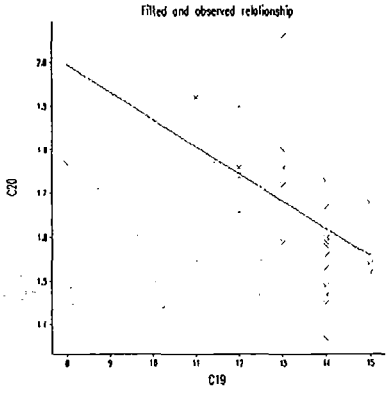

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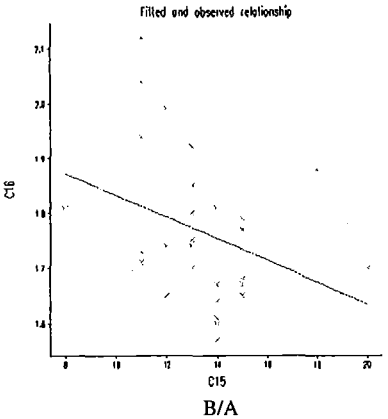

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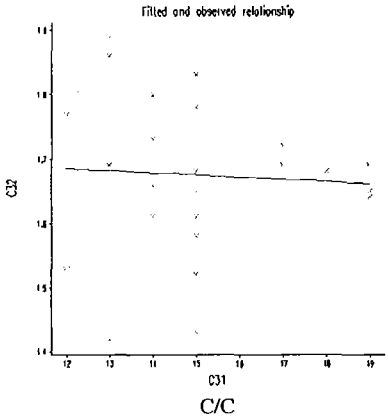

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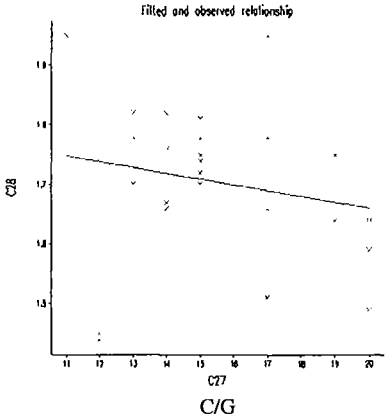

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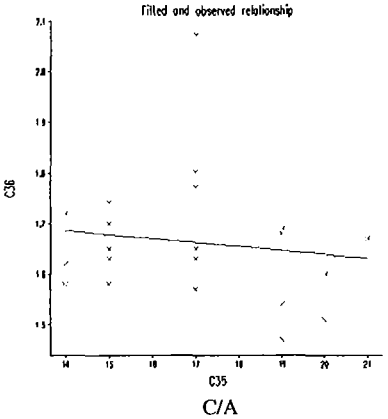

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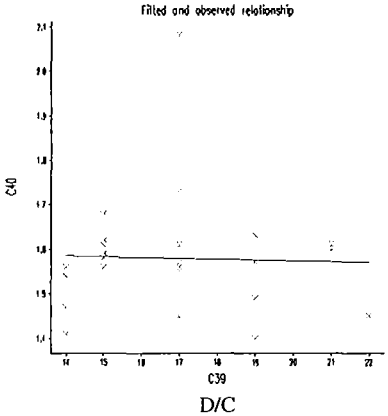
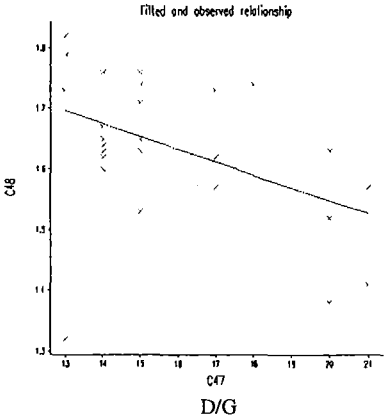


D/C



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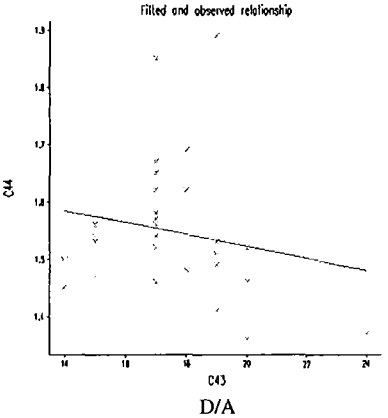

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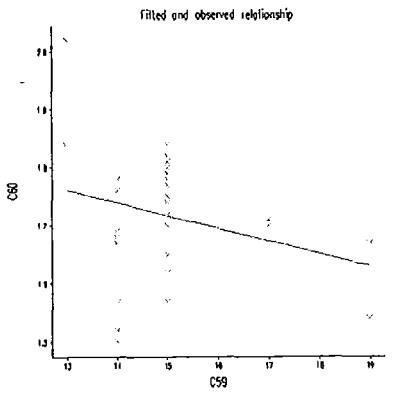
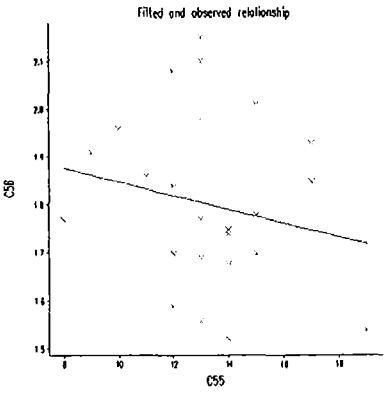


E/C



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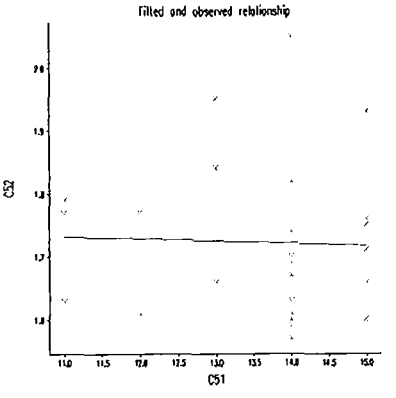

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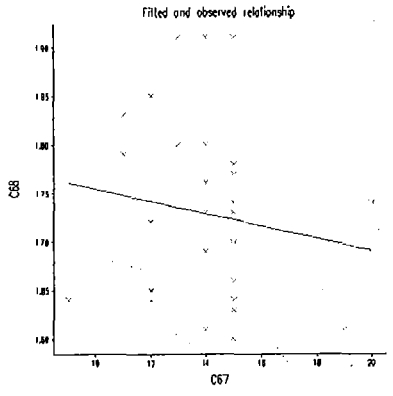

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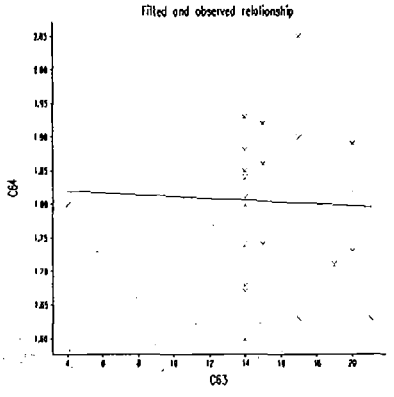

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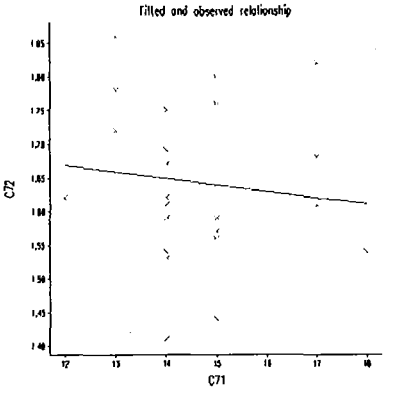

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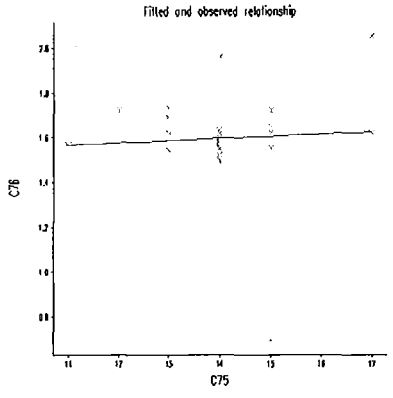

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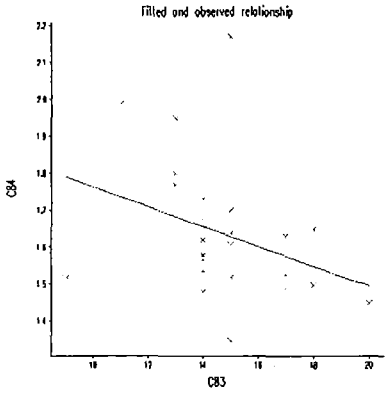

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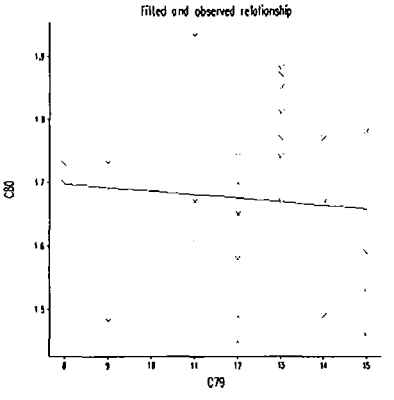

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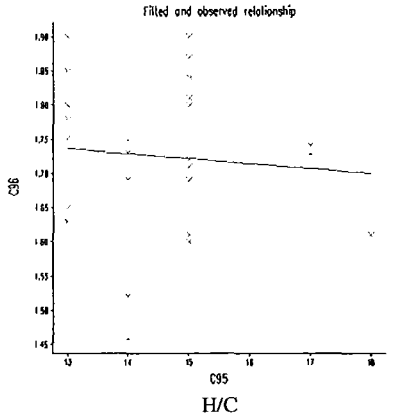

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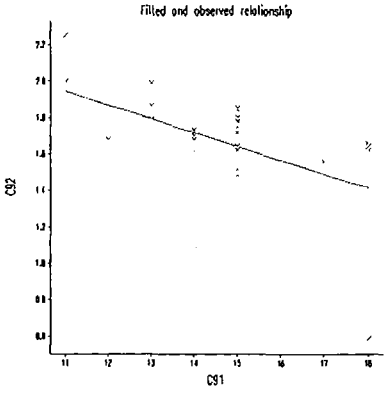

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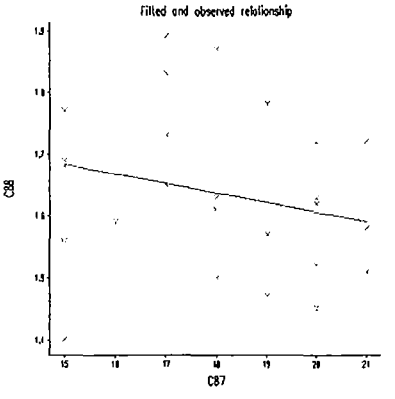

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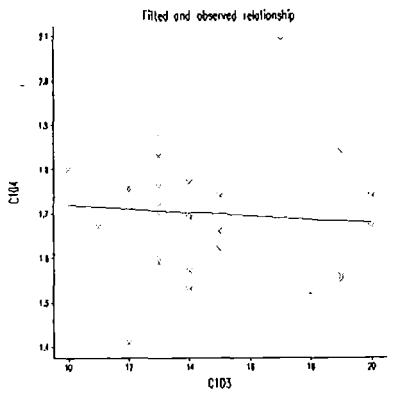

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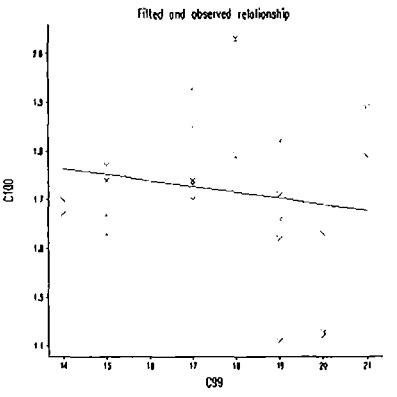

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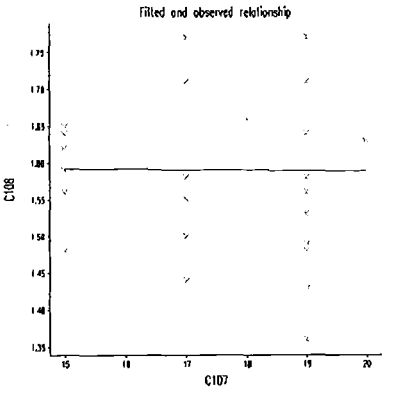

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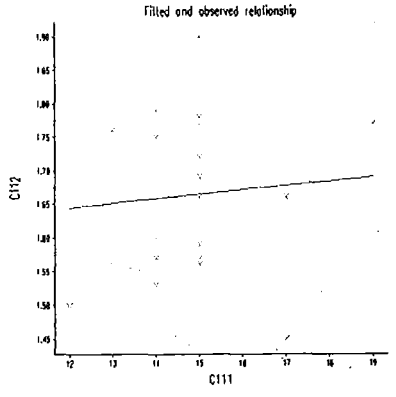

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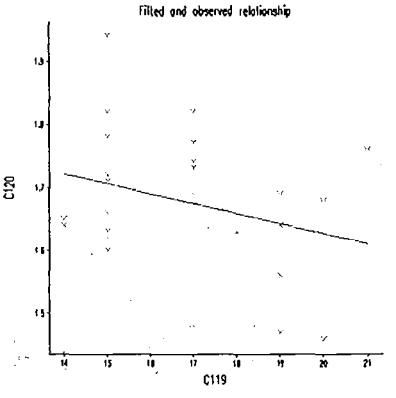

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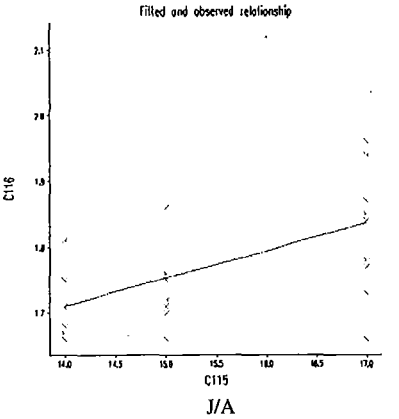
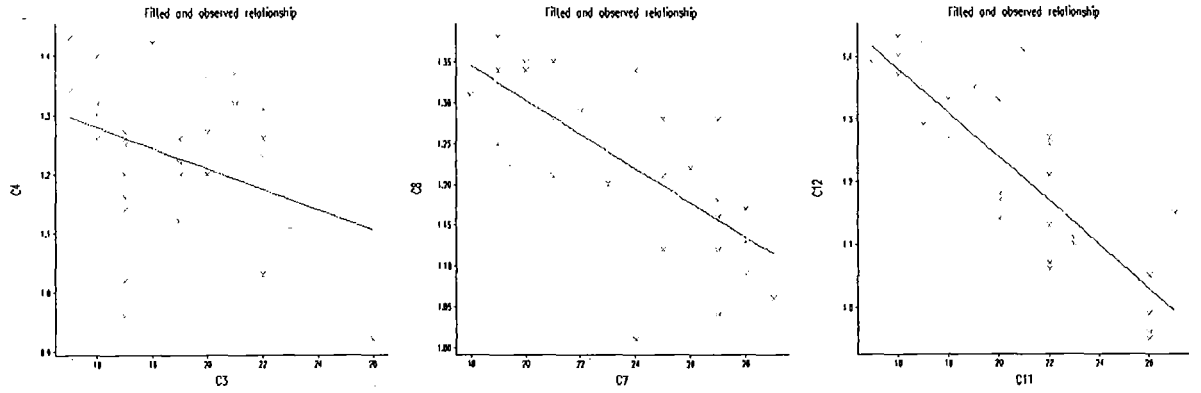


J/A

Rep /Trt	x axis	y axis	Regression line	*P* value	R^2
A/C	C3	C4	y = -0.0191 x + 2.048	0.104	6.9
A/G	C7	C8	y = -0.0003 x + 1.774	0.988	
A/A	C11	C12	y = -0.0253 x + 2.039	0.027	14.7
B/A	C15	C16	y = -0.0198 x + 2.030	0.077	7.8
B/G	C19	C20	y = -0.0624 x + 2.493	0.001	29.8
B/C	C23	C24	y = 0.0029 x + 1.629	0.795	
C/G	C27	C28	y = -0.0097 x + 1.856	0.324	0.0
C/C	C31	C32	y = -0.0035 x + 1.727	0.769	
C/A	C35	C36	y = -0.0077 x + 1.794	0.476	
D/C	C39	C40	y = -0.0020 x + 1.612	0.856	
D/A	C43	C44	y = -0.0104 x + 1.730	0.328	
D/G	C47	C48	y = -0.0212 x + 1.972	0.017	16.3
E/A	C51	C52	y = -0.0036 x + 1.771	0.856	
E/G	C55	C56	y = -0.0143 x + 1.992	0.322	0.1
E/C	C59	C60	y = -0.0220 x + 2.047	0.147	4.2
F/G	C63	C64	y = -0.0014 x + 1.826	0.837	
F/C	C67	C68	y = -0.0064 x + 1.818	0.446	
F/A	C71	C72	y = -0.0098 x + 1.786	0.557	
G/C	C75	C76	y = 0.0094 x + 1.463	0.779	
G/A	C79	C80	y = -0.0056 x + 1.742	0.671	
G/G	C83	C84	y = -0.0266 x + 2.030	0.091	7.4
H/A	C87	C88	y = -0.0154 x + 1.915	0.188	2.9
H/G	C91	C92	y = -0.0757 x + 2.779	0.002	27.0
H/C	C95	C96	y = -0.0072 x + 1.830	0.656	
I/G	C99	C100	y = -0.0123 x + 1.936	0.367	
I/C	C103	C104	y = -0.0040 x + 1.759	0.677	
I/A	C109	C108	y = -0.0007 x + 1.602	0.957	
J/C	C111	C112	y = 0.0065 x + 1.567	0.697	
J/A	C115	C116	y = 0.0415 x + 1.131	0.008	21.7
J/G	C119	C120	y = -0.0160 x + 1.946	0.124	5.4

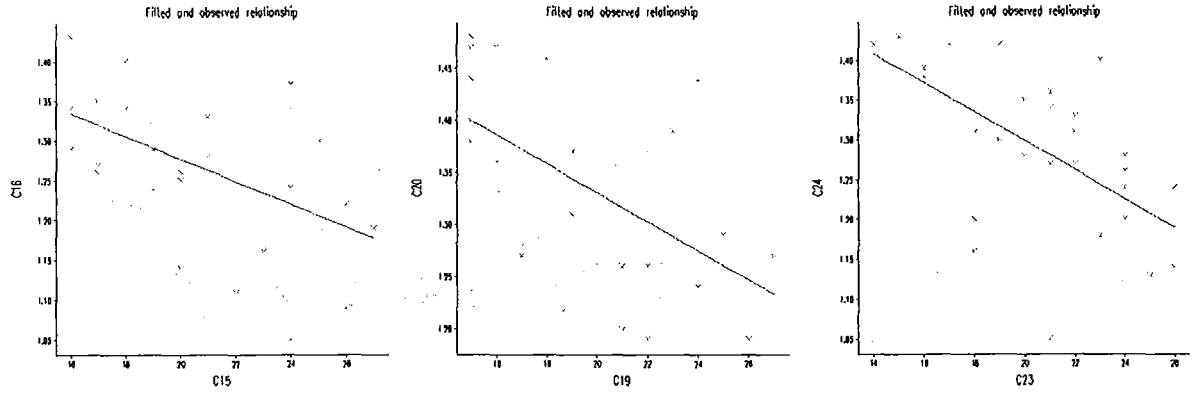
Flower size vs. capfall date on each inflorescence – Experiment Five Cabernet Sauvignon 2001



A/25

A/50

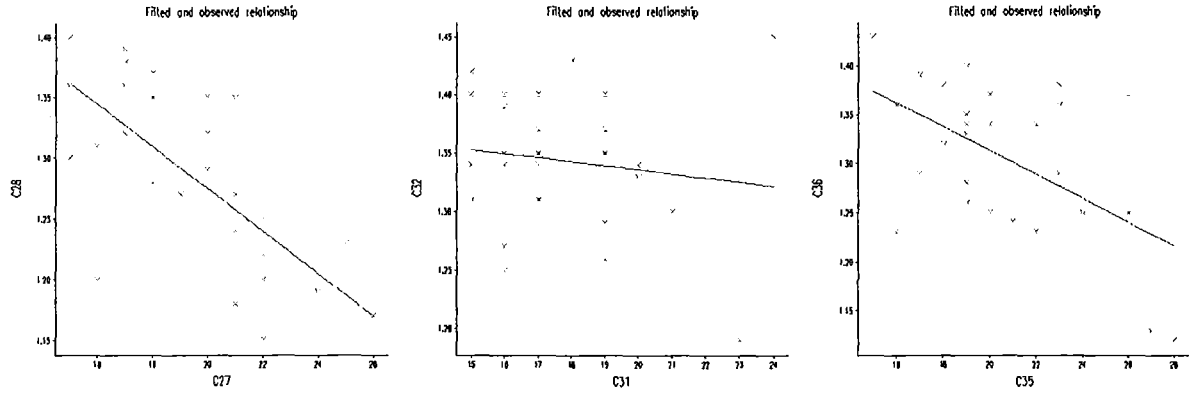
A/75



A/100

B/25

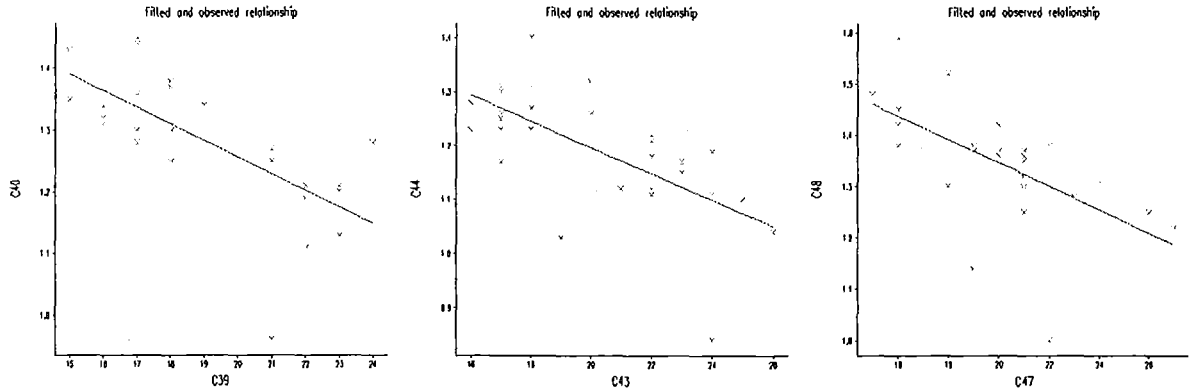
B/50



B/75

B/100

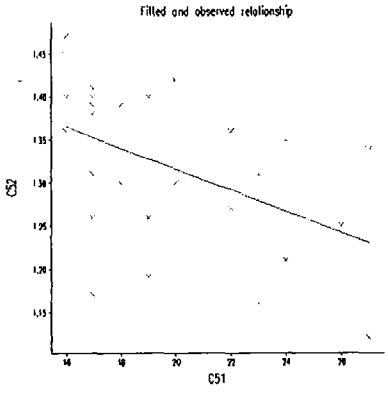
C/25



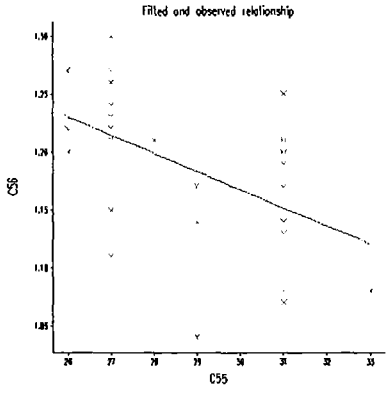
C/50

C/75

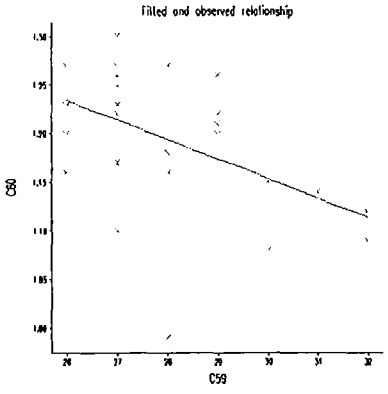
C/100



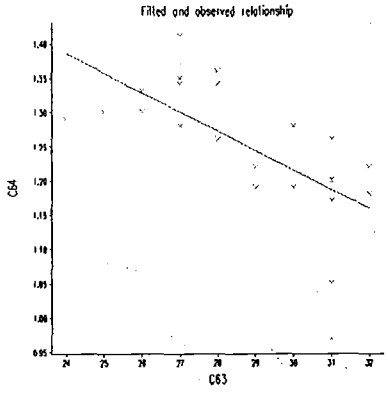
D/25



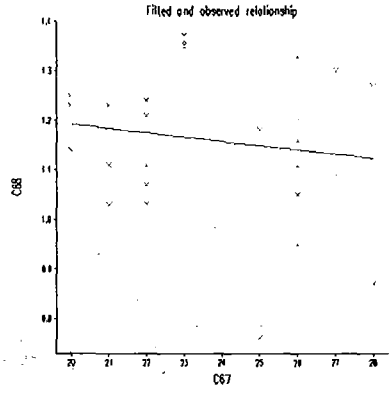
D/50



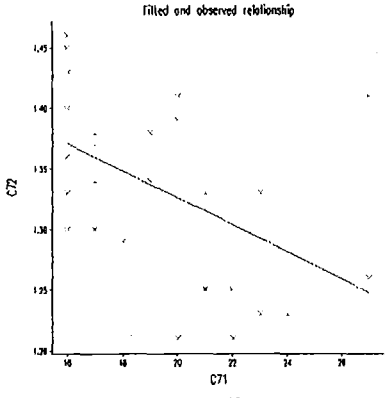
D/75



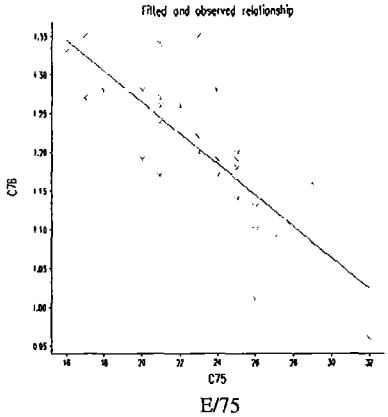
D/100



E/25



E/50



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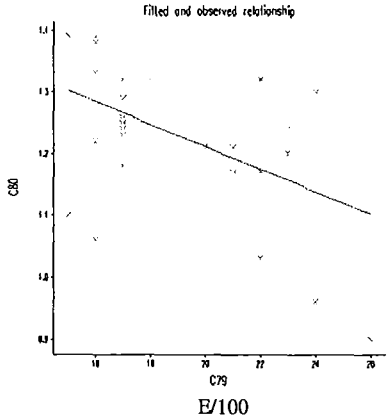
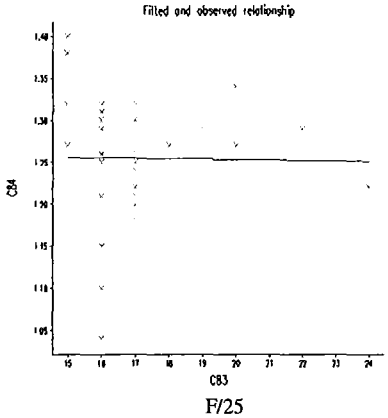


E/100



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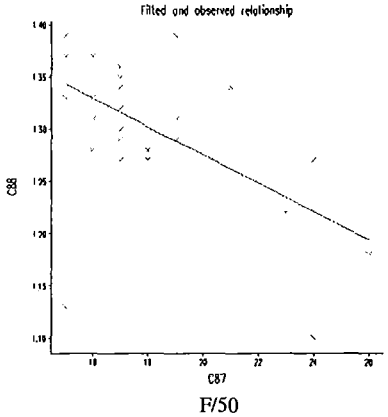
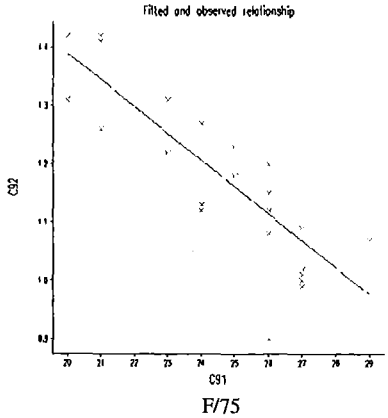


F/50



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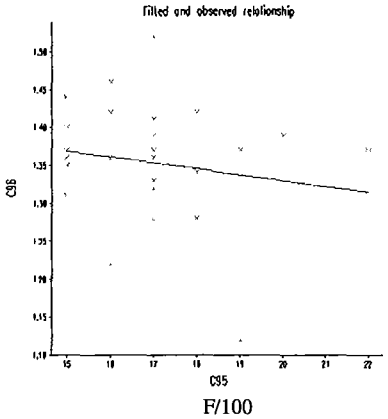

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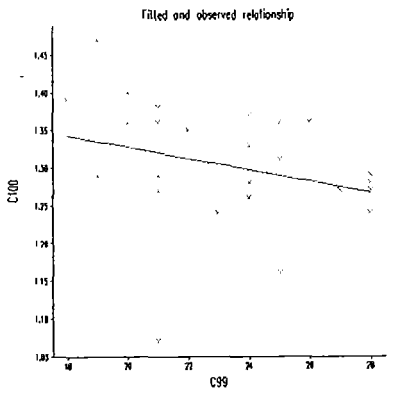

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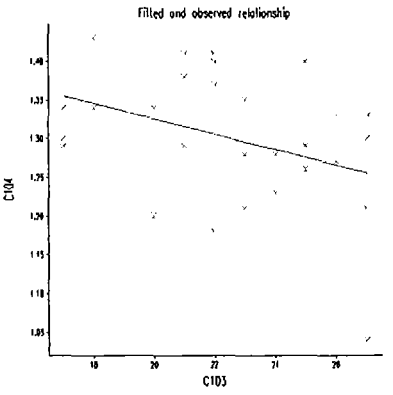

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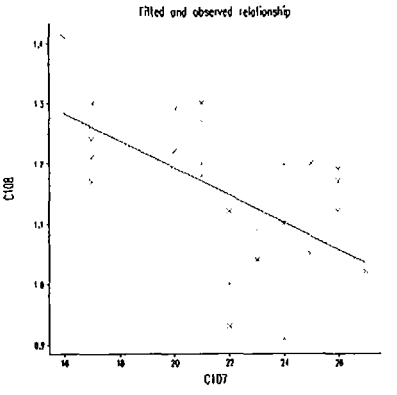

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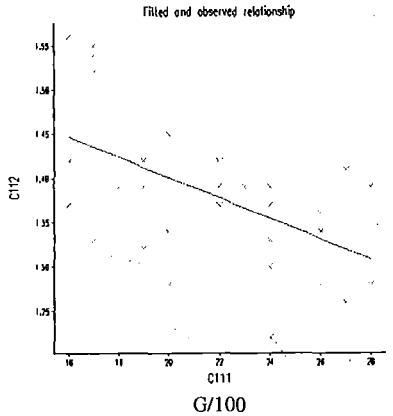


G/100

Rep/Trt	x axis	y axis	Regression line	*p* value	R^2
A/25	C3	C4	y = -0.01735 x + 1.556	0.049	10.1
A/50	C7	C8	y = -0.02101 x + 1.724	<0.001	49.6
A/75	C11	C12	y = -0.03502 x + 1.939	<0.001	70.2
A/100	C15	C16	y = -0.01429 x + 1.563	0.003	24.9
B/25	C19	C20	y = -0.01394 x + 1.609	<0.001	34.7
B/50	C23	C24	y = -0.01825 x + 1.664	<0.001	31.7
B/75	C27	C28	y = -0.01743 x + 1.623	<0.001	45.8
B/100	C31	C32	y = -0.00351 x + 1.406	0.444	
C/25	C35	C36	y = -0.01215 x + 1.556	0.002	26.8
C/50	C39	C40	y = -0.02692 x + 1.795	<0.001	48.6
C/75	C43	C44	y = -0.02431 x + 1.683	<0.001	41.3
C/100	C47	C48	y = -0.02291 x + 1.805	<0.001	36.0
D/25	C51	C52	y = -0.01225 x + 1.561	0.016	16.2
D/50	C55	C56	y = -0.01564 x + 1.637	0.005	22.3
D/75	C59	C60	y = -0.02013 x + 1.757	0.009	20.6
D/100	C63	C64	y = -0.02835 x + 2.066	<0.001	42.7
E/25	C67	C68	y = -0.00860 x + 1.365	0.441	
E/50	C71	C72	y = -0.01118 x + 1.550	0.008	20.3
E/75	C75	C26	y = -0.02008 x + 1.666	<0.001	59.0
E/100	C79	C80	y = -0.01827 x + 1.577	0.006	21.1
F/25	C83	C84	y = -0.00052 x + 1.264	0.941	
F/50	C87	C88	y = -0.01357 x + 1.547	<0.001	30.6
F/75	C91	C92	y = -0.04575 x + 2.304	<0.001	68.2
F/100	C95	C26	y = -0.00774 x + 1.484	0.363	
G/25	C99	C100	y = -0.00756 x + 1.479	0.125	4.9
G/50	C103	C104	y = -0.00998 x + 1.525	0.036	11.7
G/75	C107	C108	y = -0.02257 x + 1.644	<0.001	33.7
G/100	C111	C112	y = -0.01156 x + 1.632	0.002	26.5

APPENDIX F – Additional figures and tables for Chapter V

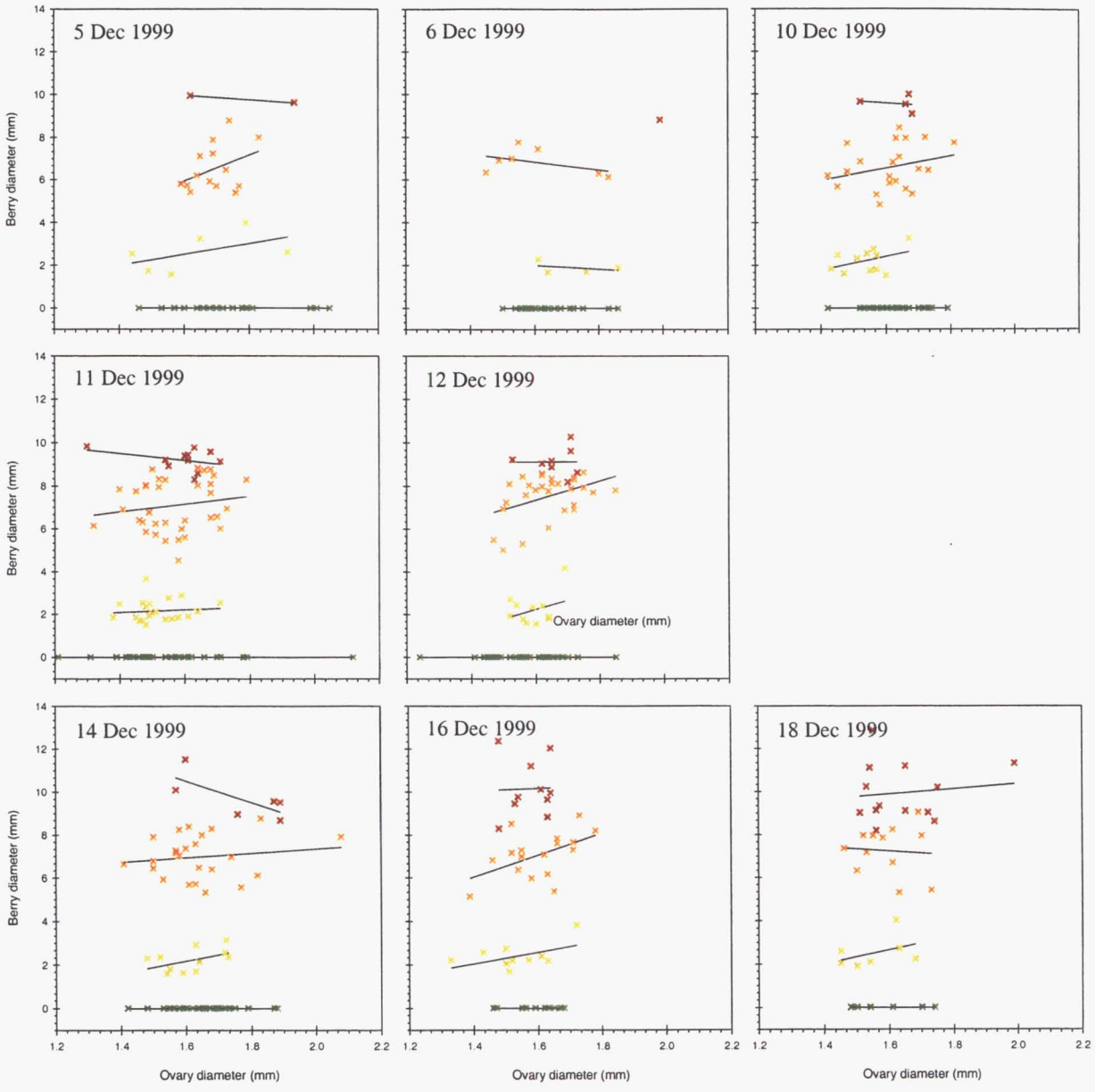


Figure 1 The relationship between flower size and berry development in Pinot noir flowers opening on a given day (Experiment Three, 1999).

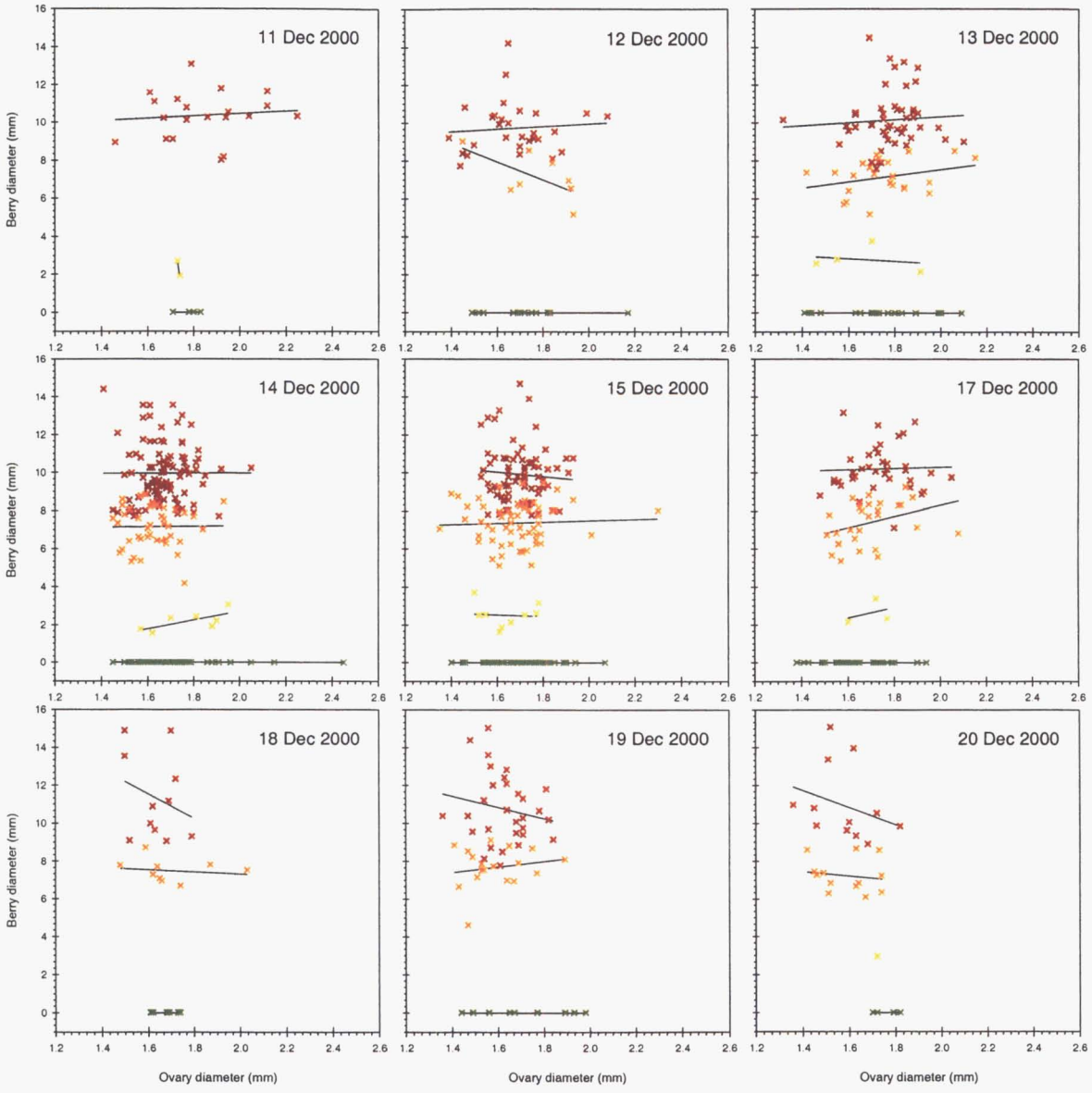


Figure 2 The relationship between flower size and berry development in Pinot noir flowers opening on a given day (Experiment Four, 2000).

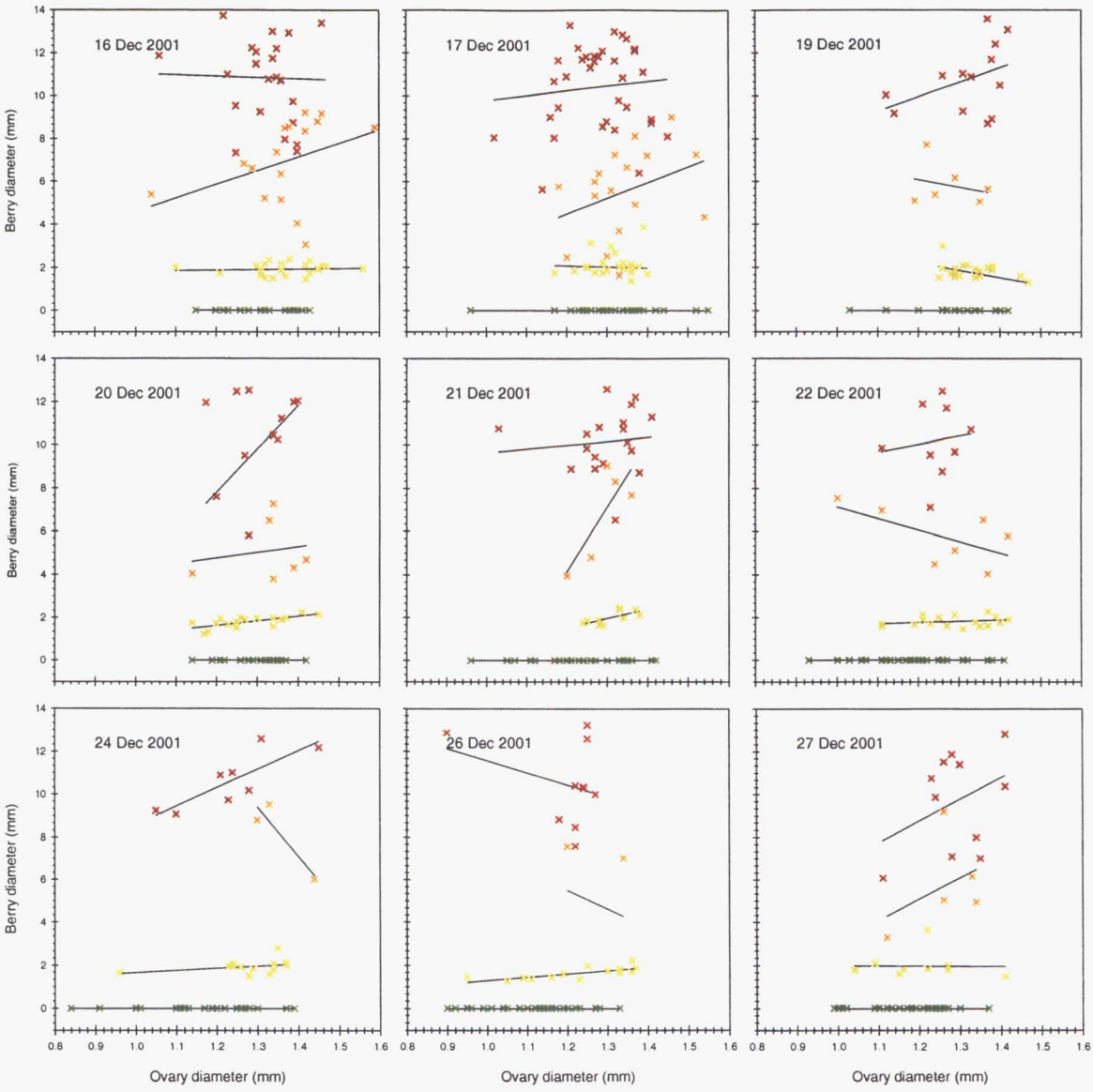


Figure 3 The relationship between flower size and berry development in Cabernet Sauvignon flowers opening on a given day (Experiment Five, 2001).

Table 1 Total calculated shoot leaf areas for Experiment Five (Cabernet Sauvignon 2001); Highlighted cells indicate abnormal leaf areas.

Plant (replicate)	Treatment leaf area	Total shoot leaf area (cm^2)	Plant (replicate)	Treatment leaf area	Total shoot leaf area (cm^2)
A	100	1448	E	100	888
A	75	1032	E	75	842
A	50	464	E	50	671
A	25	252	E	25	259
B	100	1245	F	100	1499
B	75	1076	F	75	915
B	50	636	F	50	786
B	25	307	F	25	228
C	100	1320	G	100	1573
C	75	661	G	75	1054
C	50	516	G	50	844
C	25	266	G	25	240
D	100	708			
D	75	944			
D	50	561			
D	25	209			

Table 2 Mean calculated total shoot leaf area for treatments in Experiment Five (Cabernet Sauvignon 2001)

Treatment	100%	75%	50%	25%	*P* value	LSD
Calculated total shoot leaf area (cm^2)	1241	933	640	252	<0.001	199.0

Table 3 Regression equations and statistics for the relationship between ovary diameter and berry diameter

	Regression equation	*F* value	R^2
Pinot noir 1999			
Seeded berries	y = 12.02 + -1.453x	0.142	1.9
Seedless berries	y = 3.96 + 1.864x	0.010	3.0
Shot berries	y = 0.052 + 1.420x	0.010	6.0
Abscised flowers	-	-	-
Pinot noir 2000			
Seeded berries	y = 11.44 + -0.745x	0.213	0.1
Seedless berries	y = 6.613 + 0.437x	0.361	-
Shot berries	y = 2.53 + -0.027x	0.974	-
Abscised flowers	-	-	-
Cabernet Sauvignon 2001			
Seeded berries	y = 7.61 + 2.24x	0.048	1.5
Seedless berries	y = 2.58 + 2.73x	0.063	3.1
Shot berries	y = 0.949 + 0.700x	0.010	2.8
Abscised flowers	-	-	-

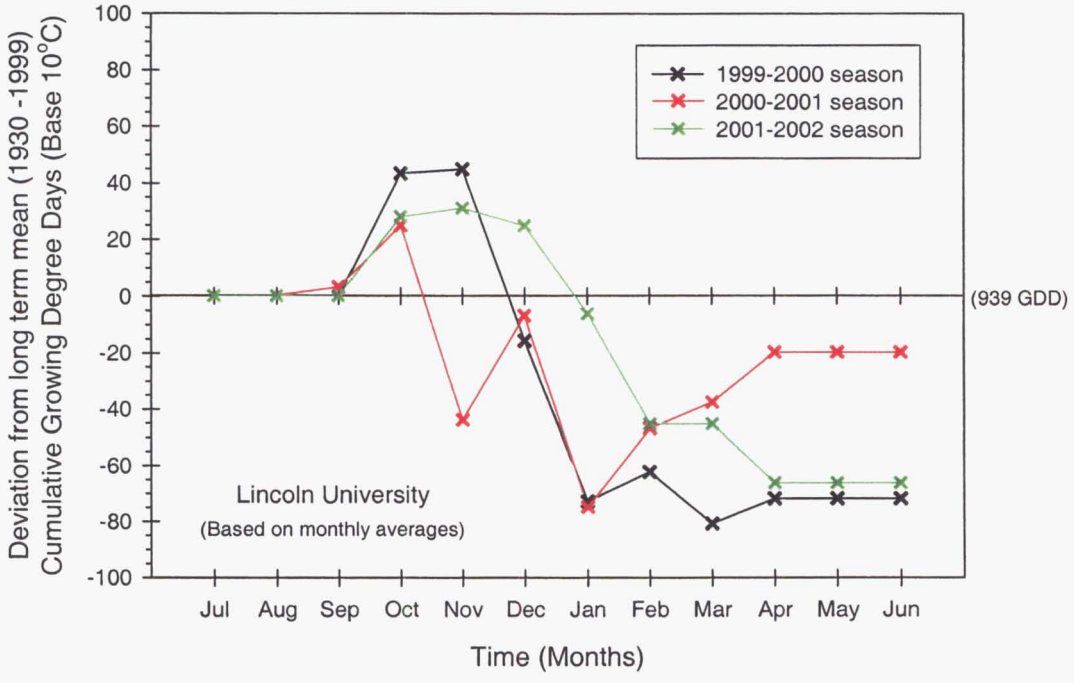


Figure 4 Deviation of cumulative Growing Degree Days (GDD) from the long term mean (1930-1999) at Lincoln University for the 1999-2000, 2000-2001, and 2001-2002 seasons.

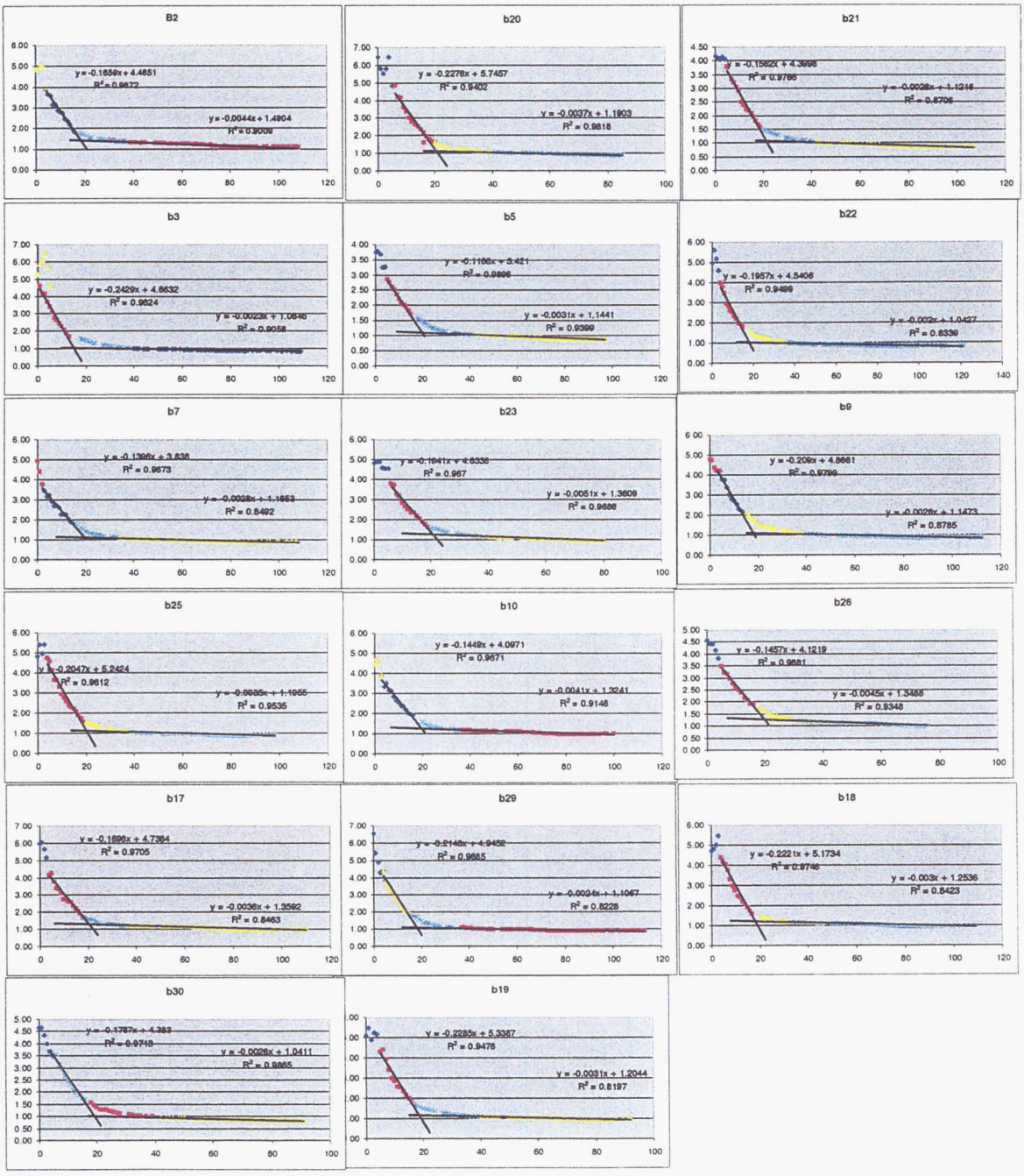
Table 4 Regression equations for Figure 5.3

Graph	Berry type	Regression equation	R^2 value	P = value
A	Seeded	y = 11.44 + -0.75x	0.04	n.s.
A	Seedless	y = 6.61 + 0.44x	0.00	n.s.
A	Shot	y = 2.53 + -0.03x	0.00	n.s.
A	Abscised	y = 0 + 0x	n/a	n/a
B	Seeded	y = 12.02 + -1.45x	0.00	n.s.
B	Seedless	y = 3.96 + 1.86x	0.00	n.s.
B	Shot	y = 0.052 + 1.42x	0.00	n.s.
B	Abscised	y = 0 + 0x	n/a	n/a
C	Seeded	y = 10.34 + -1.82x	0.00	n.s.
C	Seedless	y = 5.91 + 7.38x	0.00	n.s.
C	Shot	y = 1.88 + -7.49x	0.00	n.s.
C	Abscised	y = 0 + 0x	n/a	n/a

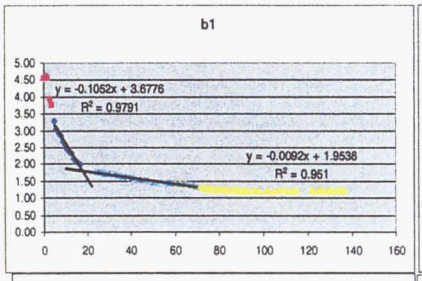
n.s. = not significant

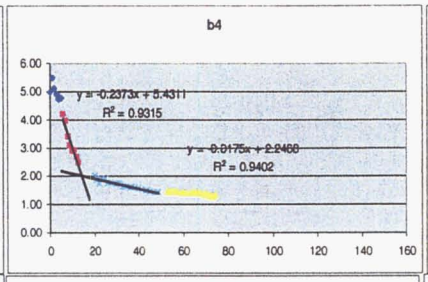
APPENDIX G

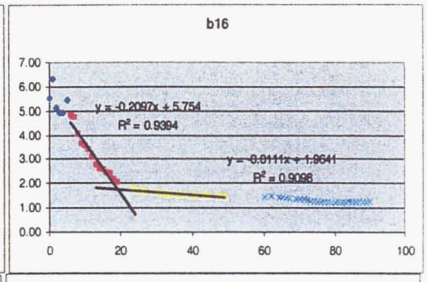
Seeded Pinot noir berries

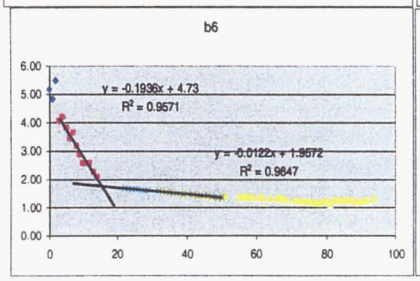


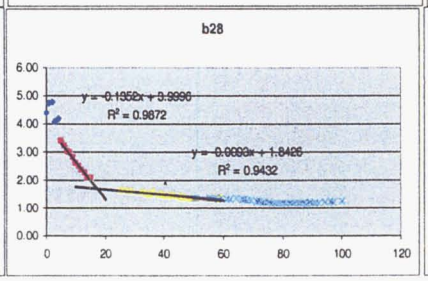
Seedless Pinot noir berries

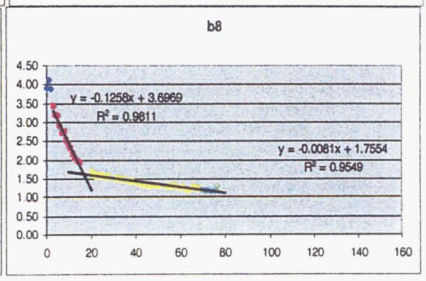




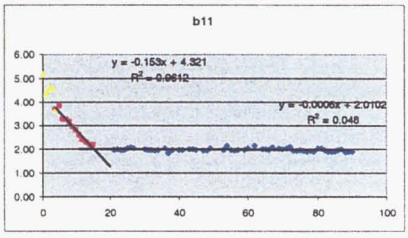


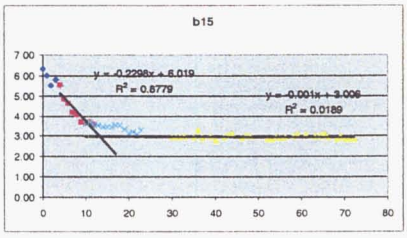


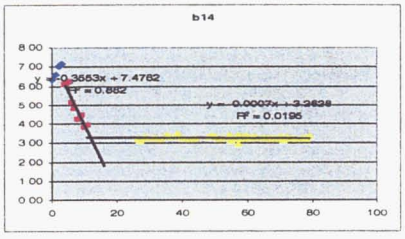


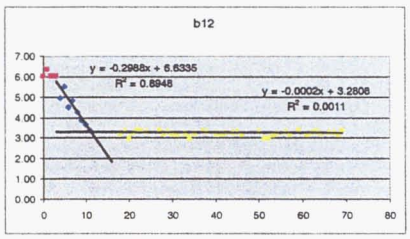


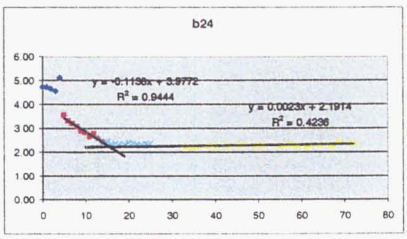
Shot Pinot noir berries

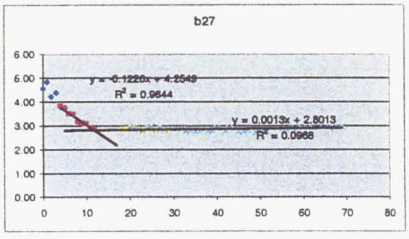


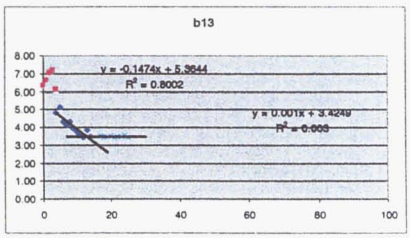




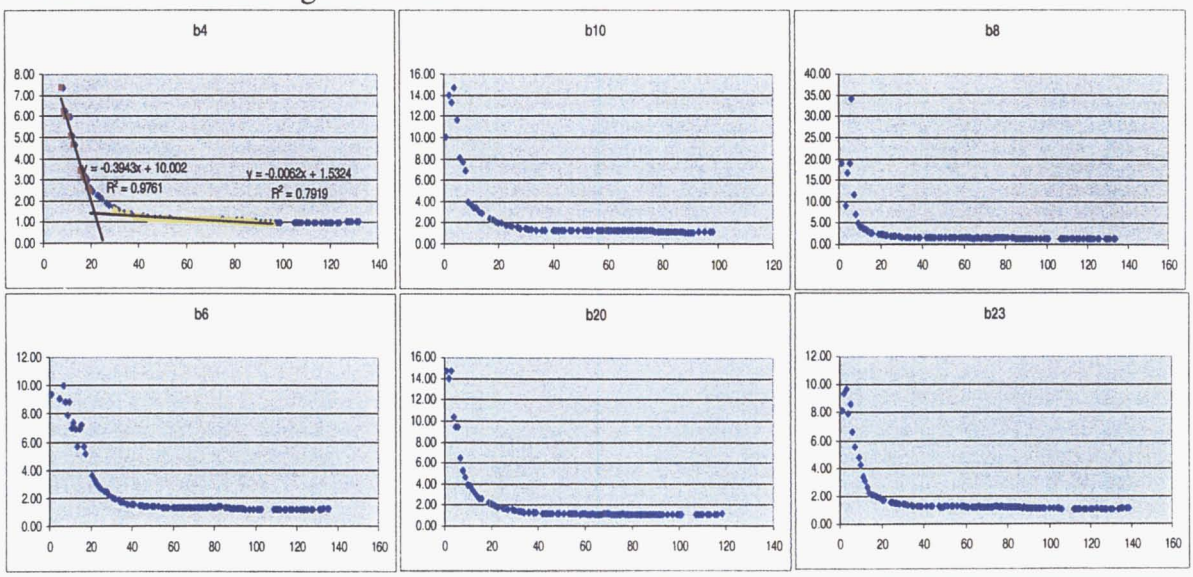




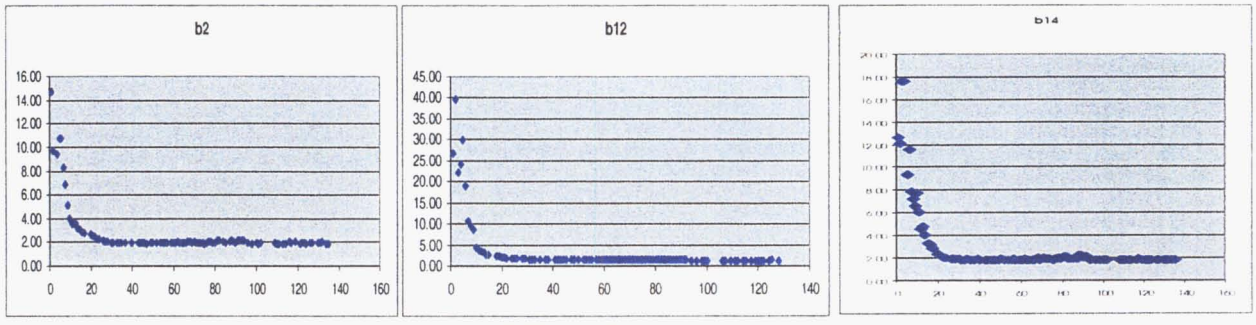




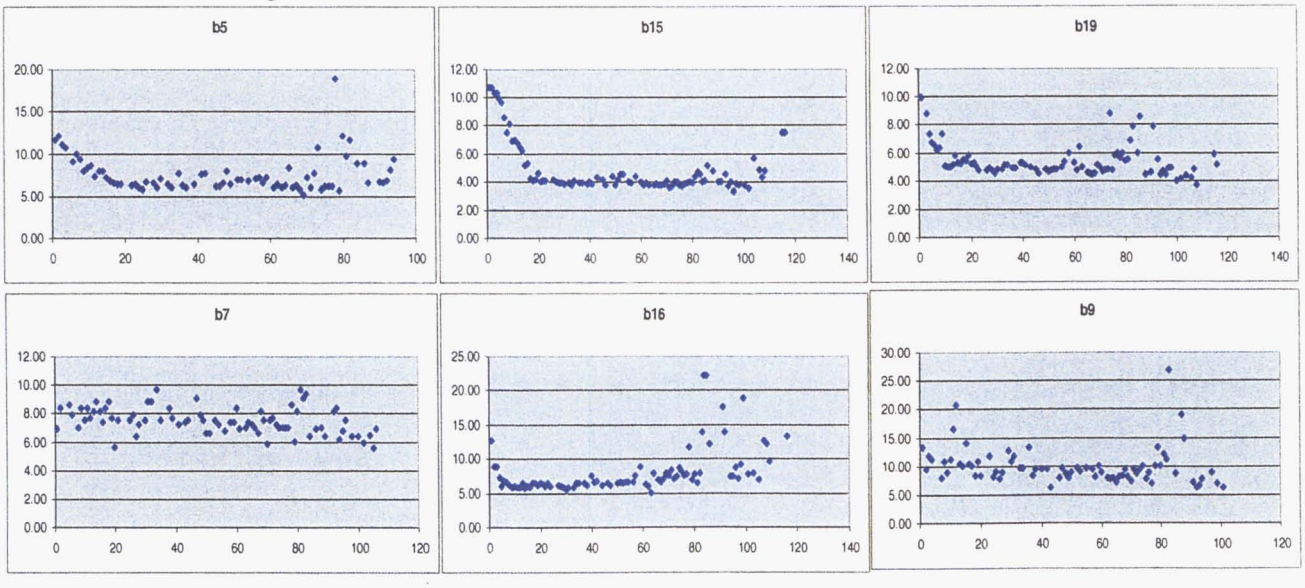
Seeded Cabernet Sauvignon berries



Seedless Cabernet Sauvignon berries

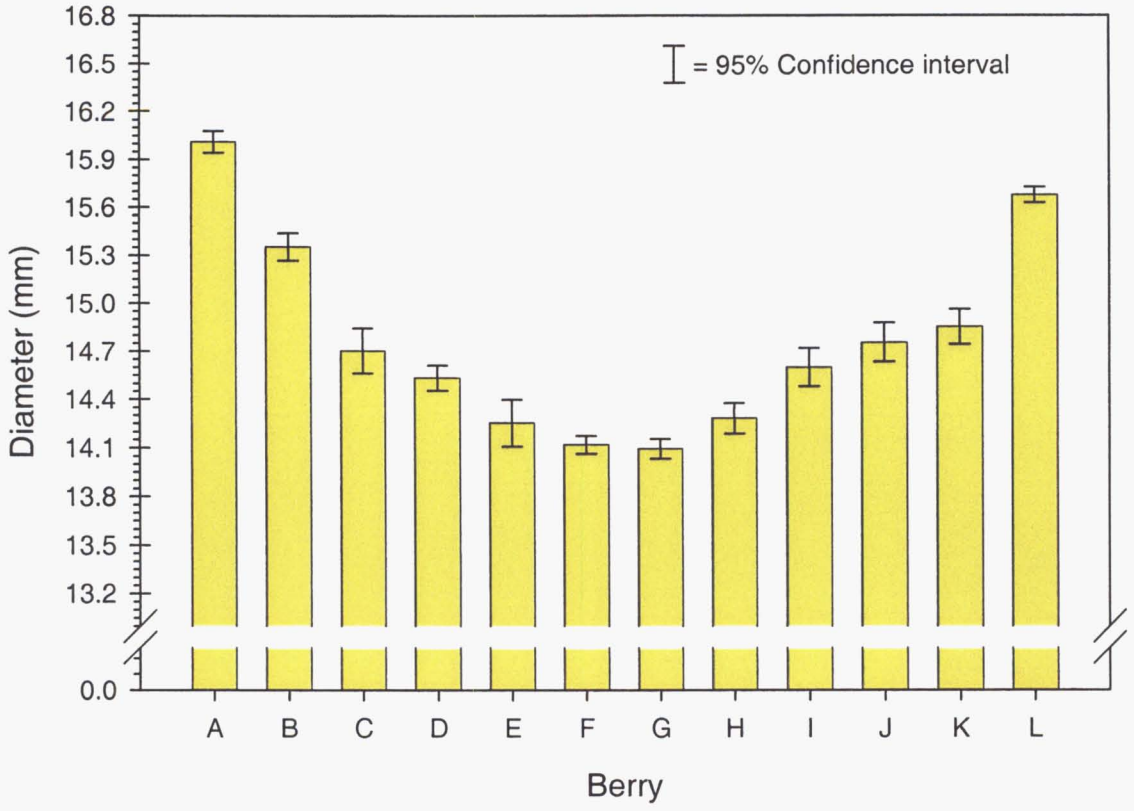


Shot Cabernet Sauvignon berries



APPENDIX H – Reliability of repetitive berry measurements

Twelve Sultana berries were randomly selected from a single bunch, and their diameters assessed. Assessments were made nine times to evaluate the accuracy of repeated measurement. Diameters were measured using a pair of digital callipers (Sylvac, Switzerland; ± 0.01mm) across the widest equator of each berry. Repeat measurements were found to be reasonably accurate with small 95% confidence intervals.



Berry	Assessment									Statistics		
	1	2	3	4	5	6	7	8	9	Mean	SEM	CI
A	16.02	16.03	15.92	15.92	15.96	16.08	15.92	16.18	16.06	16.01	0.030	0.068
B	15.26	15.27	15.42	15.35	15.50	15.45	15.15	15.32	15.45	15.35	0.038	0.086
C	14.44	14.72	14.66	14.76	14.96	14.48	14.97	14.60	14.73	14.70	0.062	0.140
D	14.58	14.60	14.63	14.40	14.72	14.48	14.46	14.50	14.43	14.53	0.035	0.079
E	13.83	14.08	14.43	14.32	14.34	14.44	14.26	14.28	14.31	14.25	0.064	0.145
F	13.86	14.10	14.09	14.07	14.06	13.99	14.05	14.04	14.01	14.12	0.024	0.054
G	13.92	14.19	14.14	14.06	14.19	14.10	14.07	14.08	14.12	14.10	0.027	0.061
H	14.47	14.36	14.08	14.37	14.20	14.21	14.38	14.17	14.32	14.28	0.042	0.095
I	14.72	14.60	14.70	14.67	14.57	14.63	14.82	14.38	14.33	14.60	0.053	0.120
J	14.51	14.62	14.95	14.86	14.94	14.90	14.78	14.65	14.61	14.76	0.054	0.122
K	14.89	14.49	14.94	14.89	15.01	14.92	14.88	14.85	14.82	14.85	0.049	0.111
L	15.71	15.79	15.67	15.61	15.75	15.71	15.61	15.67	15.60	15.68	0.022	0.050