Improving vineyard productivity through assessment of bud fruitfulness and bud necrosis

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **SAR 02/05**

Chief Investigator: **Dr Belinda Rawnsley**
Principal Researcher: **Dr Cassandra Collins**

Research Organisation: South Australian Research and Development Institute (SARDI)

Date: March 2005

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Abstract

The grapevine compound bud contains three or more buds with the primary bud producing the fruiting shoots for the following season. Primary bud necrosis (PBN) is a physiological disorder resulting in the death of the primary bud. Bud dissection used to assess bud fruitfulness and predict yield potential in vineyards has highlighted the incidence of PBN. Without bud dissection, PBN can go undetected as shoots derived from secondary buds produce fewer bunches and these are typically smaller. Consequently, yield potential is not achieved in vineyards affected by PBN. The project aims were to: assess the distribution and extent of PBN, determine timing and development of PBN and recommend appropriate management options for control. This study showed that PBN is a problem in most viticultural regions in Australia. Shiraz is the most susceptible cultivar and PBN occurred around flowering, coinciding with bud differentiation, and increased to the onset of winter. Excessive vigour contributes to high levels of PBN, and is related to the naturally produced growth hormone, gibberellic acid (GA_3). Bud position, pruning levels and irrigation influence the incidence of PBN, whereby severe pruning and water stress leads to high PBN. Balanced pruning is required to (1) reduce the incidence of PBN, (2) reduce excessive vigour and (3) reach a desired yield target with satisfactory quality.

Executive Summary

In 2001 and 2002, many grapegrowers were concerned about low fruitfulness in the vineyard, particularly in the cultivar Shiraz. Bud dissections, used for determining bud fertility to estimate yield potential, showed that many buds were necrotic. In particular, the primary bud was dead, whilst the secondary buds remained healthy. This phenomenon is known as primary bud necrosis (PBN). Although the increasing trend of analysing buds for fruitfulness in autumn/winter raised awareness of the problem, it was not known why the incidence of PBN was high in some vineyards.

Dr Belinda Rawnsley (SARDI) developed this project, in consultation with Dr Gregory Dunn and Mr Steve Martin (DPI, Tatura), Dr Peter May (ex-CSIRO and visiting research fellow, University of Adelaide) and a number of bud dissection service providers in Australia, including key participants Mr Murray Leake (Nepenthe Viticuture) and Mr Daniel Smith (DLS Horticulture). In 2003, Dr Cassandra Collins undertook the second year of research. GWRDC provided funding for a period of 2.5 years (June 2002 and December 2004). The aims of the project were to:

- Assess the distribution and quantify the impact of bud necrosis on vineyard productivity in selected Australian viticultural regions.
- Determine the cause of bud failure and time course of development of bud necrosis for major wine grape varieties in a range of climates.
- Development of appropriate management options for bud necrosis based on current best knowledge.
- Develop standard protocols for collecting and assessing dormant grapevine buds for bud fruitfulness.
- Disseminate information through workshops, and publication in industry and scientific journals. Provide an information package to consultancy and diagnostic services to validate bud fruitfulness protocols.

Within the GWRDC-funded project 'Crop control for consistent supply of quality wine grapes', Dr Greg Dunn and Mr Steve Martin (Tatura, DPI) developed guidelines for growers to predict yield targets by adjusting pruning levels in accordance with bud dissection analysis.

A national survey was formulated and distributed to grapegrowers and consultants. The survey highlighted that:

- PBN was a major concern in most viticultural regions of Australia and Shiraz showed the highest levels of PBN compared to any other cultivar.
- PBN was associated with machine harvesting and molybdenum deficiency.

- High variability existed between viticultural regions. High variability may be attributed to vine balance, climatic condition (macro and microclimate), stages of vine development, time of bud differentiation and stress during susceptible periods to development of PBN.

Shiraz is a highly vigorous cultivar and vigour is related to the naturally produced growth hormone, gibberellic acid (GA_3).

- The endogenous application of GA_3 caused an increase in PBN, whereby the most significant difference was observed before flowering. At the time of bud differentiation, GA_3 is at its highest level and is transferred to the new buds.
- PBN was found to occur around flowering, which coincided with the time of bud differentiation. This may be due to high levels of GA_3 resulting in excessive cell elongation that eventually lead to necrosis of the primary bud.
- Theoretically, the number of inflorescence formed in the bud is complete by the onset of dormancy and buds can be dissected for yield estimation after this time. However, we showed that because levels of PBN in Shiraz continued to increase throughout the season until late autumn, accurate bud dissections needed to be performed as close to pruning as possible.

Pruning level was the main influence on the incidence of PBN.

- Retaining fewer nodes per vine resulted in more vigorous growth, less bunches and higher incidence of PBN than lightly pruned vines.
- Severe pruning caused an imbalance in favour of vegetative growth at the expense of fruit production and subsequently, excessive vigour contributed to higher levels of PBN. Increasing the number of nodes per vine (light pruning) produced more shoots but caused reduced shoot length, low shoot vigour and low levels of PBN.

Irrigation method also influenced the incidence of PBN.

- Partial rootzone drying (PRD) caused a higher incidence of PBN than standard drip irrigation. In comparison, restricted deficit irrigation (RDI) showed less PBN than standard drip.
- Water stress may be the critical factor in influencing the development of PBN, especially during the period of bud differentiation.

Balanced pruning is required to (1) reduce the incidence of PBN, (2) reduce excessive vigour and (3) reach a desired yield target with satisfactory quality.

- Existing pruning strategy, nodes per vine, vine vigour, labour costs, and target yield need to be carefully considered prior to modifying pruning levels to compensate for PBN.
- Vine balance involves pruning to an appropriate node number and industry has widely adopted the guideline of a minimum 15 nodes per kg pruning weight and 15-20 shoots per metre canopy.

- In seasons of low fruitfulness and high PBN, retain more buds in the short-term, but minimise the pressure of high shoot density by keeping below 20 shoots per metre. Retaining more buds per vine will increase the number of fruitful buds but this will compensate for the high number of necrotic buds only and may not eliminate the risk of PBN in following years.

With escalating pressure on grapegrowers to produce target yields, bud dissection analysis is likely to become a common viticultural practice used for yield estimation. Knowledge of potential influences and timing of PBN will improve our ability to manage bud necrosis and bud fertility in the vineyard. By identifying characteristics of the vineyard, block-by-block variability and vine capacity, long term vine balance is ultimately the key to management of PBN.

1. Background

The increasing trend to analyse buds for fruitfulness in autumn has raised awareness of primary bud necrosis (PBN), i.e. death of the primary bud. Many vineyards have showed a high incidence of bud necrosis, with up to 70% bud necrosis detected in some vineyards in areas such as the Coonawarra and Southern Fleurieu, South Australia. In particular, there has been a high incidence of PBN detected in Shiraz. Unless dormant buds are dissected, PBN can go undetected as surviving secondary buds may shoot and produce a normal canopy. Secondary buds, however, are less fruitful and bunches tend to be smaller. Consequently, yield potential is not achieved in vineyards affected by PBN.

The impact of PBN on production is important in terms of direct crop loss and associated management costs. For example, in 2003, approximately 19, 000 tonnes of Shiraz were crushed in the McLaren Vale wine region with an estimated value of AUS$34.5 million (2003 SA Winegrape Utilisation and Pricing survey, Phylloxera and Grape Board of South Australia). In 2002/2003 PBN in Shiraz at McLaren Vale, SA was estimated at 20% (information compiled by service providers) and therefore losses were estimated at $7 million for Shiraz in that region alone. In some vineyards the incidence of PBN has been as high as 60%, seriously reducing yield potential. It is unknown why some vineyards are severely affected by PBN.

Bud fruitfulness and bud necrosis issues were raised by growers participating in viticultural workshops in a number of regional areas. This led to the development of a GWRDC-funded project "Improving vineyard productivity through assessment of bud fruitfulness and bud necrosis" (SAR 02/05) to (i) assess the distribution and quantify the impact of bud necrosis on vineyard productivity and (ii) examine the likely causes of bud failure and development of PBN with the aim to develop appropriate management options. Furthermore, knowledge of PBN will assist growers to incorporate appropriate management practices in response to bud fruitfulness data.

The grapevine bud and primary bud necrosis

The grapevine bud contains three individual buds. The main central bud is termed 'primary' and on either side of this bud are the 'secondary' and 'tertiary' buds (Figure 1.1). Generally the primary bud contains leaf and bunch primordia that produce 6-10 leaves and two bunches, respectively. If the primary bud develops into a new shoot in spring, the secondary and tertiary buds remain small. However if the shoot of the primary bud is damaged or dies, the secondary buds may develop a shoot to compensate for the loss. The death of the primary bud is termed PRIMARY BUD NECROSIS (PBN). Although secondary buds may burst, they often bear no fruit or produce smaller bunches resulting in yield loss.

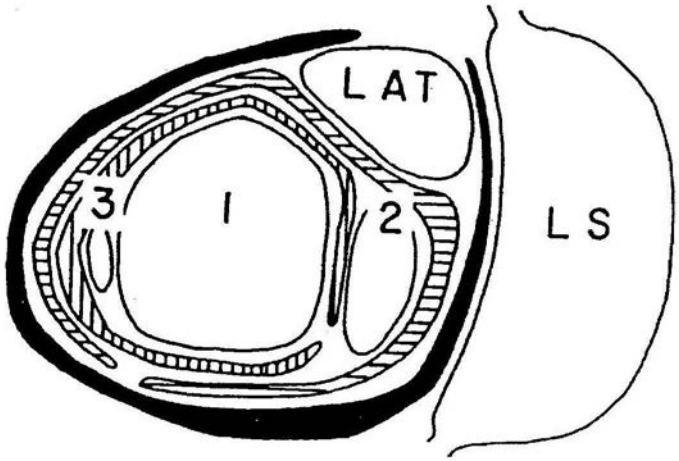


Figure 1.1. Transverse section through a compound bud of grapevine, showing relative positions of the leaf scar (LS), lateral shoot scar (LAT) and the three buds: the primary (1), secondary (2) and tertiary (3). Reprinted from Pratt, 1974.

Identification of PBN and bud dissection analysis

Visually a bud with PBN appears similar to that of a healthy bud and, therefore, difficult to detect by eye (Dry and Coombe, 1994). Although it is possible to see PBN in the field with a hand lens, bud dissections are needed to accurately detect PBN and to assess bud fruitfulness. Bud dissections involve cutting open the bud and using a microscope to observe the internal structures of the bud. The primary bud is the main central bud, and if dead (necrotic), will appear brown and dry, whilst the secondary buds are green (Figure 1.2). Bud dissection analysis for fruitfulness involves counting the number of bunch primordia in the buds without signs of necrosis. Bud necrosis usually originates from the base of the bud and affects only the primary bud. It is not clear why the secondary buds are not affected, but in severe cases, total bud death can occur.

Microscopic bud dissection is being used to assess bud fertility and predict potential yield. PBN can be easily detected in dissected buds and also whether partial or total necrosis has occurred. The information obtained from bud dissection analysis can be used to modify pruning techniques to improve fruitfulness. As a result of poor yields, some vineyard owners have, in many cases, reverted to more expensive and labour intensive cane pruning in these vineyards to improve productivity.

A

B

C

Figure 1.2. Bud dissection analysis for assessment of primary bud necrosis and bud fruitfulness. (A) primary bud healthy, count bunches in primary bud only. (B) primary bud dead, count bunch in largest secondary bud. (C) Primary and secondary bud with necrosis, count in other remaining secondary. No bunches are counted if the primary and secondary buds are dead.

Since the inflorescence primordia that produce bunches for the current season are formed during the preceding summer, buds are sampled in autumn or early winter before pruning. The timing of PBN may influence when buds can be collected. The appropriate sampling size to forecast yield potential is approximately 20 canes (Antcliff and Webster, 1955), however sampling protocols for PBN have not been developed. Variances around sample means can be calculated and if the results are inconclusive, there is enough time to assess more buds before using data to predict bud fruitfulness. However, there are no standard sampling protocols available for diagnostic facilities that offer bud dissection services. Growers are concerned about the appropriate sampling size required and the interpretation of bud fruitfulness data to ensure the maximum return on their investment.

Time of PBN Development

The time that PBN develops is dependent on cultivar. It has been reported that PBN commences soon after flowering (Lavee, 1981) and may develop up to 10 weeks after full bloom (Morrison and Iodi, 1990). Vineyards with PBN often display vigorous shoot growth as a consequence of the unfruitful secondary shoots compensating for the loss of the primary bud. PBN may also lead to development of two secondary shoots from the same node (Dry and Coombe, 1994), yet overall bunch number is reduced. Poor fruitfulness may indicate high levels of PBN in the vineyard.

Possible causes of PBN

PBN is attributed to impaired physiological or developmental processes and is generally associated with a growth surge of the bud. Despite the widespread occurrence of PBN, the principal cause is uncertain. High shoot vigour, excessive irrigation, shade (Wolf and Warren, 1995; May, 1961), high gibberellic acid levels (Ziv *et al.,*1981), and reduction in bud carbohydrates (Vasudevan *et al*., 1998a) have all been associated with bud necrosis.

Vigorous shoot growth, shoot topping and thinning

High shoot vigour, expressed as cane diameter, internode length and growth rate, has been associated with a high incidence of PBN. For example, Shiraz is a highly vigorous cultivar and is prone to PBN. Dry and Coombe (1994) showed that PBN was highest in shoots greater than 12mm diameter and in cv. Queen of Vineyard, canes with diameter greater than 10mm had significantly more PBN than thinner canes (Lavee *et al*., 1981). However, in cv. Kyoho, no association was found between vigour and PBN. The correlation between vigour and the incidence of PBN may be associated with rapid shoot growth in spring. A rapid growth surge is related to increased levels of growth hormones causing abnormal tissue development. Dry (1986) showed that the level of PBN is directly proportional to the severity of shoot topping, defoliation or shoot thinning. Severe shoot thinning increased the incidence of PBN in Shiraz, whereby removal of shoots promotes increased vigour of remaining shoots. However, studies in Chile showed that a modest level of shoot thinning

reduced the incidence of bud necrosis in Sultana (Perez and Kliewer, 1990), while in Riesling the effect of shoot thinning on the incidence of PBN was variable between seasons (Wolf and Warren, 1995).

Gibberellic acid

Gibberellic acids (GA_3s) are naturally produced plant growth hormones that affect cell division and cell elongation in stems and leaves. Levels of GA_3s are higher in vigorously growing shoots than regular growing shoots, particularly after flowering (Lavee, 1987). It was suggested that high levels of GA_3 in vigorous vines induced PBN.

GA_3 is often applied to table grapes to increase berry size. It was shown to cause an increase in PBN when applied before or soon after anthesis but had little effect when applied after bloom (Ziv *et al*., 1981). Buds were insensitive to GA_3-induced necrosis following bud differentiation. The timing of GA_3 application also influenced which buds were affected along the shoot. When applied prior to flowering, PBN was found in the lower part of the shoot, whereas application after flowering caused higher incidence of PBN in buds higher along the shoot. Whether applied or occurring naturally, the amount of GA_3 in the vine may influence the incidence of PBN.

Shading

Bud fruitfulness and the incidence of PBN is affected by light penetration into the canopy, with shaded vines having fewer bunches due to a lower percentage of fruitful shoots (May and Antcliff, 1963). Morrison and Iodi, (1990) reported that the overall bud burst percentage and bud fruitfulness decreased with increasing levels of shading and PBN was higher on shoots located in the shade. In Riesling, shade applied at three-week intervals until veraison did not increase PBN (Wolf and warren, 1995; Vasudevan *et al.*, 1998b). Shaded vines have a higher incidence of PBN than non-shaded vines in susceptible cultivars, but this may also be directly correlated to vigorous growth.

Carbohydrates

Carbohydrates are essential for mitochondrial growth and multiplication during the vegetative and floral induction processes in the bud. Analysis of carbohydrates showed that vines subjected to shade had low levels of sucrose, glucose, fructose and starch (Vasudevan *et al.,* 1998b), and a higher incidence of PBN. Low light can cause a reduction in carbohydrate resources and impede supply to the bud. This may impair axillary bud development. Although low carbohydrate levels have been attributed to PBN, it is unknown what starch or sugar concentrations contribute to susceptibility of a bud to necrosis. Carbohydrate levels may be a contributing factor, but not the primary cause of PBN.

Confusion with pests and disease

Bud necrosis and bud death is often confused with mite infestation and fungal diseases. Rust mites feed predominantly in the outer bud scales and bud mites feed on internal bud tissue. Although mites can cause considerable damage leading to possible death of the bud, there is little evidence to suggest that mites cause PBN. Likewise, fungal and bacterial diseases do not cause PBN. The fungus *Diaporthe perjuncta* (formerly *Phomopsis* type 1) was associated with delayed budburst and bud death, but this was found to be untrue (Rawnsley *et al*., 2002). Cultural practices and environmental conditions seem to play the most important role in determining the incidence of bud necrosis rather than pests and disease.

Distribution and susceptible varieties

Data compiled from bud dissection analysis from a range of cultivars indicates Shiraz has the highest incidence of PBN in most viticultural regions in Australia. Other varieties, such as Cabernet Sauvignon, Riesling and Chardonnay also show significantly high levels of PBN in some regions. In general, PBN occurs in most viticultural regions of Australia although limited information is available from WA, Tasmania and QLD. Most research on PBN has focused on cvs. Sultana, Queen of Vineyard, Kyoho and Riesling, therefore there is a need to determine varietal susceptibility in a range of red and white grapes grown in Australia.

2. Project Aims

The project focussed on two main areas: bud necrosis and bud fruitfulness. At the commencement of the project, it was unknown if bud necrosis was a result of increased use of bud dissection analysis or if seasonal or cultural conditions were contributing to a higher incidence of PBN detected in vineyards. Although the project aimed to address the immediate problem, the knowledge gained would also be of benefit for long-term vineyard production systems. This will assist growers in decisions on vineyard management that will help maximize return on their investment, including cost of the bud dissection service provided.

The aims of the project were to:

1) Assess the distribution and quantify the impact of bud necrosis on vineyard productivity in selected Australian viticultural regions.
2) Determine the cause of bud failure and time course of development of bud necrosis for major wine grape varieties in a range of climates.
3) Development of appropriate management options for bud necrosis based on current best knowledge.
4) Develop standard protocols for collecting and assessing dormant grapevine buds for bud fruitfulness.
5) Disseminate information through workshops, and publication in industry and scientific journals. Provide an information package to consultancy and diagnostic services to validate bud fruitfulness protocols.

Drs Belinda Rawnsley and Cassandra Collins worked collaboratively with other researchers and bud dissection service providers around Australia to address objective 4 (see Appendix 3). Within the GWRDC-funded project 'Crop control for consistent supply of quality wine grapes', Dr Greg Dunn and Mr Steve Martin (Tatura, DPI) developed guidelines for growers to predict yield targets by adjusting pruning levels in accordance with bud dissection analysis.

3. National Survey

INTRODUCTION

Little is known about the extent and distribution of PBN in Australia. In recent years, the use odf bud dissection analysis has shown that some vineyards experience high levels of PBN which can ultimately reduce yield potential. It is unknown if the incidence of PBN is increasing or if more growers are aware of the problem. Data compiled from a number of bud dissection service providers has shown that Shiraz has a high incidence of PBN in most viticultural regions. Significantly high levels of PBN have also been observed in other varieties such as Cabernet Sauvignon, Riesling, Viognier and Chardonnay (Dry *et al*., 2003; Dry and Coombe, 1994; Rawnsley, 2003). While PBN occurs in most viticultural regions there is limited information about this problem from Western Australia, Tasmania and Queensland. Determining the impact of PBN on a number of different red and white wine grape cultivars would be valuable to the wine industry, as most research overseas has been focused on table grapes such as Sultana, Flame Seedless, Thompson Seedless, Queen of the Vineyard and Kyoho (Naito *et al*., 1986; Lavee, 1981; Morrison and Iodi, 1990; Dry *et al*., 2003). Hence some of the information available may not be applicable to wine grape production.

A survey was conducted to investigate the extent and distribution of PBN in vineyards around Australia (Collins and Rawnsley, 2004). Survey participants were asked to supply details about their vineyard including information on soil, water and canopy management and bud fertility. This information was analysed and correlated to levels of PBN in the vineyard over several seasons where possible.

National Survey Design

To gain a better understanding of the impact of PBN, a survey to growers, consultants and managers was designed to determine the significance and the distribution of PBN in most grape-growing areas in Australia. Information compiled from this survey included the following:

- Locality (eg. state, region)
- Vineyard details (eg. soil type, age)
- Vineyard management (eg. pruning and harvest strategy)
- Water management (eg. irrigation type, water quality)
- Canopy management (eg. shoot and bunch thinning, nutrient deficiencies)
- Fruitfulness (eg. bud fruitfulness and PBN levels)

In most cases, participants in the survey supplied bud fertility data from bud dissection analysis performed by service providers. Participants were also asked to comment about possible management strategies in response to PBN and whether they saw PBN as a problem in their vineyard. Information from the surveys has been collated and relationships with PBN assessed.

This was achieved by statistical analysis on the survey data, which was performed by BiometricsSA (Adelaide) and involved the conversion of the raw data to a statistical form for analysis. The survey comprised data where in some cases, the estimates were based on very few observations and a high amount of variability was found between vineyards. For these reasons, the results indicate aspects of vineyard management that could be investigated further in controlled experiments, rather than concluding that one factor typically caused high levels of PBN. Relatively less data was collected from 2002 than for 2003 and this should be considered when interpreting the results. Results have been presented using the raw data unless otherwise stated.

RESULTS

Seventy-eight growers, consultants and managers responded to the survey, providing information on 288 vineyard blocks. Information was received from South Australia, Victoria, New South Wales, Western Australia and Tasmania. **Fifty-five percent** of participants responded that **PBN was a problem** in their vineyard, while **33% were uncertain** and the remainder of respondents did not think PBN was a problem or did not respond. In 2003, vineyards with a higher incidence of PBN were found to be less fruitful.

State and regional variation

PBN was reported to occur in all states that were represented in the survey. There were no significant differences in the levels of PBN between states. Regionally there were higher levels of PBN in McLaren Vale than in the Clare Valley, Barossa (SA), Nannup (WA), Young (NSW). Mudgee (NSW), Margaret River (WA), Southern Fleurieu and South-east (SA) also indicated higher levels of PBN then the Clare Valley and Nannup when analysed.

When the effect of cultivar and region on the incidence of PBN was compared, it was found that:

- Shiraz grown in the Southern Fleurieu had higher levels of PBN than when it was grown in Mudgee, the Adelaide Hills, the Barossa, or the SE of South Australia. Similarly Shiraz grown in McLaren Vale had higher levels of PBN than when grown in Nannup.
- Chardonnay grown in the Adelaide Hills had less PBN than when it was grown in the SE of South Australia, Barossa or Margaret River.
- Likewise, Riesling in the Clare Valley had less PBN than the SE of South Australia or the Barossa.

This implies that region and cultivar were closely related to the incidence of PBN.

Effect of cultivar and rootstock

Cultivars; Shiraz, Pinot Gris, Riesling, Petit Verdot, Gewürztraminer, Chardonnay, Sauvignon Blanc, Semillon, Merlot, Pinot Noir and Cabernet Sauvignon had greater than 20% PBN in some

vineyards in 2003 (Figure 3.1). In 2003, there were significant differences in the levels of PBN between cultivars. Petit Verdot and Pinot Gris had higher levels of PBN than Sangiovese, Zinfandel

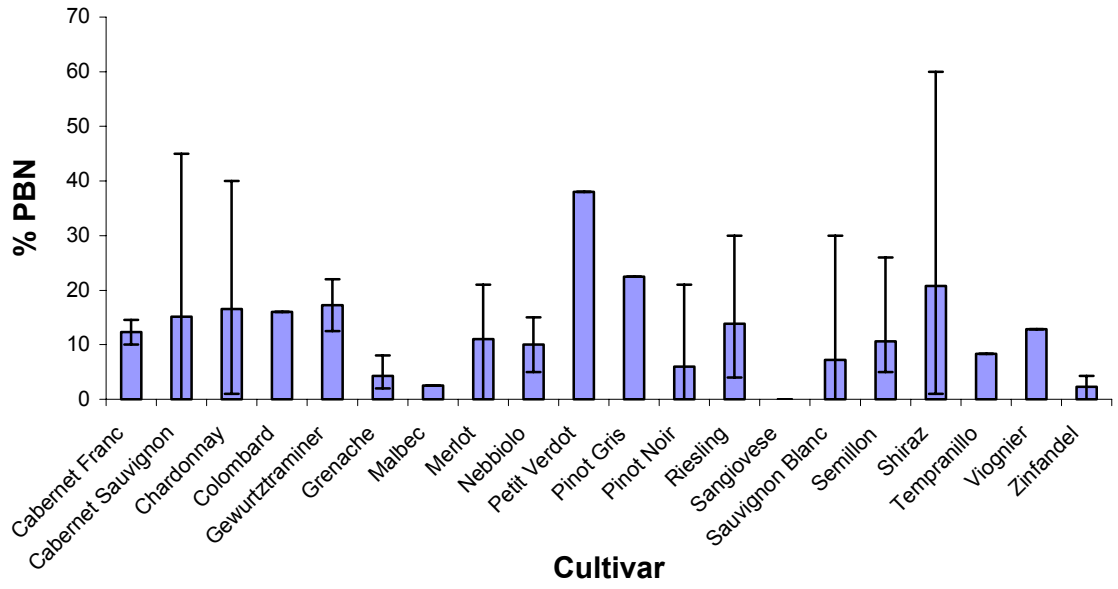


Figure 3.1. Average percentage of primary bud necrosis (PBN) for different cultivars present in PBN survey of Australian vineyards, 2003. Error bars indicate maximum and minimum percentage PBN for each cultivar.

and Malbec when planted on their own roots. Petit Verdot also had higher levels of PBN compared to Tempranillo, Colombard and Semillon on their own roots, and Gewürztraminer on Cabernet Sauvignon rootstock in 2003.

Some of the cultivars featured in the survey on more than one rootstock displayed significant differences in PBN levels among the rootstocks. Rootstock significantly affected the incidence of PBN with the cultivars Cabernet Sauvignon and Shiraz (Table 3.1). Cabernet Sauvignon on rootstocks Schwarzmann and Richter110 or on Schwarzmann alone had higher levels of PBN in 2003 when compared to Paulsen rootstock. A similar finding was observed in Shiraz where the use of Schwarzmann rootstock resulted in higher levels of PBN than when compared to the use of the rootstock Riesling1654. These results suggest a higher level of PBN can result when the rootstock Schwarzmann is used for cultivars Cabernet Sauvignon and Shiraz. No effect on the level of PBN was found amongst the rootstocks for the other cultivars.

Table 3.1. Effect of rootstock on the percentage of primary bud necrosis (PBN) in 2003 for cultivars Cabernet Sauvignon and Shiraz.

Cultivar	Rootstock	%PBN**
Cabernet Sauvignon	Paulsen	0.0
Cabernet Sauvignon	own roots	5.0
Cabernet Sauvignon	unknown*	5.5
Cabernet Sauvignon	Schwarzmann and Richter	12.5
Cabernet Sauvignon	Schwarzmann	13.9
Shiraz	Riesling 1654	8.1
Shiraz	own roots	9.6
Shiraz	Schwarzmann and Richter 111	12.5
Shiraz	Doradillo	21.6
Shiraz	unknown*	32.6
Shiraz	Schwarzmann	37.2

*Indicates where participants did not know rootstock type.

**Data transformed for the comparison of cultivar and rootstock and its effect on PBN levels.

Effect of pruning, harvest and irrigation strategy

Greater than 64% of vineyards in the survey were machine pruned in 2002 and 2003 and of those, following hand-clean up was common. In 2003, vines were predominantly spur-pruned (78%) but there was little difference in the incidence of PBN between cane and spur pruning. PBN levels were lower when vines were cane pruned rather than spur pruned with a kicker cane (Figure 3.2) No other significant differences were found among pruning strategies. A high proportion (>80%) of growers used mechanical means for harvesting fruit and in 2003, the results suggested that machine harvesting caused higher levels of PBN compared to those that were hand harvested (Figure 3.3). In vineyards with high levels of PBN, modification of pruning and harvesting methods should be considered to achieve greater fruitfulness.

The majority of vineyards were drip-irrigated in all years, with standard drip used more commonly than restricted deficit irrigation (RDI). The application of RDI to vineyards resulted in significantly lower levels of PBN compared to standard drip irrigation, 4.8% and 11.8% PBN respectively (Figure 3.4). Only 3 respondents in the survey reported use of partial rootzone drying (PRD) as a form of irrigation in 2003.

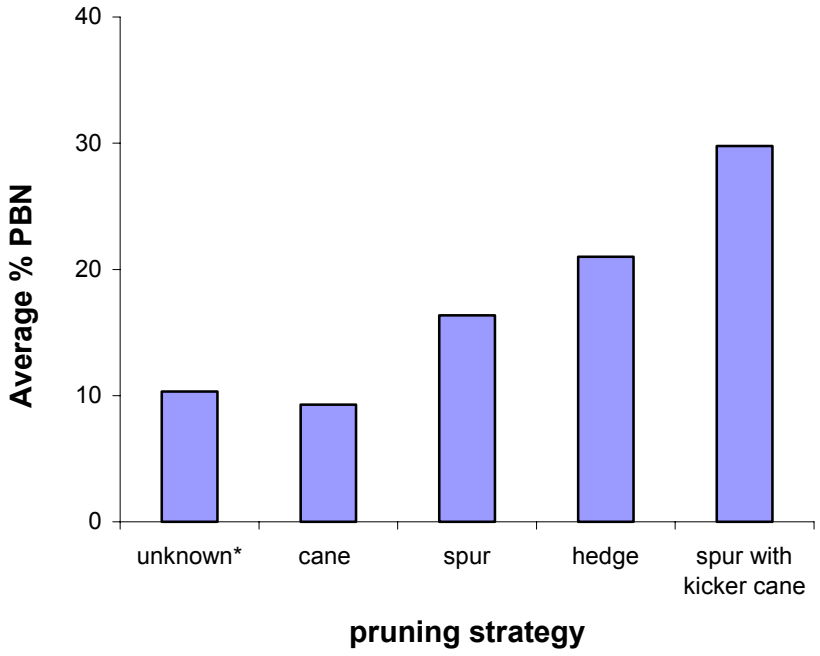


Figure 3.2. Average percentage of primary bud necrosis (PBN) in 2003 for various pruning strategies in vineyards.*Indicates where no response by participants was provided.

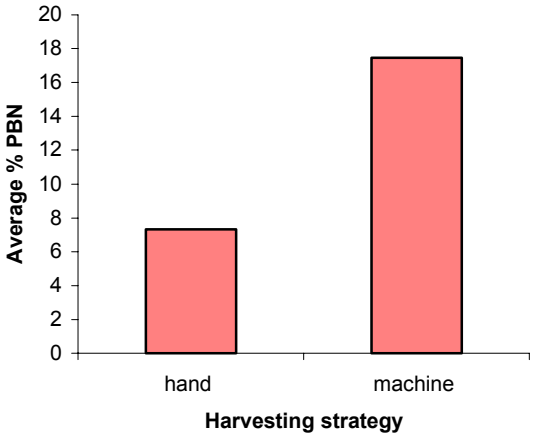


Figure 3.3. Average percentage of primary bud necrosis (PBN) in 2003 for hand and machine harvested vineyards.

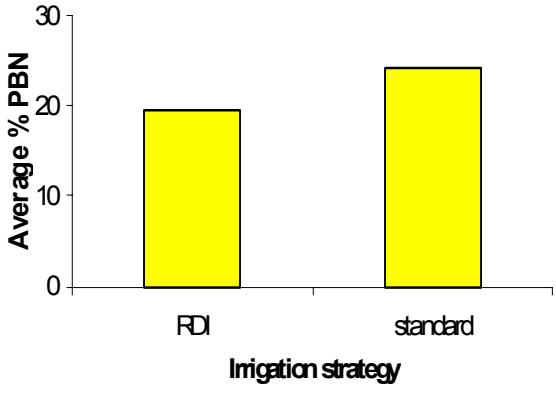


Figure 3.4. Average percentage primary bud necrosis (PBN) in 2002 in vineyards using standard drip and restricted deficit irrigation (RDI).

While only a small number of vineyards were reported to be molybdenum (Mo) deficient these vineyard blocks had significantly higher levels of PBN for both 2002 and 2003 than those vineyards not Mo deficient. No other nutrient deficiencies were found to have an effect on PBN levels. Other factors in this survey that did not appear to have an effect on the incidence of PBN include; state, soil type, salinity, rainfall, water source, trellis system, canopy management (shoot thinning, bunch trimming), and yield. Growers were more likely to modify management practices if they knew PBN was present.

DISCUSSION

This survey highlighted that PBN is a problem in most Australian viticultural regions and it affects a number of wine varieties, in particular Shiraz, Petit Verdot, Pinot Gris, Gewürztraminer, Riesling, Sauvignon Blanc, Semillon, Chardonnay and Cabernet Sauvignon (however some cultivars were represented by only a small sample number). Feedback from this survey illustrated that the viticultural industry is aware of PBN as a potential vineyard problem, and as such, grape-growers are changing management strategies to compensate for high levels of PBN.

In contrast to past research (Dry, 1986; Perez and Kliewer, 1990; Wolf and Cook, 1992; Wolf and Warren, 1995), there was no relationship observed between canopy management strategies such as shoot thinning, shoot trimming, bunch thinning and levels of PBN. This may be due to high data variance from the survey data.

The correlation between high PBN levels and low fruitfulness is important in terms of crop forecasting to meet target yields (Lavee *et al*., 1981; Dry and Coombe, 1994). It has been suggested that factors leading to a reduced number of inflorescences also instigate the process leading to PBN (Lavee *et al*., 1981). High variability was found between vineyards indicating that it would be difficult to estimate PBN regionally and may be more reliant on other factors. This implies that each vineyard would need to be managed on a case-by-case basis and factors such as seasonal changes and block variability would need to be considered to accurately estimate PBN levels.

The interaction between the cultivars Cabernet Sauvignon and Shiraz with rootstock may warrant further investigation, as results contrast with previous findings by Dry *et al*. (2003). Harvest, pruning and irrigation strategy, as well as molybdenum deficiency, are factors highlighted in the survey as having a potential impact on the levels of PBN. Higher levels of PBN were observed in mechanically harvested vineyards, which may be due to mechanical damage and hence stress on vines, affecting the nutrient and water flow to the primary bud. Previous studies on mechanically harvested Pinot Noir showed an increase in bud damage and a subsequent reduction in fruit potential when compared to hand harvesting (Dry *et al*., 1990). Past research has suggested that vine vigour can affect the levels of PBN (Lavee *et al*., 1981; Naito *et al*., 1986; Wolf and Cook,

1992; Dry and Coombe, 1994) and that different pruning levels affect vine vigour (Smart, 2001; Clingeleffer and Sommer, 1995). These findings and the results of the survey suggest that adjusting pruning levels to control vine vigour may be an effective way to manage PBN. In addition, vine vigour is reduced through the implementation of RDI. When RDI was used in vineyards in 2002, a reduction in the incidence of PBN was observed compared to vineyards using standard drip irrigation. Water stress in grapevines has been found to have an effect on shoot growth (Smart and Coombe, 1983; Williams and Mathews, 1990) and results from the survey indicate that PBN levels are influenced by different irrigation systems. Overall, increased knowledge of bud necrosis and bud fruitfulness will play an important role in overall management of the vineyard.

4. Timing of primary bud necrosis and relationship with vine vigour

INTRODUCTION

Grapevine fruit production occurs over 2 years with PBN becoming evident in the first year. Research has suggested that necrosis commences soon after flowering (Lavee, 1981; Dry, 1986; Morrison and Iodi, 1990) and may develop up to 10 weeks after full bloom (Dry, 1986), ceasing after the onset of dormancy (Morrison and Iodi, 1990). If PBN occurs early, the remaining secondary buds show greater development than normal, commonly enlarging to fill the space occupied by the dying primary bud (Morrison and Iodi, 1990; Dry, 1986). Subsequently, secondary buds are responsible for producing the shoot in the growing season, and often bear no or little fruit.

The high incidence of bud necrosis has been highlighted by the increasing use of bud dissection services to determine bud fruitfulness as an early indicator of potential yield. Several vineyard owners in South Australia consider that spur-pruned Shiraz is particularly susceptible to PBN. It is unknown if vigour is the major influence in the severity of PBN and if the incidence of PBN is seasonal. As a consequence of poor yields, owners have, in many cases, reverted to more expensive and labour intensive cane pruning in these vineyards to improve productivity. It is important to determine when PBN occurs to find ways to minimise the problem.

Field research was conducted in vineyards with known problems of poor fruitfulness. The information gained in this research will make significant progress in understanding the high incidence of bud death observed, and whether bud fruitfulness assessment is a worthwhile investment in predicting crop potential. The aims of this study were to investigate the effect of shoot vigour on the incidence of PBN and to determine when PBN commences in cv. Shiraz.

MATERIALS AND METHODS

Field trials

Trials were conducted in commercial vineyards (cv. Shiraz) with a history of PBN. Field sites were established at McLaren Vale, Southern Fleurieu and Eden Valley, South Australia (Figure 4.1). Vineyard details of each site are provided in Table 4.1. Vines were spur pruned however at Southern Fleurieu and the grower retained more nodes per vine than in previous seasons to combat the high incidence of PBN and hence type of pruning has been designated as “minimal”.

Figure 4.1. Vineyards (cv. Shiraz) in South Australia used for the assessment of primary bud necrosis. (A) Southern Fleurieu. (B) Eden Valley. (C) McLaren Vale.

A

B

C

Table 4.1. Details of vineyards in South Australia selected for assessment of primary bud necrosis.

Site	Year planted	Trellis	Pruning type	Row spacing (m)	Vine spacing (m)	Vines per ha	No. vines per row	No. vines per panel
McLaren Vale	1998	Single cordon (VSP)	Spur	3	1.5	2222	113	4
Southern Fleurieu	1995	Single cordon (VSP)	Minimal	2.8	1.5	2380	151	3
Eden Valley	1918	Scott Henry	Spur	3.66	3.66	771	45	3

At McLaren Vale and Southern Fleurieu, 100 two-bud spurs were randomly selected from 100 vines in 10 rows and tagged prior to bud burst. Fifty vines were selected at Eden Valley from 5 rows and the total 100 spurs where divided into 50 spurs on the upper cordon and 50 spurs on the lower cordon. Cane-pruned vines were also monitored at two vineyards whereby 50 cane-pruned vines were randomly selected over two rows at McLaren Vale and 100 cane-pruned vines where tagged in 10 rows at Eden Valley. The following measurements were taken throughout the 2002/2003 season:

- Nodes per vine
- Shoots per vine
- Stage of development
- Shoots per node
- Shoot length
- Shoot diameter
- Bunches per node
- Bunches per vine
- Bunch weight at harvest

Shoot length was measured weekly between October and November and growth rate expressed as the length increase (cm) between sample dates and final length recorded. Shhot length was recorded at all sites in 2002 and 2003. The incidence of PBN was assessed on 20 shoots randomly selected in the trials in the following winter. The bud dissection method described in chapter 1 was used. Simple estimation of yield was described as:

$$\text{Yield (t/ha)} = \text{bunch weight (kg)/vine} \times \text{no. vines /ha}$$

(where kg/vine = average no. bunches/vine × av. bunch weight (g))

Timing of PBN development was assessed at the Southern Fleurieu vineyard in 2003. Timing of bud necrosis was determined by randomly collecting 20-40 shoots weekly commencing just prior to flowering. The severity of PBN was evaluated on a scale of 0-5, whereby 0 = healthy, 1 = 25% of the bud showing necrotic tissue, 2 = 25-50% necrotic, 3 = 50-75% necrotic, 4 = >75% necrotic and 5 = dead bud. Shading was scored visually as: exposed, partly shaded or shaded. Shoot length, internode length and diameter was measured and green buds (nodes 1 to 10) visually assessed for necrosis.

RESULTS

McLaren Vale vineyard

Bud dissection analysis

2001

Bud dissections were performed by Dr Peter May in June 2001 (cv. Shiraz). Six canes were assessed only. Overall fruitfulness across 10 buds was 1.4 inflorescence primordia (bunches) per bud. Fruitfulness for 2-bud spurs was 1.0 bunches per bud. Of the 60 buds assessed, 26.7% of these were dead. The highest levels of necrosis were observed at buds 1 and 9 (Figure 4.2).

2002

The viticultural manager performed bud dissections in June 2002 (cv. Shiraz). If the primary was dead, secondary buds were included in the calculation of fruitfulness. Thirteen canes were sampled for each analysis set (Figure 4.3). Rows 32-33 were spur-pruned, rows 16 and 17 cane-pruned. Overall fruitfulness up to 5 buds along the shoot for spur-pruned vines was 1.4 bunches per bud (50% dead). Fruitfulness for cane-pruned vines was 1.57 bunches per bud (61% dead). This year showed exceptionally high levels of PBN.

2003

Spur-pruned – Of the 100 tagged two-bud spurs, 145 shoots were collected for bud dissection analysis. Fruitfulness was lowest in buds from basal nodes and increased along the shoot, with an average 1.57 bunches per bud derived from buds 1-10 (Figure 4.7). Spur-pruning retains the first two buds hence adjusted fruitfulness using buds 1 and 2 (1.22 bunches per bud) would provide more accurate information for yield estimation. Bud fertility (number of buds containing one or more bunches) in the vineyard was 78%, including bunch counts in the primary and secondary buds. However, if primary buds only were assessed, fruitfulness was reduced to 71%. This shows that counting bunches in secondary buds influence the estimation of overall fruitfulness.

Overall, PBN was 24%. The distribution of PBN was highest at bud 1 (37%) with an increase again at nodes 5-6 (Figure 4.7). PBN levels were lower than the previous year although a similar trend along the shoot was observed. Bud dissection analysis showed that node position on the spur

influenced the distribution of PBN along the shoot. The incidence of necrotic buds was higher on bud 2 than on bud 1, 17.5% and 22.1% respectively (Figure 4.8).

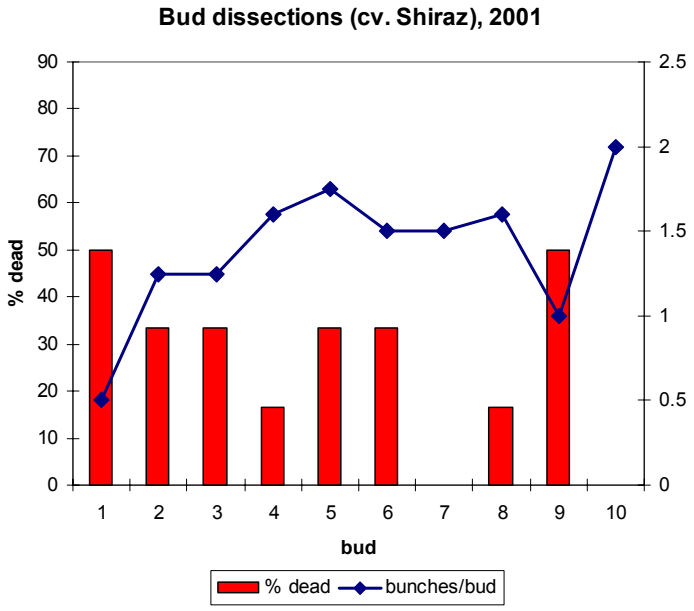


Figure 4.2. Percentage dead buds and number of bunches (inflorescence primordia) per bud in June 2001 at McLaren Vale (cv. Shiraz).

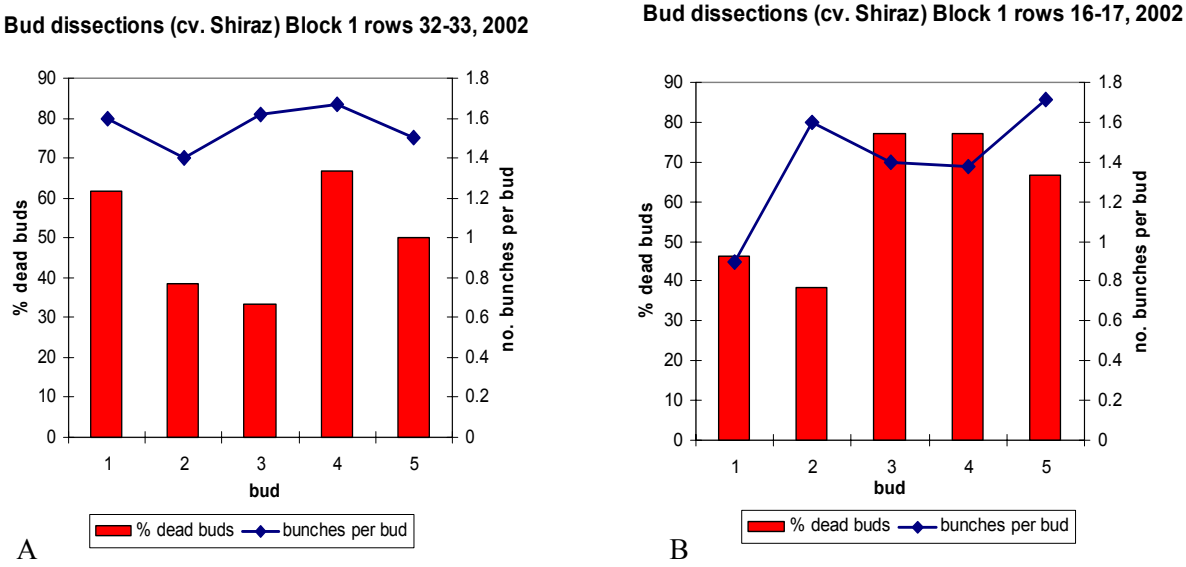


Figure 4.3. Percentage dead buds and number of bunches (inflorescence primordia) per bud assessed in June 2002 for (A) spur-pruned vines and (B) cane-pruned vines at McLaren Vale (cv. Shiraz).

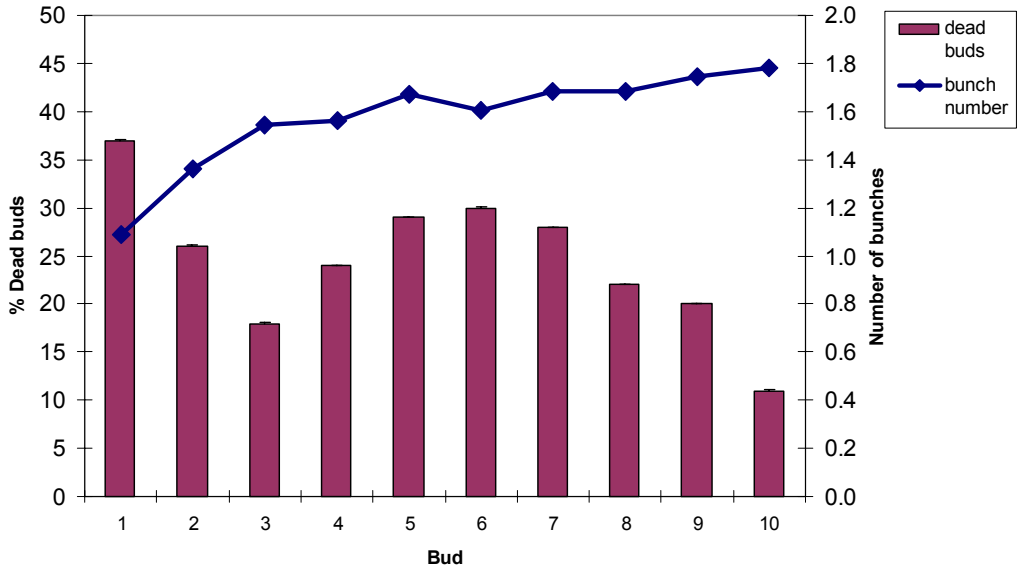


Figure 4.4. Number of inflorescence primordia (bunches) and incidence of dead buds (including primary bud necrosis) per bud at different positions along shoots derived from spur-pruned vines in 2003 (cv. Shiraz, McLaren Vale)**.**

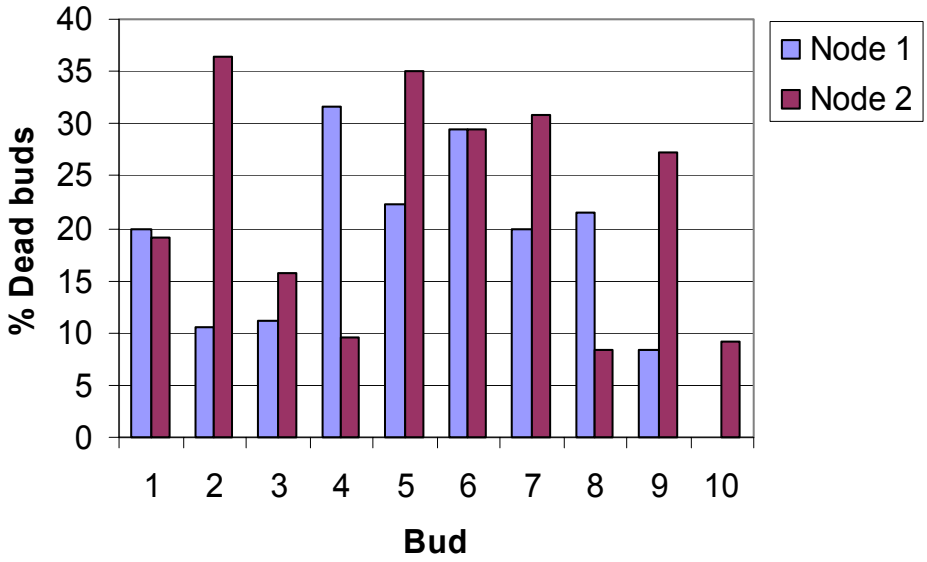


Figure 4.5. Comparison of PBN on shoots derived from a 2-bud spur, whereby node 1 represents the lower bud position on the spur and node 2 the upper position, 2003 (cv. Shiraz, McLaren Vale).

Cane-pruned – From cane-pruned vines, 126 shoots were assessed. Fruitfulness (1.05 bunches per bud) was lower than spur-pruned vines and did not consistently improve along the shoot. Bud fertility was 66.1%, but if primary buds were considered only, bud fertility decreased to 55.2%. Again, this shows the importance secondary buds have in estimation of yield potential. More buds on cane-pruned vines were dead (34.3%) than those on spur-pruned vines. However, a similar trend was observed along the shoot, where bud 1 had the highest incidence of PBN (Figure 4.9).

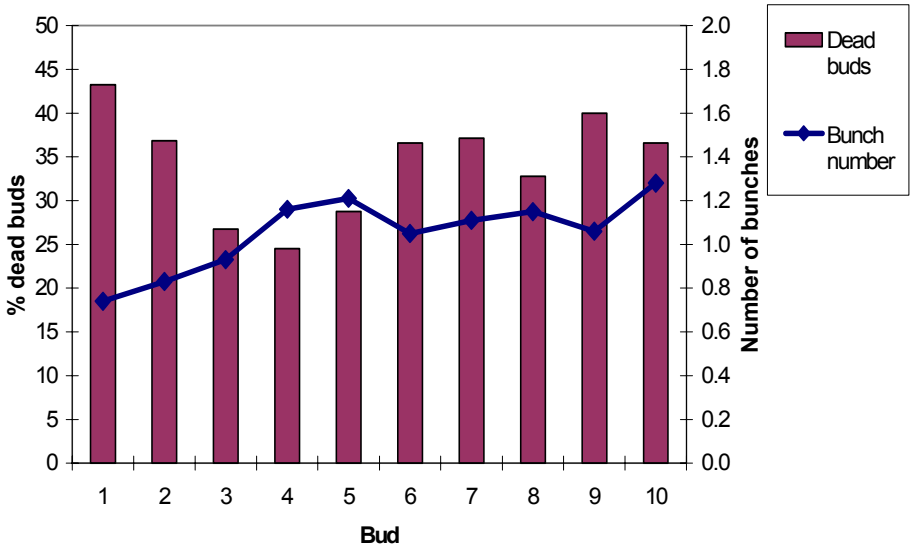


Figure 4.6. Number of inflorescence primordia (bunches) and incidence of dead buds (including primary bud necrosis) per bud at different positions along shoots derived from cane-pruned vines (cv. Shiraz, McLaren Vale).

Vine assessment

In the McLaren Vale vineyard, vines were spur-pruned to 40 buds/vine. High budburst (>100%) was observed in both seasons. In 2002, shoots on spur-pruned vines grew quickly (typically known as the "grand period of growth") and ceased rapid growth on the onset of flowering. In comparison, shoot growth in 2003 slowed after budburst and showed high variability between samples (Figure 4.7). Although shoots developed more quickly in 2002, flowering time was similar in both years. Shoots from cane-pruned vines were shorter and did not display exponential growth (data not shown). There was no correlation between shoot growth rate and the incidence of PBN. Statistical analysis of shoot growth rate at various times of the season and final PBN percentage showed there was no evidence to suggest that shoot vigour was associated with high levels of PBN (Figure 4.8).

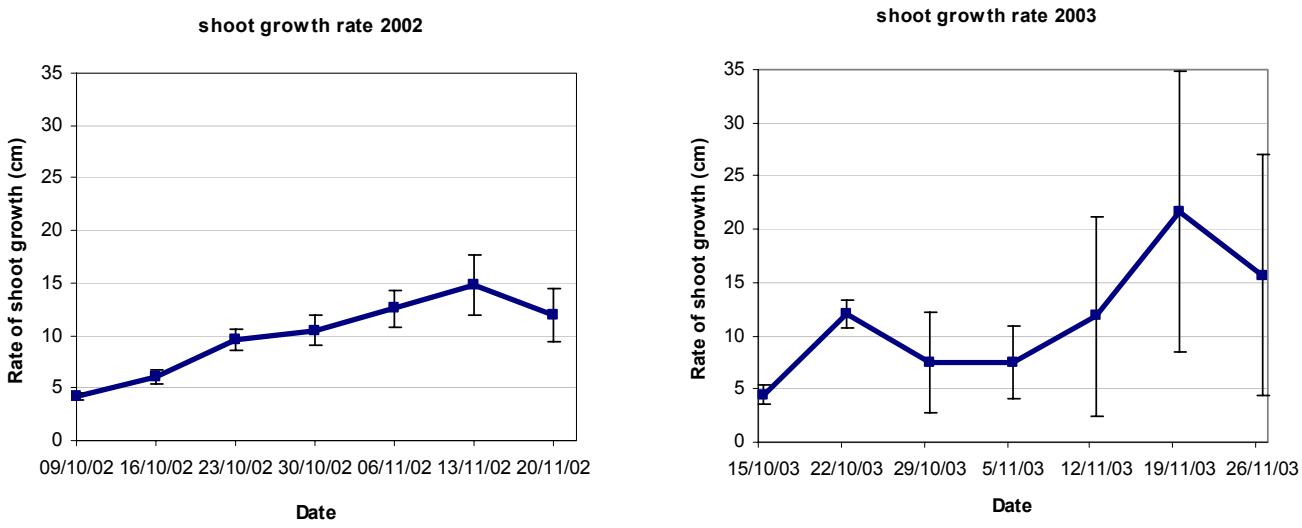


Figure 4.7. Shoot growth rate for spur-pruned vines at McLaren Vale, 2002. (A) 2002. (B) 2003. Shoot growth rate was calculated as the length increase per day between sample dates.

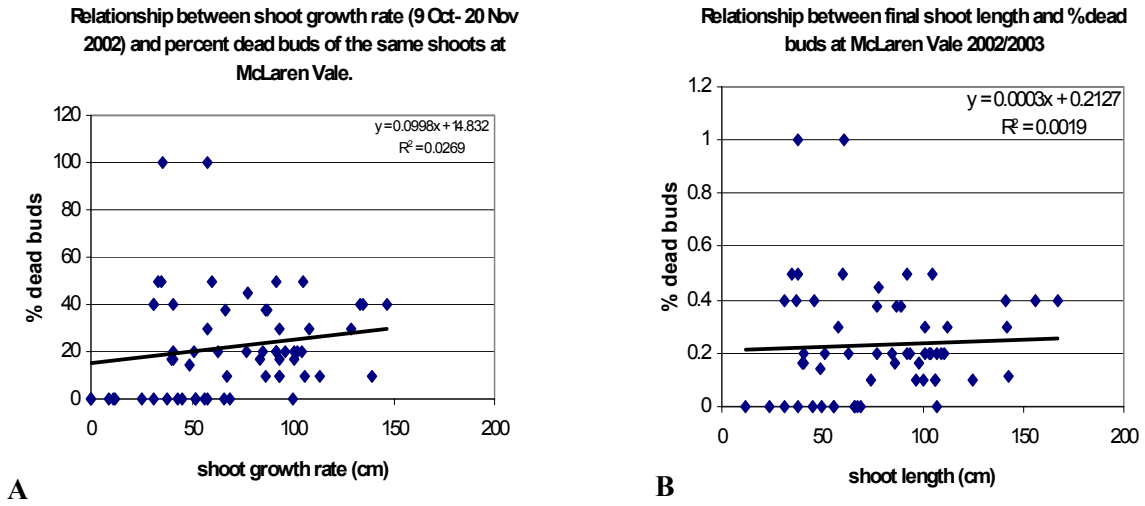


Figure 4.8. Relationship between (A) shoot growth rate between 9 October and 20 November 2002 and (B) final shoot length at 20 November 2002 with the incidence of PBN on spur-pruned cv. Shiraz, McLaren Vale.

Following harvest in 2003, measurement of shoot diameter indicated that shoots were thicker on spur-pruned vines than on cane-pruned vines, but there was no correlation between PBN and shoot diameter. Statistical analysis showed that the number of bunches significantly increased with number of shoots (Figure 4.9) yet there was no relationship between the number of necrotic buds with number and weight of bunches. Of all vine components measured, only the total number of buds retained per vine significantly affected the incidence of PBN (P<0.005). The response was exponential, indicating that as the number of buds per vine increased, the incidence of PBN increased (Figure 4.10).

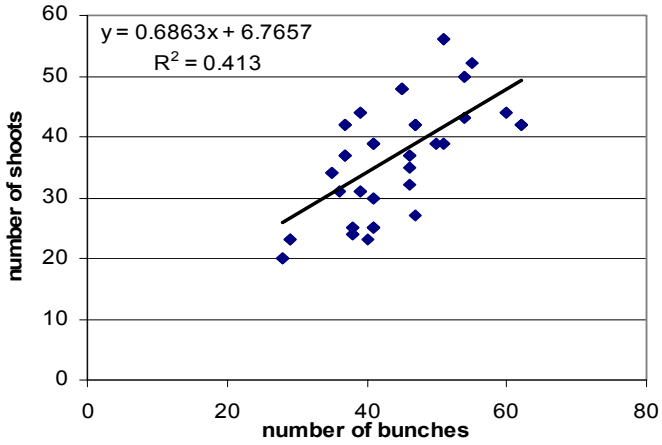


Figure 4.9. Relationship between number of shoots and number of bunches per vine at harvest 2003, cv. Shiraz, spur-pruned vines, McLaren Vale.

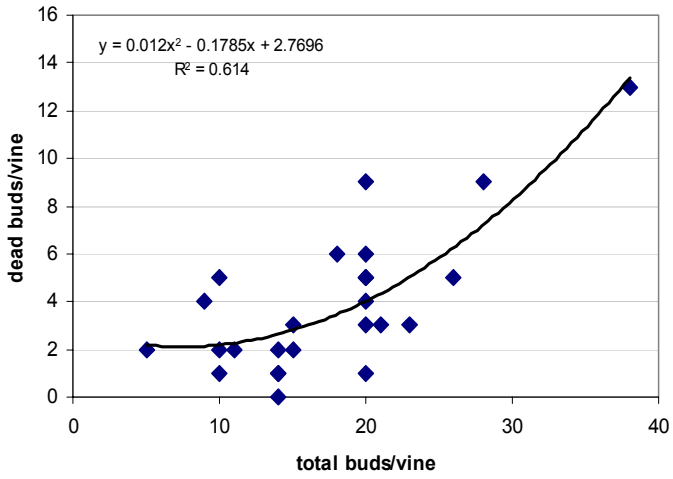


Figure 4.10. Relationship between number of dead buds and total number of buds retained per vine (2003, cv. Shiraz, spur-pruned vines, McLaren Vale).

Yield estimation

Initial yield estimates (7.7 t/ha) were made in late October based on a bunch count of 35.2 bunches/vine using an approximate 100 g bunch weight. Overall, bunch weight was lower (81.6 g) than anticipated and final yield achieved was 8.4 t/ha. Based on bud dissection data and actual yield components (1.22 inflorescence primordia per bud at 40 nodes/vine) 9.25 t/ha could have been achieved. This does not take into consideration percentage budburst or extra shoots per vine. Also bunch weight is critical in estimation of yield potential, therefore increased bunch size will alter the actual tonnage obtained.

Vines were cane-pruned (average 2.4 canes per vine) in rows 16 and 17 only, therefore t/ha reflects the potential yield achievable. Yield was higher than spur-pruned vines as a result of more nodes/vine resulting from increased shoot and bunch number. However, the increased number of nodes resulted in a reduction of bunch weight.

Bud dissection analysis was used to determine the pruning level required to achieve a desired yield for the following season. For example, based on 1.22 bunches per bud and bunch weight of 85 g, a desired yield of 10 t/ha would be achieved by leaving 22 spurs/vine (44 nodes/vine). It is important to understand that the actual number of bunches per vine that develops is affected by percent budburst, environmental (eg. temperature) and to a lesser extent, vineyard practices (eg. pruning time), and therefore a 15% error level should be considered.

Table 4.2. Comparison of pruning level and yield components per vine at McLaren Vale 2002/2003.

	No. nodes/vine retained at pruning	**No. shoots/ vine 30/10/02**	**No. bunches/ vine 30/10/02**	**No. bunches/ vine 18/3/03**	**Av. bunch weight/vine (kg) 18/3/03**	**Av. bunch weight (g) 18/3/03**	**Approx. t/ha**
Spur-pruned	39.4	43.1	35.2	49.9	4.08	81.6	9.0
Cane-pruned	61.5	58.8	57.6	80.0	5.42	64.2	11.4

Table 4.3. Yield components used to predicted t/ha (Block 1 cv. Shiraz) based on an average 1.22 inflorescence primordia (bunches) per bud, vines spur-pruned to 40 nodes/vine and estimated bunch weight of 85 grams.

Spur No./ Vine	Bud No./ Spur	Shoots/ vine	Spurs /metre	Shoots /metre	Bunches /metre	Yield/Vine (kg)	Vine Distance	Row Distance
20	2	40	13.33	26.67	32.65	4.16	1.5	3

Row Length	Vines in Row	Number of Rows	Vines/Ha	Vines/Ac	Bunches/Ha	Bunch Weight (Kg)	**Tonnage/ha**	**Tonnage/ac**
100	66.67	33.33	2222.22	899.32	108848	0.085	**9.25**	**3.75**

Southern Fleurieu vineyard

Bud dissection analysis

Of the 100-tagged spurs, 68 shoots were collected for bud dissection analysis in 2003. Fruitfulness was relatively constant along the cane with overall fruitfulness (buds 1 –10) at 1.58 inflorescence primordia (bunches) per bud. This reduced to 1.42 bunches per bud if the first two buds were retained only. Bud fertility (number of buds containing one or more bunches) in the vineyard was 58% and 44% of the buds were necrotic. Node position on the spur influenced the distribution of PBN along the shoot. The incidence of PBN was highest in buds 5 and 9 (59% and 62%, respectively) than in the more terminal buds on a shoot (Figure 4.11).

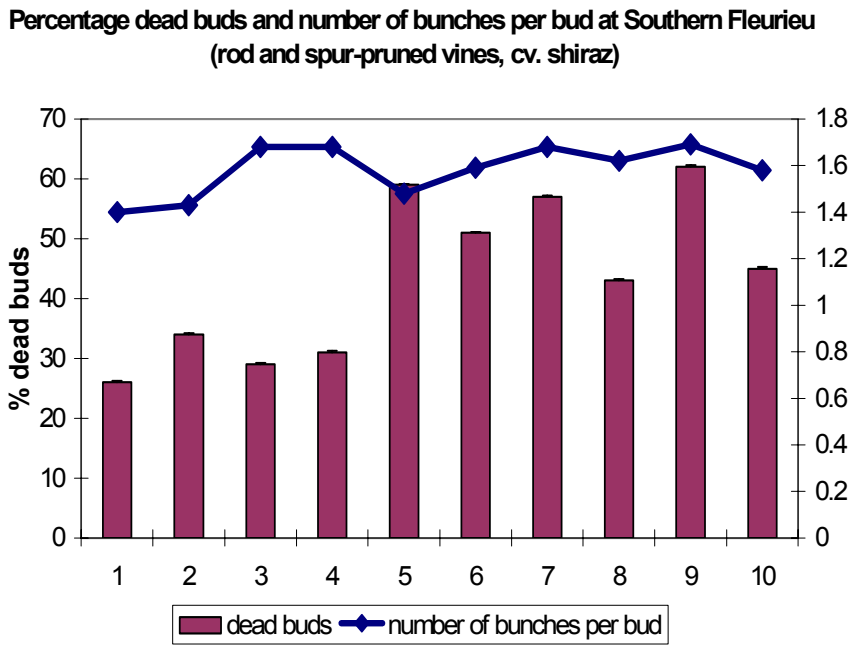


Figure 4.11. Number of inflorescence primordia (bunches) and incidence of dead buds (including primary bud necrosis) per bud at different positions along shoots derived from 7-bud canes in 2003 (cv. Shiraz, Southern Fleurieu).

Vine assessment

Overall budburst was 64%. Rate of growth was extremely variable as budburst was not consistent along the cane. Buds 1-2 did not commonly burst at all during the season and a higher number of shoots arose from the terminal buds (Figure 4.12). Shoots grew exponentially until flowering. The "grand period of growth" was observed and the greatest increase in growth occurred between 13-20 November (E-L stage 15). Shoots from this vineyard grew slower and were relatively shorter compared to those at McLaren Vale.

There was no correlation between shoot growth rate and the incidence of PBN. Statistical analysis of shoot growth rate at various times of the season and final PBN percentage showed there was no evidence to suggest that shoot vigour was associated with high levels of PBN. Also there was no association between shoot diameter and number of necrotic buds. The number of bunches significantly increased with the number of shoots (Figure 4.13), however mean bunch weight was low (46.1g). As the number of bunches increased, bunch weight decreased. The incidence of PBN was not correlated to bunch factors.

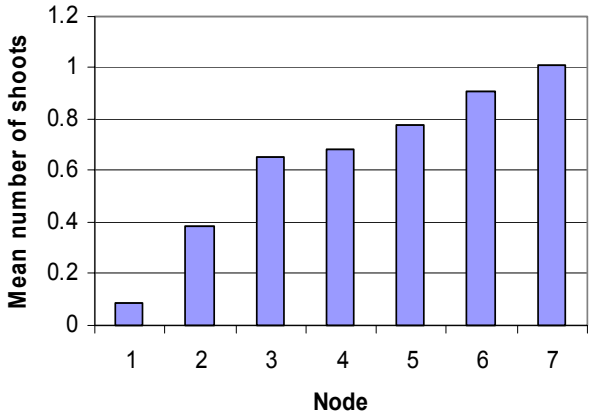


Figure 4.12. Mean number of shoots per node on 7-bud canes. Southern Fleurieu, 2002, cv. Shiraz (n=100).

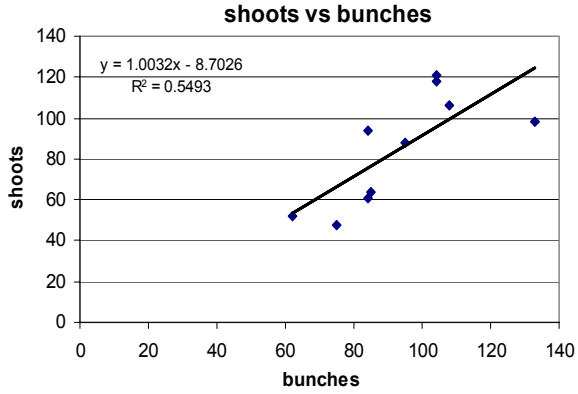


Figure 4.13. Relationship between number of shoots and number of bunches per vine (cv. Shiraz, Southern Fleurieu)

Yield estimation

Initial yield estimates (20 t/ha) were made in late October based on 85 bunches/vine using a 100 g bunch weight. Final yield (9.5 t/ha) was calculated after harvest using average bunch weight per vine from ten vines whereby overall bunch weight was lower than anticipated (Table 4.4).

Bud dissection and yield components were used to predict the yield potential based on current practices. Vines pruned to 80 nodes/vine and 1.43 inflorescence primordia per bud, 12.68 t/ha could be achieved (Table 4.5). This does not take into consideration percentage budburst or extra shoots per vine. Ideally, a higher bunch weight would be desirable in future seasons. A desired yield of 10 t/ha would be achieved by leaving 29 spurs/vine (58 nodes/vine). This is based on the results of bud dissection data, whereby 1.43 bunches per bud were estimated at nodes 1 and 2, and using an average bunch weight of 50 g. Increasing the bunch weight to 80 g/bunch would increase yield to 16t/ha.

Table 4.4. Comparison of minimal pruning and yield components per vine at Southern Fleurieu, 2002/2003.

No. nodes/vine retained at pruning	**No. shoots/ vine 30/10/02**	**No. bunches/ vine 30/10/02**	**No. bunches/ vine 18/3/03**	**Av. bunch weight/ vine (kg) 18/3/03**	**Av. bunch weight (g) 18/3/03**	**Approx. t/ha**
107.5	93.4	85.0	86.1	4.1	46.1	9.5

Table 4.5. Determination of pruning level (spurs per vine) using yield components, bunch weight of 50 grams and 1.43 inflorescence primordia (bunches) per bud to achieve desired yield, indicated in the below example as 10 t/ha.

Block	Row Width	Vine Spacing	Vines/ha	Vines per acre	Desired Yield kg/acre	Desired yield/vine (kg)	**Desired t/ha**
Shiraz	2.8	1.5	2380	964	4050	4.2	**10**

Average Fruitfulness at nodes 1 &2	Average bunch weight (kg)	Shoots per Vine	**2-n Spurs per vine**	2-n Spurs per Metre
1.43	0.05	58.9	**29**	19.6

Timing of PBN

Primary bud necrosis was first evident at the beginning of flowering, including buds showing any signs of necrotic tissue (Table 4.6). By berry development, about 30% of the buds sampled had developed symptoms of PBN. As the season progressed, the incidence of PBN increased, as did the severity of PBN. By January 50% of the primary bud tissue was necrotic, as indicated by severity rating 3 (Figure 4.14) and by late autumn, most primary buds were either nearly or completely dead. It was anticipated that PBN would occur until the onset of bud dormancy, but bud dissection analysis showed a further increase in the incidence of PBN after this time.

The incidence of PBN was higher in the basal buds than in the more distal buds on a shoot (Figure 4.15) and there appeared to be a correlation between internode length and PBN. There was a significant difference in internode length along the shoot ($P<0.005$). The shorter internodes had higher levels of PBN than longer internodes. There was no significant difference in the incidence and severity of PBN from shoots collected in the shade, partial shade or exposed to full sunlight. Shoot thinning prevented comparison of shoot length. In April, 43% of primary buds assessed in the present experiment were found to be necrotic and this corresponded with bud dissection analysis for the vineyard.

Table 4.6. Percentage of primary bud necrosis at different stages of grapevine development from shoots derived from canes and spurs (cv. Shiraz).

Date	Stage	% PBN spur	% PBN cane	Severity of PBN
27 Nov	Inflorescence developed	0	2	0
4 Dec	Start of flowering	14	11	1
11 Dec	Flowering	15	22	2.1
26 Dec	10% caps fall	19	26	3.4
16 Jan	Berries pea-sized	31	N/A	3.1
25 April	Beginning leaf fall	43	N/A	4.3

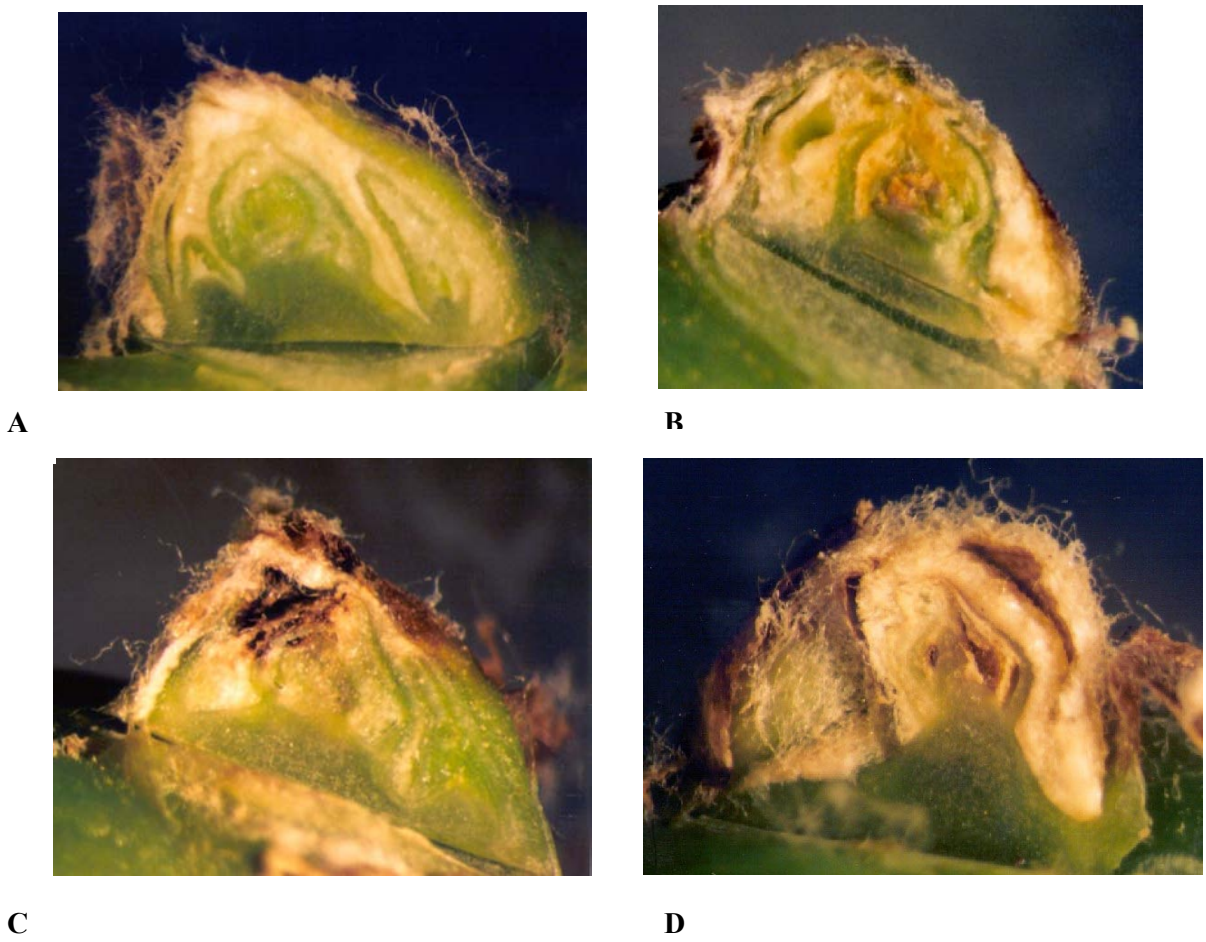


Figure 4.14. Development and severity of PBN of primary bud necrosis in Spring at Southern Fleurieu, South Australia. (A) Healthy bud, (B) 25% or less necrotic bud, (C) 25-50% necrotic bud and (D) 50-75% necrotic bud.

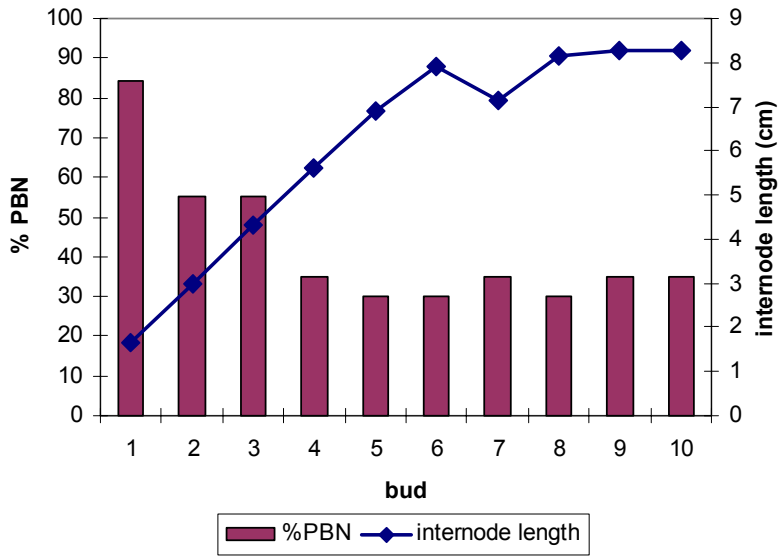


Figure 4.15. Percentage of buds showing necrosis in the primary bud and corresponding internode length (cm) on April 24 2003, Southern Fleurieu, cv. Shiraz.

Eden Valley vineyard

Bud dissection analysis

2002

The viticultural assistant manager performed bud dissections in June 2002 (cv. Shiraz). Ten canes were assessed up to 12 buds. Fruitfulness was not assessed, only the presence of healthy and necrotic buds. In total, 43% of buds were necrotic. The highest level of PBN was observed at buds 4 and 5 (80% and 50%, respectively).

2003

Spur-pruned – In the vineyard, 21% of the buds were dead. PBN levels were higher than the previous year (14%). The incidence of PBN was higher in bud 1 (45%) than in the more distal buds on a shoot (Figure 4.16). Fruitfulness was lowest in buds from basal nodes and increased along the shoot. Overall fruitfulness (buds 1 –10) was 1.58 inflorescence primordia (bunches) per bud. The fruitfulness for 2-bud spurs was 1.15 bunches per bud. Bud fertility (number of buds containing one or more bunches) in the vineyard was 89%, including bunch counts in the primary and secondary buds. However, if primary buds only were assessed, fruitfulness was reduced to 79%.

Cane-pruned – Less buds on cane-pruned vines were necrotic (14.6%) than those on spur-pruned vines (Figure 4.17). The incidence of PBN was considered low. Fruitfulness (1.38 bunches per bud) was lower than spur-pruned vines. Fruitfulness was lowest at bud 1 and slightly increased along the shoot. Bud fertility was 97.7%, but if primary buds were considered only, bud fertility decreased to 84.2%.

Vine assessment

Budburst was high for both spur and cane-pruned vines (96% and 103%, respectively). A higher number of nodes were retained on the upper cordon of spur-pruned vines than on the lower cordon., but there was no significant difference between budburst. Flowering occurred at the end of November. Shoot vigour (measured by length and diameter) did not vary between spur positions. Shoots on 2-bud spurs grew significantly quicker than shoots on cane-pruned vines. The “grand period of growth” was observed on spur-pruned vines, whereas shoots from cane-pruned vines were shorter and did not display exponential growth (Figure 4.18). Shoot diameter measured at the time of bud dissection was slightly greater for spur-derived shoots. There was a positive correlation between the number of shoots per vine and the number of bunches. As shoot number increased, bunch number increased (Figure 4.19). Bunch weight however was exceptionally low for spur-pruned vines.

There was no correlation between shoot growth rate, shoot diameter and the incidence of PBN. Bunch number and weight also did not influence PBN. There was no significant difference between

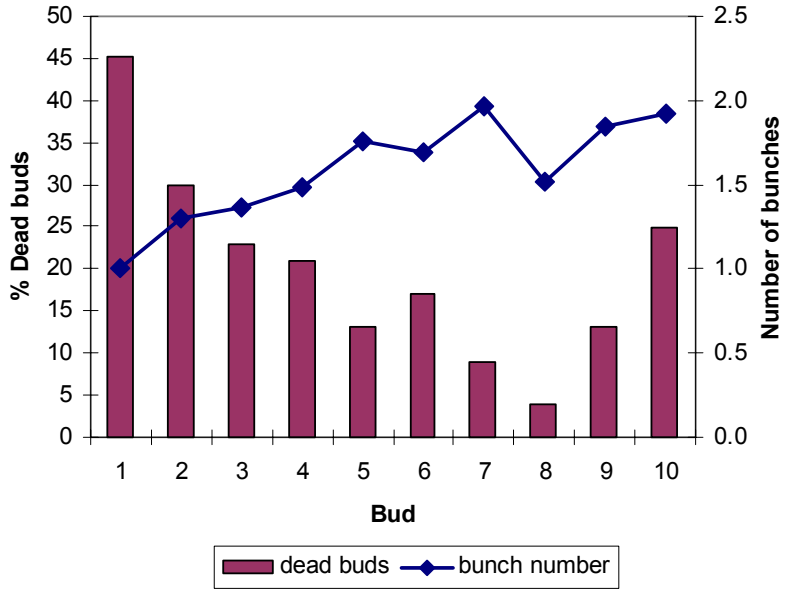


Figure 4.16. Number of inflorescence primordia (bunches) and incidence of dead buds (including primary bud necrosis) per bud at different positions along shoots derived from spur-pruned vines (cv. Shiraz, Eden Valley).

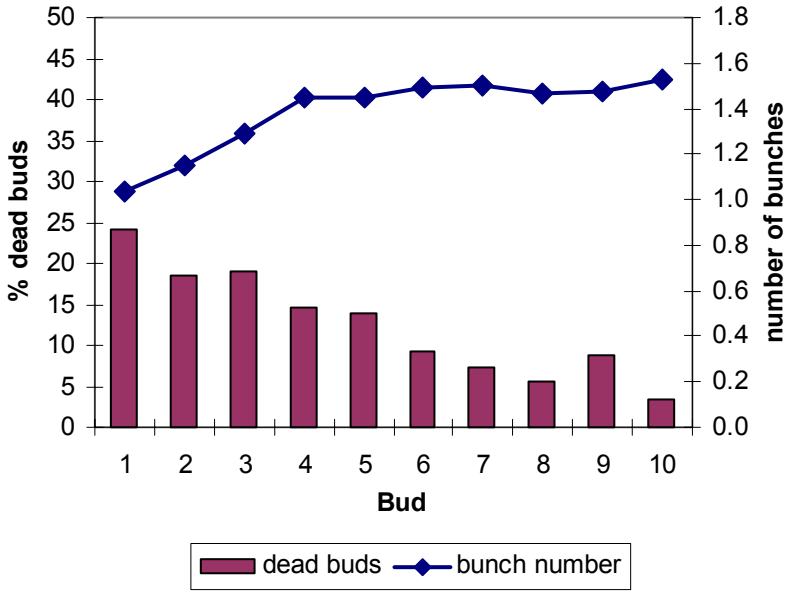


Figure 4.17. Number of inflorescence primordia (bunches) and incidence of dead buds (including primary bud necrosis) per bud at different positions along shoots derived from cane-pruned vines (cv. Shiraz, Eden Valley).

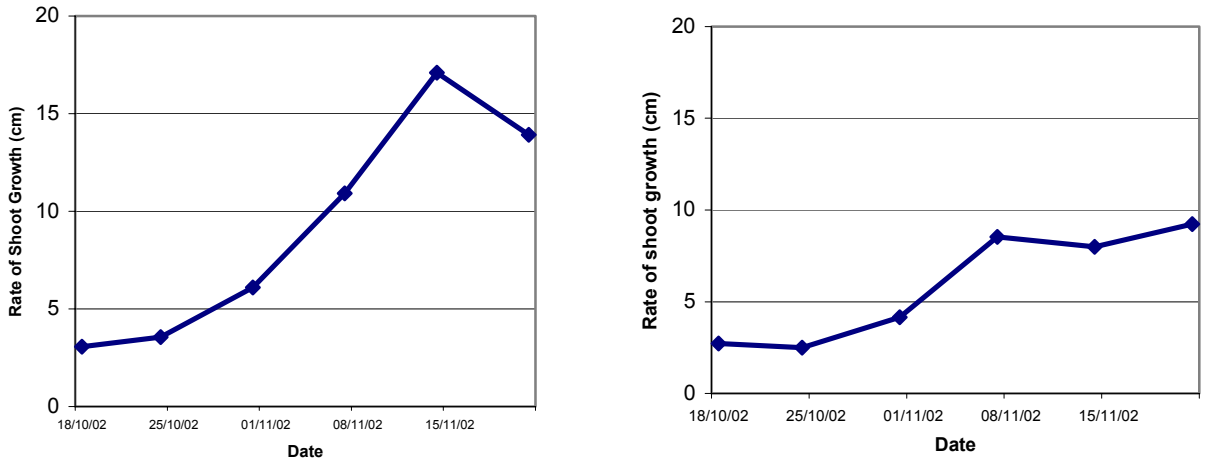


Figure 4.18. Shoot growth rate of Shiraz at Eden Valley, 2002. (a) Spur-pruned vines, (b) cane-pruned vines. Shoot growth rate was calculated as the length increase per day between dates.

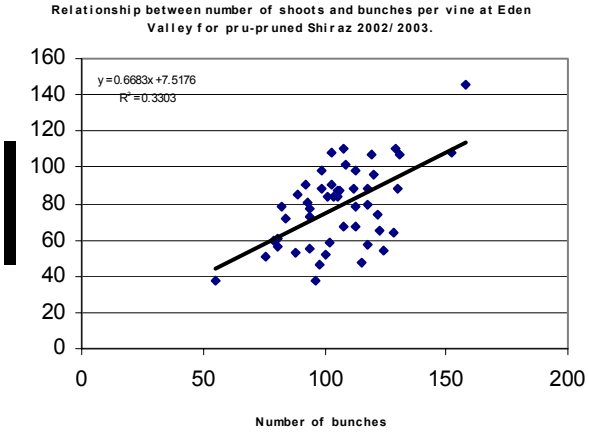


Figure 4.19. Relationship between number of shoots and number of bunches per vine at harvest (cv. Shiraz, spur-pruned, Eden Valley).

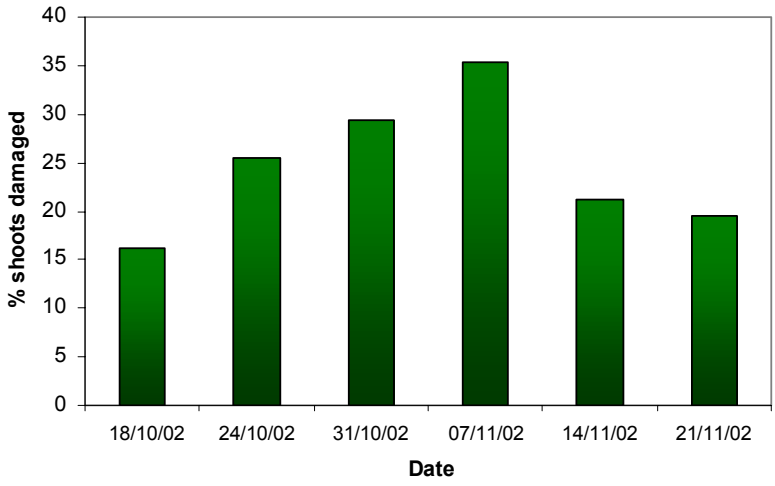


Figure 4.20. Percentage of shoots damaged by birds between 18 October and 21 November 2002 on spur-pruned cv. Shiraz, Eden Valley.

the incidence of PBN on the upper and lower cordon of spur-pruned vines (23% and 19%, respectively). The relationship between shoot growth and PBN could not be assessed in the Eden Valley vineyards for the cane-pruned Shiraz block due to the amount and extent of bird damage present. For this reason, 5 rows were excluded from the trial later in the season. Bird damage was monitored during the season and the highest incidence was observed on 7 November 2002 (Figure 4.20). Bird damage was over 54% for rows 6-10 alone.

Yield estimates

Initial yield estimates (5.9 t/ha) were made in late October based on a bunch count of 78 bunches/vine and 100 g bunch weight. Final yield (0.77 t/ha) was adjusted after harvest using average bunch weight per vine from ten vines. Overall, bunch weight was severely lower than anticipated (13 g), whereby many berries did not reach full development and shrivelled early. Final yield was 0.77 t/ha (Table 4.7). Bud dissection and yield components were used to predict the yield potential for the following season. Based on 1.15 inflorescence primordia per bud with vines pruned to 60 nodes/vine and 50 g bunch weight, 1.28 t/ha could be achieved.

Yield was higher in cane-pruned vines than spur-pruned vines, predominantly due to a higher bunch weight. Bunch counts in October and at harvest were similar, yet bird damage reduced the overall yield potential in this block. The level of PBN was low, therefore pruning level in the following season may be adjusted according to bud fruitfulness.

Table 4.7. Comparison of pruning level and yield components per vine at Eden Valley 2002/2003.

	No. nodes/vine retained at pruning	**No. shoots/ vine 30/10/02**	**No. bunches/ vine 30/10/02**	**No. bunches/ vine 18/3/03**	**Av. bunch weight/vine (kg) 18/3/03**	**Av. bunch weight (g) 18/3/03**	**Approx. t/ha**
Spur-pruned	69.9	103.6	79	76.6	1.08	13.4	0.77
Cane-pruned	50.7	55.5	42.4	49.9	4.08	81.6	3.04

SUMMARY

Bud dissection is a valuable tool to predict yield and determine the number of dead buds. Without bud dissection, PBN can go unnoticed as the canopy can appear normal but have less fruit. Bud dissection analysis showed bud fruitfulness and incidence of PBN can vary significantly between vineyards. Acceptable bud fruitfulness was experienced at Southern Fleurieu, however a high level of PBN was observed. On the other hand, the incidence of PBN was low at Eden Valley. In some instances where PBN levels were extremely high, retention of more nodes per vine reduced the problem in the following season.

There was no correlation between vigorously growing shoots and the incidence of PBN. Past studies (cv. Sultana and Queen of the Vineyard) have shown that thick, long shoots have more dead buds than weaker ones (Lavee *et al*., 1981; Morrison and Iodi, 1990). This trend was not detected for cv. Shiraz. Longer and thicker shoots did not have higher levels of PBN than other shoots. Also bunch size and number of bunches per shoot did not affect the incidence of PBN. Cane-pruned vines generally showed lower levels of PBN compared to spur-pruned vines. In addition to high levels of PBN, low fruitfulness on cane-pruned vines could be attributed to low light in the canopy caused by shading.

PBN can vary significantly from block to block. One block may show low levels of PBN, and the neighbouring block of the same cultivar may show high PBN. We do not fully understand why this is so, but high variability exists most commonly in Shiraz than any other cultivar. High seasonal variability may be a result of vine balance, climatic conditions or modification of cultural practices. Due to block variability, it is difficult to determine seasonal trends and if PBN levels will be high in a particular region. A good bud dissection service provider can assist in interpretation of the data and offer recommendations to obtain desired yields.

Theoretically, the number of inflorescence formed in the bud is complete by the onset of dormancy and buds can be dissected for yield estimation after this time. However, this study showed that levels of PBN in Shiraz continue to increase until autumn. Subsequently, the resulting bunch number can be significantly reduced. Buds should therefore be dissected as close to pruning as possible to predict yield potential. Bud fruitfulness was often lower if inflorescence primordia in the primary bud were only considered. Counting bunches in the secondary buds provided a more accurate estimation of overall fruitfulness. Although it is speculated that bunches arising from secondary buds are smaller, this highlights the importance of the secondary buds in analysis of fruitfulness.

A change in one or more yield components drastically affects the estimation of yield. Pruning level has the most influence on components such as shoot number, vigour, bunch and berry weight. Light

pruning levels (high number nodes per vine) can result in a greater number of shoots and bunches but lower bunch weight. Increased shoots per vine can lead to crowding and shading in the canopy, reducing bunches per shoot and bunch weight (Tassie and Freeman, 2001). Bunch weight plays a critical role in yield estimation. There can also be discrepancies observed between bunches observed in spring, and bunch count at harvest. Generally more bunches are harvested than counted earlier in the growing season. Bud dissection is a reliable method to determine bud fertility and knowing the incidence of PBN is critical in more accurate forecasting of yield.

Balanced pruning is the concept of equating the nodes retained at pruning with vine capacity. The aim is to maintain a balance between vegetative growth and fruit production (Tassie and Freeman, 2001). Following the present study, pruning trials were established to determine best management practices to reduce the incidence of PBN (chapter 8). Based on percent PBN after bud dissection analysis, the current recommendations are to leave extra buds per vine if PBN levels exceed 20%. The vine will then put energy into bursting the extra primary buds rather than the secondary buds. The number of buds retained will depend on a number of factors including trellis type, existing pruning strategy, vine vigour, labour costs, and target yield. Recommendations are therefore different for every block assessed and must be tailored for individual vineyards.

5. Cultivar and Clonal Susceptibility

INTRODUCTION

The problem of primary bud necrosis is most evident in the cultivar Shiraz. Shiraz is one of the most vigorous growing cultivars and generally has longer shoots, extensive lateral growth and hence a higher probability of shading problems compared to other cultivars. The role of canopy management is important in Shiraz to control vigorous shoot growth and fruitfulness. With an increasing awareness of PBN and poor fruitfulness in Shiraz, more information is required to assist in reducing the incidence of PBN.

The incidence of PBN appears to be cultivar-specific. Research on PBN and fruitfulness has been widely conducted on table grapes, such as Thompson seedless (Sultana) and Queen of Vineyard (Lavee *et al*., 1981). There are limited studies on the effect of PBN on wine grapes cultivars. In the USA, the most susceptible wine grape cultivars are Viogner, Riesling and Shiraz (Wolf, 2001). Chardonnay is not sensitive to PBN. In Australia, natural levels of PBN are highest in Shiraz (Dry, 1986), but studies on the incidence of PBN in a range of cultivars have not been conducted. The development of PBN occurs during the period between bloom and the onset of bud dormancy for Flame Seedless and Thompson Seedless. The timing of PBN can be variable within cultivars, eg. PBN commenced at 3 weeks after flowering in Thompson Seedless (Lavee *et al.,* 1981), whereas Vasudevan *et al*., (1998a) reported PBN to occur 6 to 10 weeks after flowering in the same cultivar. Similar to the pattern of PBN incidence in table grapes, PBN in Riesling occurs 3 weeks after flowering. Severity of PBN increases up to 9 weeks after full bloom (Morrison and Iodi, 1990) and ceases after the onset of bud dormancy. In most reports, sampling for PBN ceased at the onset of dormancy. Knowledge of the timing of PBN may have implications for future management of the problem.

The aim of this study was to determine the timing and progression of PBN in different cultivars and clones of grapevine in two viticultural regions of South Australia.

MATERIALS AND METHODS

To monitor the development of PBN in a number of cultivars, shoots were collected from seven cultivars in the Adelaide Hills and four cultivars from Padthaway, South Australia (Table 5.1). Twenty shoots (1 shoot per vine) were randomly collected from each cultivar every fortnight in the 2003/2004 growing season commencing at E-L stage 14 (7 leaves separated, shoots approx 15cm and new buds evident on shoots). A total of 15 collections were made between 26 November 2003 and 8 June 2004 in the Adelaide Hills and 17 collections between 28 October 2003 and 8 June 2004 at Padthaway. Shoots were cut below the first bud (node one) and taken to a maximum of 10 buds. Shoots were packaged in plastic bags with moisture for transportation to the laboratory for analysis.

Shoot diameter, length and internode length was measured and buds assessed for presence, severity and location (top, middle, base of bud) of PBN.

Dormant canes were collected from a clonal trial at Nepenthe vineyards, Adelaide Hills to assess clonal variation of the cultivar Cabernet Sauvignon. Nine clones were selected: SA125, G9V3, LC10, LC6, LC14, CW44, LC84, R2V11 and Reynella. Twenty canes, consisting of 10 buds, were collected from each clone in June 2004 and assessed as above.

Data was analysed using a standard generalised linear model (GLM) with the logit link function. In order to analyse the PBN response, the data was collapsed to become a count of buds with proportion of PBN from the total number of buds per shoot.

Table 5.1. List of cultivars monitored at two sites in South Australia for development and severity of primary bud necrosis.

Site	Cultivar	Clone
Adelaide Hills	Cabernet Sauvignon	G9V3
Adelaide Hills	Zinfandel	C11V7
Adelaide Hills	Chardonnay	I10V1
Adelaide Hills	Pinot Noir	D5V12
Adelaide Hills	Sauvignon Blanc	5385
Adelaide Hills	Shiraz	1127
Adelaide Hills	Semillon	*
Padthaway	Shiraz	*
Padthaway	Chardonnay	*
Padthaway	Riesling	*
Padthaway	Cabernet Sauvignon	*

* Unknown

RESULTS

Cultivar susceptibility to PBN - Adelaide Hills

Many of the cultivars had similar levels of PBN throughout the growing season (Figure 5.1). In Shiraz, however, PBN levels fluctuated throughout the season, with rises in PBN at full bloom, berry ripeness and following harvest. There were no trends to suggest that any of the other six cultivars were more or less susceptible than the other. On June 8, Zinfadel had significantly lower levels of PBN than Pinot Noir, Sauvignon Blanc and Shiraz. Semillon, Cabernet Sauvignon and, Chardonnay were only significantly different to Shiraz.

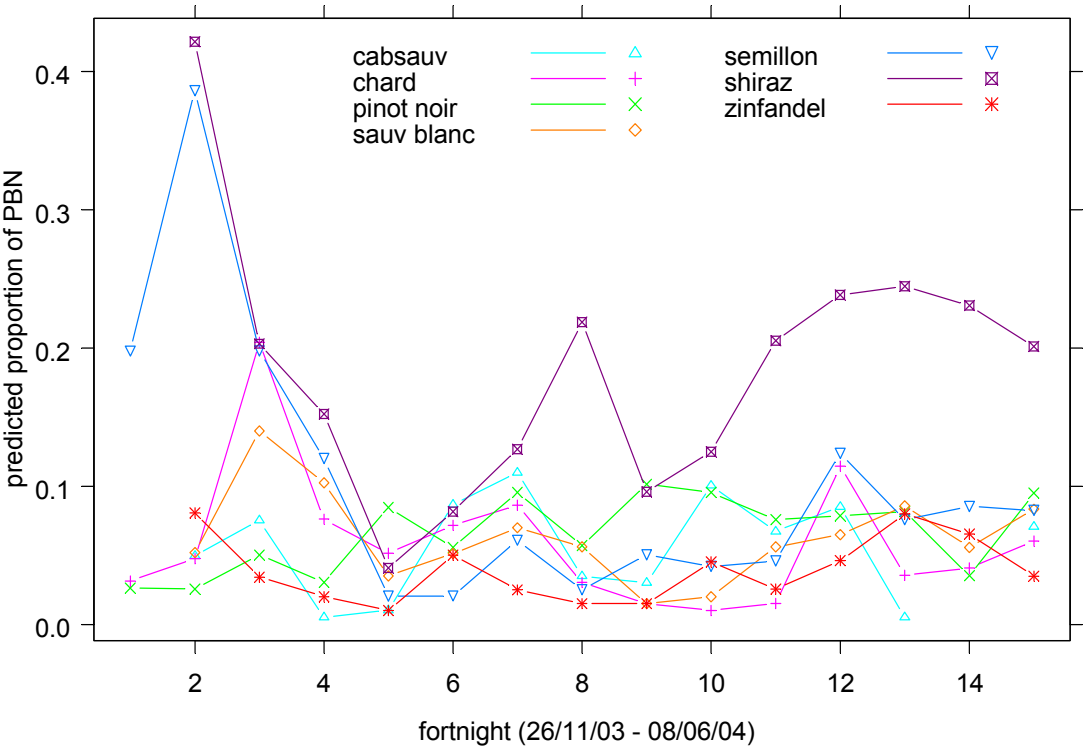


Figure 5.1. Means of PBN for seven cultivars over time based on fortnightly observations (26/11/2003-8/6/2004) in the Adelaide Hills, South Australia.

Table 5.2. Predicted proportions of primary bud necrosis between cultivars at various dates from vines sampled in the Adelaide Hills, South Australia.

	Chardonnay	Cab Sauv	Pinot Noir	Sauv Blanc	Semillon	Shiraz	Zinfandel
26-Nov-03	0.0314^{b}	0.0000^{1}	0.0263^{a}	*	0.1979^{b}	*	0.0000^{1}
9-Dec-03	0.0476^{ab}	0.0497^{ab}	0.0256^{a}	0.0518^{ab}	0.3862^{c}	0.4219^{c}	0.0806^{b}
23-Dec-03	0.2042^{b}	0.0754^{a}	0.0503^{a}	0.1400^{b}	0.1979^{b}	0.2031^{b}	0.0343^{a}
6-Jan-04	0.0761^{bc}	0.0051^{a}	0.0305^{ab}	0.1026^{cd}	0.1200^{cd}	0.1523^{d}	0.0201^{a}
20-Jan-04	0.0515^{bc}	0.0102^{a}	0.0847^{c}	0.0352^{ab}	0.0203^{a}	0.0408^{abc}	0.0102^{a}
3-Feb-04	0.0718^{b}	0.0867^{b}	0.0558^{ab}	0.0513^{ab}	0.0205^{a}	0.0816^{b}	0.0500^{ab}
17-Feb-04	0.0863^{bc}	0.1100^{bc}	0.0955^{bc}	0.0700^{bc}	0.0609^{ab}	0.1269^{c}	0.0250^{a}
2-Mar-04	0.0303^{ab}	0.0350^{ab}	0.0570^{b}	0.0561^{b}	0.0253^{ab}	0.2188^{c}	0.0151^{a}
16-Mar-04	0.0151^{a}	0.0302^{a}	0.1015^{b}	0.0151^{a}	0.0505^{ab}	0.0960^{b}	0.0151^{a}
30-Mar-04	0.0102^{a}	0.1005^{d}	0.0955^{cd}	0.0201^{ab}	0.0419^{ab}	0.1250^{d}	0.0452^{bc}
13-Apr-04	0.0153^{a}	0.0674^{bc}	0.0758^{c}	0.0561^{bc}	0.0459^{abc}	0.2053^{d}	0.0255^{ab}
27-Apr-04	0.1146^{bc}	0.0850^{abc}	0.0785^{abc}	0.0650^{ab}	0.1237^{c}	0.2386^{d}	0.0462^{a}
11-May-04	0.0355^{b}	0.0051^{a}	0.0816^{bc}	0.0859^{c}	0.0758^{bc}	0.2448^{d}	0.0800^{bc}
25-May-04	0.0408^{ab}	0.0000^{1}	0.0354^{a}	0.0558^{ab}	0.0854^{b}	0.2308^{c}	0.0653^{ab}
8-Jun-04	0.0603^{ab}	0.0707^{ab}	0.0950^{b}	0.0833^{b}	0.0825^{ab}	0.2011^{c}	0.0350^{a}

The interaction between cultivars and date was significantly different (P<0.001) indicating PBN differed between cultivars over time. Of the shoots collected on 26 November, only Chardonnay, Pinot Noir and Semillon had detectable levels of PBN (Table 5.2). Flowering commenced around 9 December 2003, at which PBN was observed in all cultivars. Shiraz had significantly higher levels of PBN than all other cultivars on 2 March and at each fortnight from 13 April to 8 June 2004. The results indicate the variability in the incidence of PBN throughout the season.

In all cultivars, internodes were shorter at buds 1-3 and then length was consistent up to bud 10. Comparison of shoot growth around the time of flowering showed that internodes were longer in Shiraz than in any other cultivar (Figure 5.2). Semillon appeared least vigorous. Although internode length at buds 1, 2 and 3 were significantly (P<0.005) shorter than other internodes further along the shoot in Shiraz, there was no interaction between internode length and PBN (Figure 5.3).

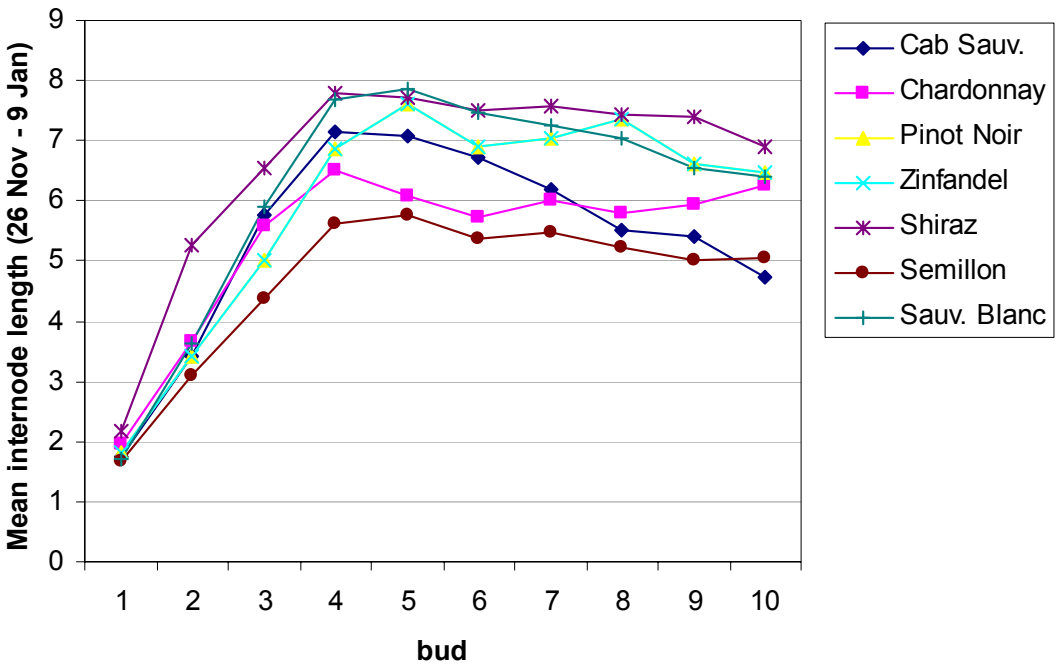


Figure 5.2. Mean internode length of shoots comprising up to ten buds from seven cultivars between 26 November 2004 and 6 January 2005 at Nepenthe vineyard, Adelaide Hills, South Australia.

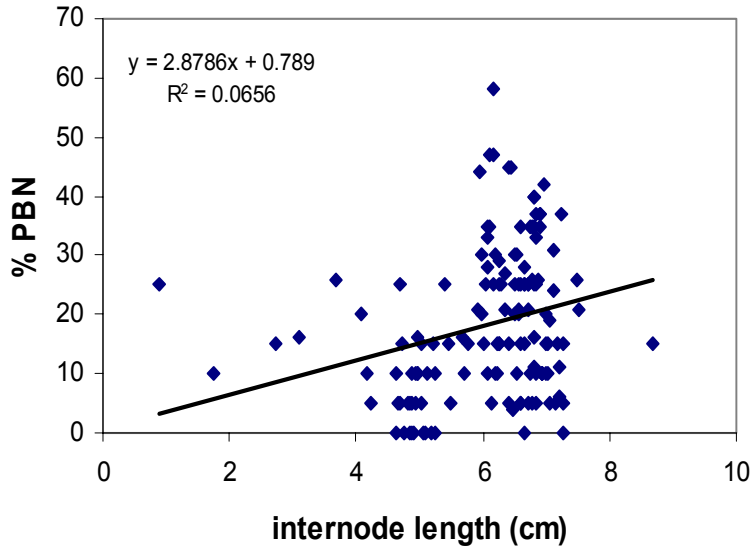


Figure 5.3. Relationship between internode length and percent primary bud necrosis in cv. Shiraz at Nepenthe vineyards, Adelaide Hills, South Australia.

Cultivar susceptibility to PBN - Padthaway

Although shoot sampling at Padthaway commenced in late October, most cultivars did not have PBN present until 25 November. In Padthaway, flowering occurred in late November. The commencement of PBN coincided with the onset of flowering.

The incidence of PBN significantly differed between cultivars over time ($P<0.001$). The proportion of PBN was similar between Cabernet Sauvignon, Chardonnay and Riesling throughout the growing season, whereas Shiraz was highly susceptible (Figure 5.4). The highest incidence of PBN was observed on May 11 when 46% of Shiraz buds were necrotic. Similar to vines at Nepenthe, the incidence of PBN in Shiraz decreased following leaf fall. Shiraz displayed the highest incidence of PBN compared to other cultivars from 3 February to 8 June 2004, with one exception on April 27 where Shiraz and Riesling were not significantly different (Table 5.3). Riesling appeared least susceptible to PBN on 8 June, however this trend was not consistently observed at other dates. Overall, the incidence of PBN for Cabernet Sauvignon, Chardonnay and Riesling was low.

Similarly to vines grown in the Adelaide Hills, Shiraz was the most vigorous throughout the growing season compared to other cultivars assessed (Figure 5.5). However internode length continued to increase along the shoot until buds 5-6. There was no association between internode length and incidence of PBN (Figure 5.6).

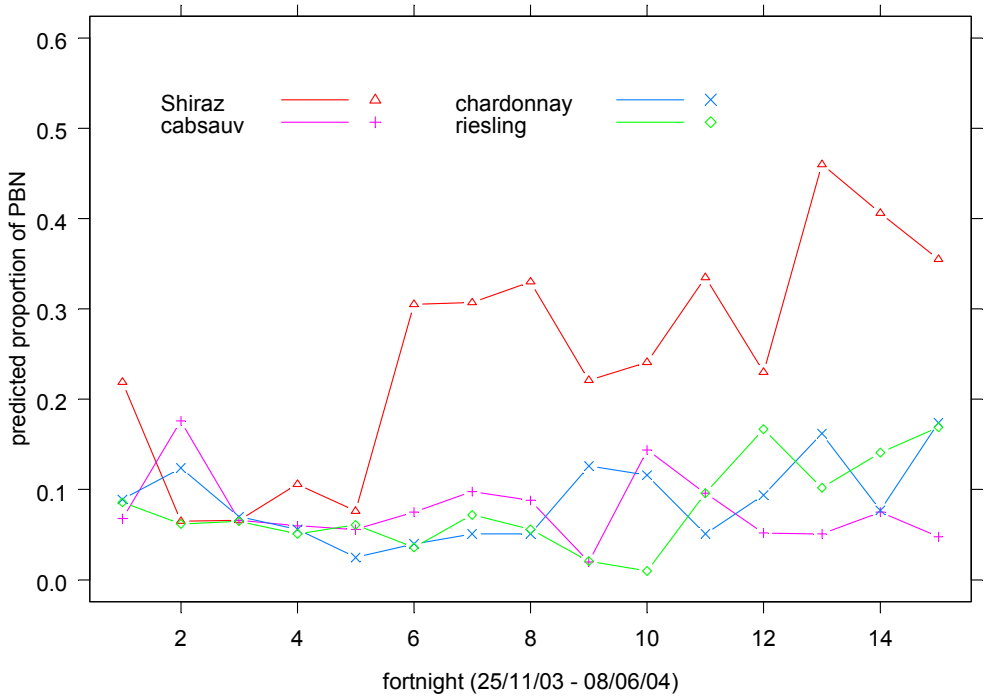


Figure 5.4. Means of PBN for seven cultivars over time based on fortnightly observations (26/11/2003-8/6/2004) at Padthaway, South Australia.

Table 5.3. Comparison of predicted proportions between cultivars within each date at Padthaway, South Australia.

	Cab Sauv	Chardonnay	Riesling	Shiraz
25-Nov-03	0.068^{b}	0.089^{b}	0.086^{b}	0.219^{a}
9-Dec-03	0.176^{a}	0.124^{ab}	0.062^{c}	0.065^{bc}
23-Dec-03	0.066^{a}	0.070^{a}	0.065^{a}	0.066^{a}
6-Jan-04	0.060^{ab}	0.056^{ab}	0.051^{b}	0.106^{a}
20-Jan-04	0.056^{ab}	0.025^{b}	0.061^{ab}	0.076^{a}
3-Feb-04	0.075^{b}	0.040^{b}	0.036^{b}	0.305^{a}
17-Feb-04	0.098^{b}	0.051^{b}	0.072^{b}	0.307^{a}
2-Mar-04	0.088^{b}	0.051^{b}	0.056^{b}	0.330^{a}
16-Mar-04	0.020^{c}	0.126^{b}	0.021^{c}	0.221^{a}
30-Mar-04	0.144^{b}	0.116^{b}	0.010^{c}	0.241^{a}
13-Apr-04	0.096^{b}	0.051^{b}	0.096^{b}	0.335^{a}
27-Apr-04	0.052^{b}	0.094^{b}	0.167^{a}	0.230^{a}
11-May-04	0.051^{c}	0.162^{b}	0.102^{bc}	0.460^{a}
25-May-04	0.075^{c}	0.077^{c}	0.141^{b}	0.406^{a}
8-Jun-04	0.048^{c}	0.174^{b}	0.169^{b}	0.355^{a}

Means with same letter are not significantly different at 5% significance level.

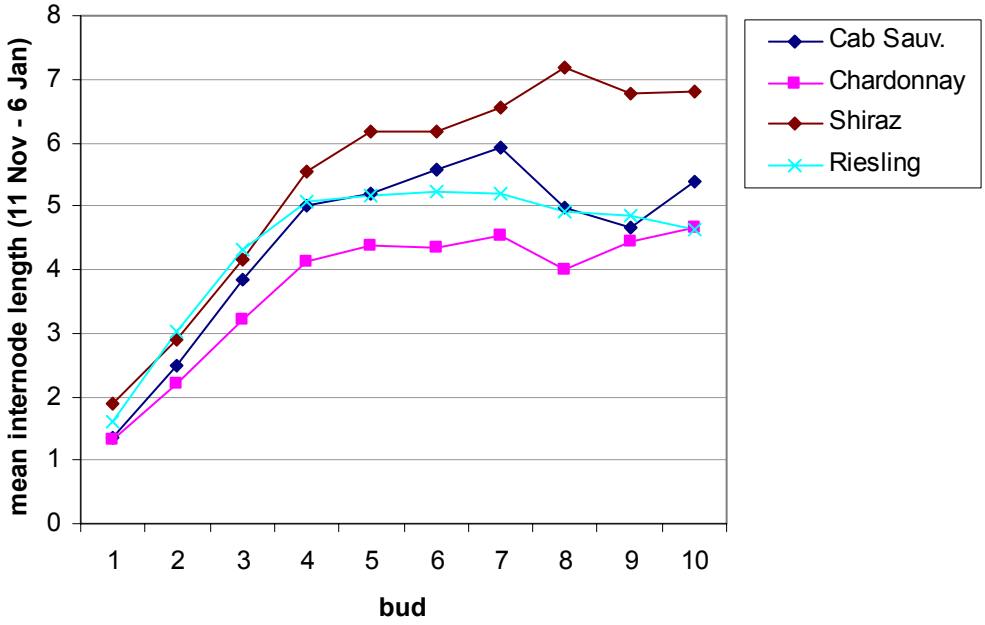


Figure 5.5. Mean internode length of shoots comprising up to ten buds from four cultivars between 11 November 2004 and 6 January 2005 at Padthaway, South Australia.

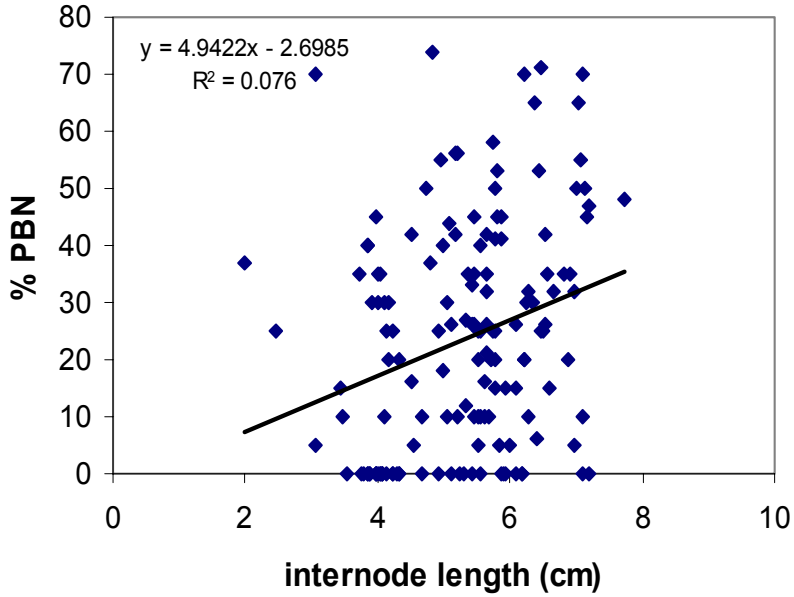


Figure 5.6. Relationship between internode length and percent primary bud necrosis in cv. Shiraz at Padthaway, South Australia.

Effect of clonal variability on the incidence of PBN

The incidence of PBN did not significantly differ (*P*=0.063) between clones of Cabernet Sauvignon at the Adelaide Hills vineyard (Figure 5.3). No clones were more susceptible to PBN than others. Although the highest levels of PBN were observed in clones LC10, LC6, RZVII and SA125, no clone had greater than 21% necrotic buds. It was unlikely that clonal selection would influence the incidence of PBN in Cabernet Sauvignon and the levels of PBN observed would not significantly reduce yield.

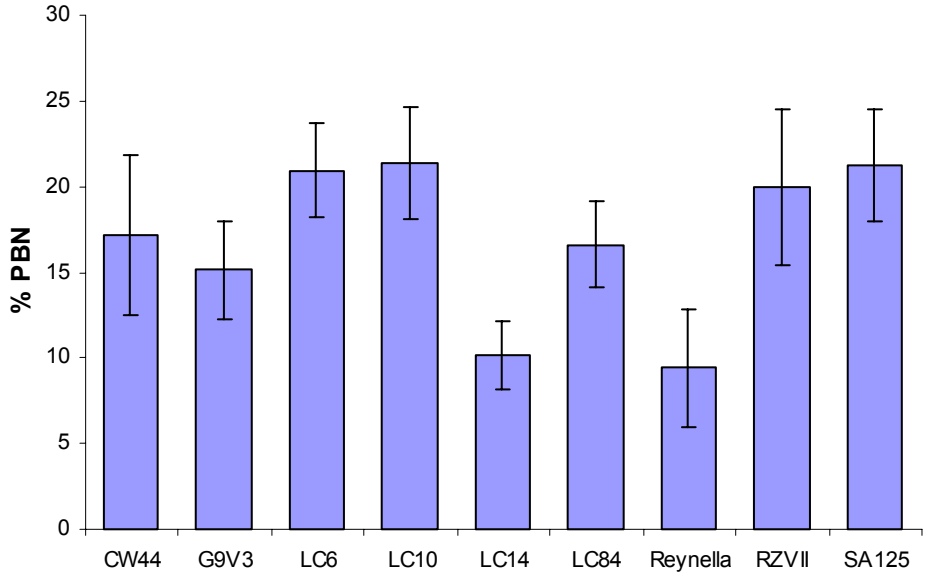


Figure 5.7. Incidence of PBN on different clones of Cabernet Sauvignon collected on 8 June 2004 in the Adelaide Hills, South Australia.

DISCUSSION

Shiraz was most susceptible to PBN in the Adelaide Hills and Padthaway, South Australia. Although Riesling has been identified as a susceptible cultivar to PBN in the USA (Vasudevan *et al.,* 1998b), the incidence of PBN in Riesling at Padthaway was low. Other cultivars displayed significantly lower proportions (<20%) of PBN compared to Shiraz at both sites. In general, <20% PBN may not cause significant yield loss. Shiraz showed the highest variability of PBN throughout the growing season. Although it was expected that PBN levels would increase during the season, the incidence of PBN fluctuated. This verifies that, regardless of cultivar, bud fruitfulness must be assessed as close to pruning as possible to ensure pruning levels are modified accordingly.

PBN commenced at flowering for all cultivars. Flowering and initiation of inflorescence occur at about the same time generally in the latter stage of the grand period of shoot growth (Coombe, 2001). In this process, the undifferentiated anlagen (uncommitted primordia) develop either as inflorescence or tendril primordia and, shortly thereafter, the latent buds enter dormancy (Mullins *et al*., 1992). The processes involved in floral initiation appear to coincide with the commencement of PBN. Research has shown in table grapes that levels of PBN increase upon application of the growth hormone, gibberellic acid (GA_3). Although not used in wine grape production, GA_3's are still produced naturally and are involved in both anlagen formation and determination of anlagen development. Even though GA_3 is necessary for initiation, the role of GA_3 in grapevines varies with the stage of the latent bud. In vines with excessive GA_3, formation of inflorescence primordia can be inhibited (Mullins *et al*., 1992; Boss and Thomas, 2002) and in apples, seed-produced GA_3's move to the spur buds where they inhibit flower induction (Stephan *et al*. 1999). Hence, it is possible that the commencement of PBN occurs around flowering under the influence of excessive GA_3. Vigorous shoot growth is a consequence of excessive GA_3 activity, and this correlates with the high incidence of PBN in Shiraz.

Shoot vigor is often positively correlated with PBN. In Australia, Dry (1998) reported that thicker shoots (>12 mm) had up to 40% more PBN than thinner shoots (<12 mm). In Riesling, the more rapid elongating shoots exhibited more necrotic buds (Wolf and Warren, 1995). Lavee *et al.*, (1981) proposed that elevated GA_3 levels associated with vigorous shoots lead to PBN. In this study, we found little correlation between internode length and incidence of PBN. However, there was a notable difference in shoot internode length between Shiraz and other cultivars.

Previous reports indicated that PBN increased to the onset of bud dormancy (Morrison and Lodi, 1981; Lavee *et al*., 1981; Vasudevan *et al*., 1998b). Although the results here supported this assumption, sampling throughout the entire season revealed the incidence of PBN could increase later. PBN levels increased around flowering and again on the onset of dormancy. Interestingly, these times coincided with peaks of GA_3 activity in grapevine.

From this study, it appeared that timing of PBN and cultivar susceptibility is correlated to the activity of GA_3. Many studies have shown that application of GA_3 to table grapes increases PBN. Since seeds are reported to be natural sources of endogenous gibberellin, excessive levels of GA_3 in wine grapes appear to have a similar effect. The role of GA_3 in the induction of PBN was investigated and reported in the following chapter.

6. Influence of Gibberellic Acid

INTRODUCTION

Gibberellins are plant growth hormones involved in cell division and elongation (Takahashi *et al*., 1991). Exogenous application of Gibberellin A_3, also known as gibberellic acid (GA_3), increases berry size in table grapes. Applications of GA_3 (100mg/L^{-1}) to cv. Kyoho before and after flowering increased PBN to almost 100 percent in nodes 5 –20, compared to non-treated vines (Naito *et al*., 1986). Ziv *et al*. (1981) also reported increases in PBN at lower concentrations of GA_3 (20mg/L^{-1}). PBN frequency was increased when GA was applied up to 2 weeks after bloom (Ziv, 1981) and buds were insensitive to GA once the bud was well differentiated, indicating a dependence on stage of bud development.

Endogenous GA_3 is greater in buds from vigorous vines than buds from normal vines (Lavee *et al.*, 1981). It was proposed that elevated GA_3 levels associated with vigorous shoot growth led to PBN. Plant growth regulators, such as paclobutrazol and SADH, been found to reduce the rate of shoot growth and the level of PBN (Naito *et al*., 1986; Wolf and Warren, 1995). Growth retardants block the synthesis of gibberellins and reduce the rate of cell division. Hence the application of paclobutrazol may be a means of controlling excessive vigour in vines susceptible to PBN.

The aim of the investigation was to assess the effect of GA_3 and paclobutrazol on the incidence of PBN in cv. Shiraz when applied at different times of bud development.

MATERIALS AND METHODS

A trial was established in October 2003 at the Nuriootpa Research Station, Barossa Valley to assess the incidence of PBN on vines following application of Gibberellic Acid (GA_3) and paclobutrazol. Vines (cv. Shiraz) were planted in September 1988. Vines in one row were randomly allocated to receive either no treatment (control) or one treatment of GA_3 or paclobutrazol (100 mg/L^{-1}). Shoots were treated at one week prior to flowering, at flowering and one week after flowering (Figure 6.1). Ten vines were used per treatment at the different times. Two methods of application were trialled to assess rate of translocation. On each vine, two methods (drop or paint) were used to apply GA_3 or paclobutrazol to every buds on the shoot. For the drop method, a pipette was used to apply approximately 10µl solution onto the bud and was subsequently wrapped in Parafilm overnight to assist penetration. Alternatively, a paintbrush was dipped in GA_3 solution and the bud was painted with each of the treatments. Shoot length and bunch number was recorded for each shoot at the time of application. A total of 20 shoots per treatment were assessed for the incidence, severity and location of PBN in the bud and bud fruitfulness in June 2004.

Vine No.	Treatment	Shoot	Application	Bunch No.
11	Control	1	control	2
11	Control	2	control	1
12	PAC before	1	D	1
12	PAC before	2	P	2
13	GA flowering	1	D	2
13	GA flowering	2	P	2
14	GA before	1	P	2
14	GA before	2	D	2
15	GA after	1	P	1
15	GA after	2	D	2
16	PAC after	1	D	2
16	PAC after	2	P	2
17	PAC flowering	1	D	2
17	PAC flowering	2	P	2
18	PAC after	3	P	3
18	PAC after	4	D	2
19	control	3	control	1
19	control	4	control	2
20	PAC flowering	3	D	2
20	PAC flowering	4	P	2
21	GA flowering	3	D	2
21	GA flowering	4	P	2
22	PAC before	3	D	3
22	PAC before	4	P	3
23	GA after	3	P	2
23	GA after	4	D	1
24	PAC before	5	D	3
24	PAC before	6	P	2
25	GA flowering	5	D	2
25	GA flowering	6	P	2
26	GA before	3	D	2
26	GA before	4	P	2
27	GA before	5	P	2
27	GA before	6	D	2
28	GA after	5	P	2
28	GA after	6	D	2
29	PAC flowering	5	D	2
29	PAC flowering	6	P	2
30	control	5	control	2
30	control	6	control	2
31	PAC after	5	D	2
31	PAC after	6	P	2
32	GA before	7	D	2
32	GA before	8	P	2
33	GA after	7	P	2
33	GA after	8	D	2
34	PAC before	7	D	2
34	PAC before	8	P	2
35	PAC after	7	D	2
35	PAC after	8	P	2
36	PAC after	9	D	2
36	PAC after	10	P	2
37	control	7	control	2
37	control	8	control	2
38	control	9	control	2
38	control	10	control	2
39	PAC flowering	7	D	0
39	PAC flowering	8	P	2
40	GA flowering	7	D	2
40	GA flowering	8	P	2
41	GA before	9	D	2
41	GA before	10	P	2
42	PAC after	11	D	2
42	PAC after	12	P	2
43	PAC after	13	D	2
43	PAC after	14	P	1
44	PAC flowering	9	D	2
44	PAC flowering	10	P	2
45	PAC after	15	D	2
45	PAC after	16	P	2

Vine No.	Treatment	Shoot	Application	Bunch No.
46	GA after	9	P	1
46	GA after	10	D	2
47	GA before	11	P	2
47	GA before	12	D	2
48	control	11	control	2
48	control	12	control	2
49	PAC before	9	D	2
49	PAC before	10	P	3
50	PAC before	11	P	2
50	PAC before	12	D	2
51	GA after	11	P	1
51	GA after	12	D	2
52	GA flowering	9	D	2
52	GA flowering	10	P	2
53	GA before	13	D	2
53	GA before	14	P	2
54	PAC after	17	D	2
54	PAC after	18	P	2
55	control	13	control	2
55	control	14	control	2
56	GA after	13	P	2
56	GA after	14	D	2
57	PAC flowering	11	D	2
57	PAC flowering	12	P	2
58	GA flowering	11	D	2
58	GA flowering	12	P	2
59	GA flowering	13	D	2
59	GA flowering	14	P	2
60	GA before	15	D	2
60	GA before	16	P	2
61	PAC flowering	13	D	2
61	PAC flowering	14	P	2
62	PAC before	13	D	2
62	PAC before	14	P	2
63	GA after	15	P	2
63	GA after	16	D	2
64	control	15	control	2
64	control	16	control	2
65	PAC flowering	15	D	1
65	PAC flowering	16	P	2
66	GA flowering	15	D	2
66	GA flowering	16	P	3
67	GA after	17	P	2
67	GA after	18	D	2
68	PAC after	19	D	2
68	PAC after	20	P	3
69	GA before	17	D	3
69	GA before	18	P	2
70	PAC before	15	P	2
70	PAC before	16	D	2
71	PAC flowering	17	D	2
71	PAC flowering	18	P	2
72	GA after	19	P	2
72	GA after	20	D	2
73	control	17	control	2
73	control	18	control	2
74	GA flowering	17	D	2
74	GA flowering	18	P	2
75	PAC flowering	19	D	2
75	PAC flowering	20	P	2
76	PAC before	17	P	2
76	PAC before	18	D	2
77	GA before	19	D	2
77	GA before	20	P	2
78	PAC before	19	D	2
78	PAC before	20	P	2
79	control	19	control	2
79	control	20	control	2
80	GA flowering	19	D	2
80	GA flowering	20	P	2

Figure 6.1. Experimental design for the application of Gibberellic Acid (GA), Paclobutrazol (PAC) at three time intervals: before flowering, at flowering and after flowering and untreated vines (control) to vines (cv. Shiraz) at Nuriootpa Research Centre, Barossa Valley, South Australia.

RESULTS

The distribution of PBN along the shoot showed buds at nodes 3-6 had higher levels of PBN than other buds in all treatments, including those untreated. Prior to flowering, GA_3 caused higher levels of PBN in most buds along the shoot compared to the control (Figure 6.2). With the exception of bud 1 and 10, greater than 40% of buds treated with GA_3 along the shoot were necrotic. The highest recorded incidence of PBN was 95%. PBN was markedly reduced at nodes 8-10.

The level of PBN naturally occurring on Shiraz was 42% (control). Exogenous application of GA_3 to buds by the drop and paint method significantly increased the incidence of PBN when applied before flowering (Figure 6.3A and B). At this time, 60% of buds treated with GA_3 were necrotic. Levels of PBN also increased marginally when GA_3 was applied at flowering (47%). Drop application of GA_3 after flowering caused a slight increase in PBN however the paint method of GA_3 did not have an effect at this time.

The growth retardant, paclobutrazol, significantly decreased PBN (27%) when applied by a pipette (drop method) before flowering (Figure 6.3A). When a paintbrush applied GA_3, a slight increase in PBN was observed when applied at the same time (Figure 6.3B). Paclobutrazol did not have an effect when applied at and after flowering. In all experiments, shoots treated with paclobutrazol had lower incidence of PBN than those treated with GA_3. The mean incidence of PBN when data for each method of application was combined indicated that GA_3 increased PBN (Figure 6.2C). Overall, regardless of treatment, the vines assessed had high levels of PBN. PBN levels greater than 20% are deemed unsatisfactory for fruiting potential in the following season.

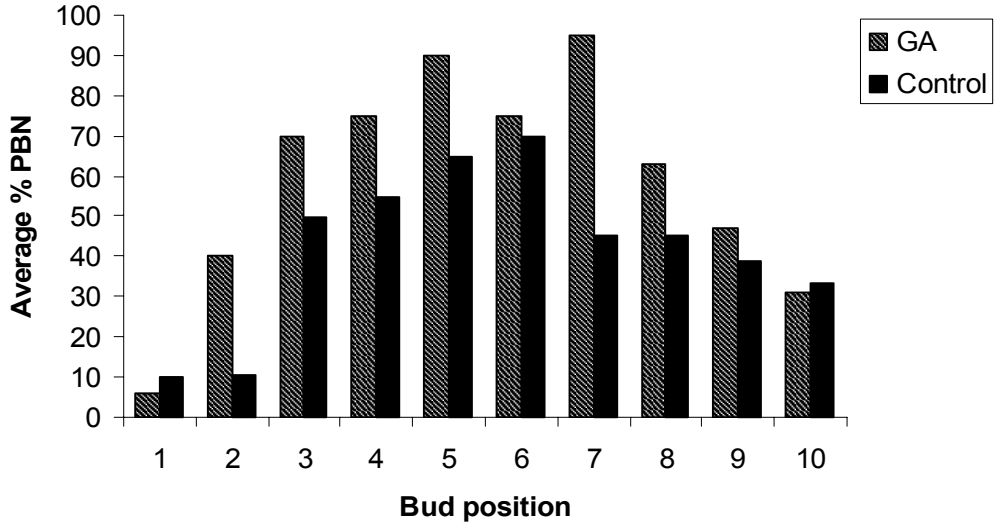


Figure 6.2. Distribution of primary bud necrosis along shoots (cv. Shiraz) following treatment with Gibberellic Acid (GA_3) via drop application and compared with untreated (control) shoots one week prior to flowering at Nuriootpa, South Australia.

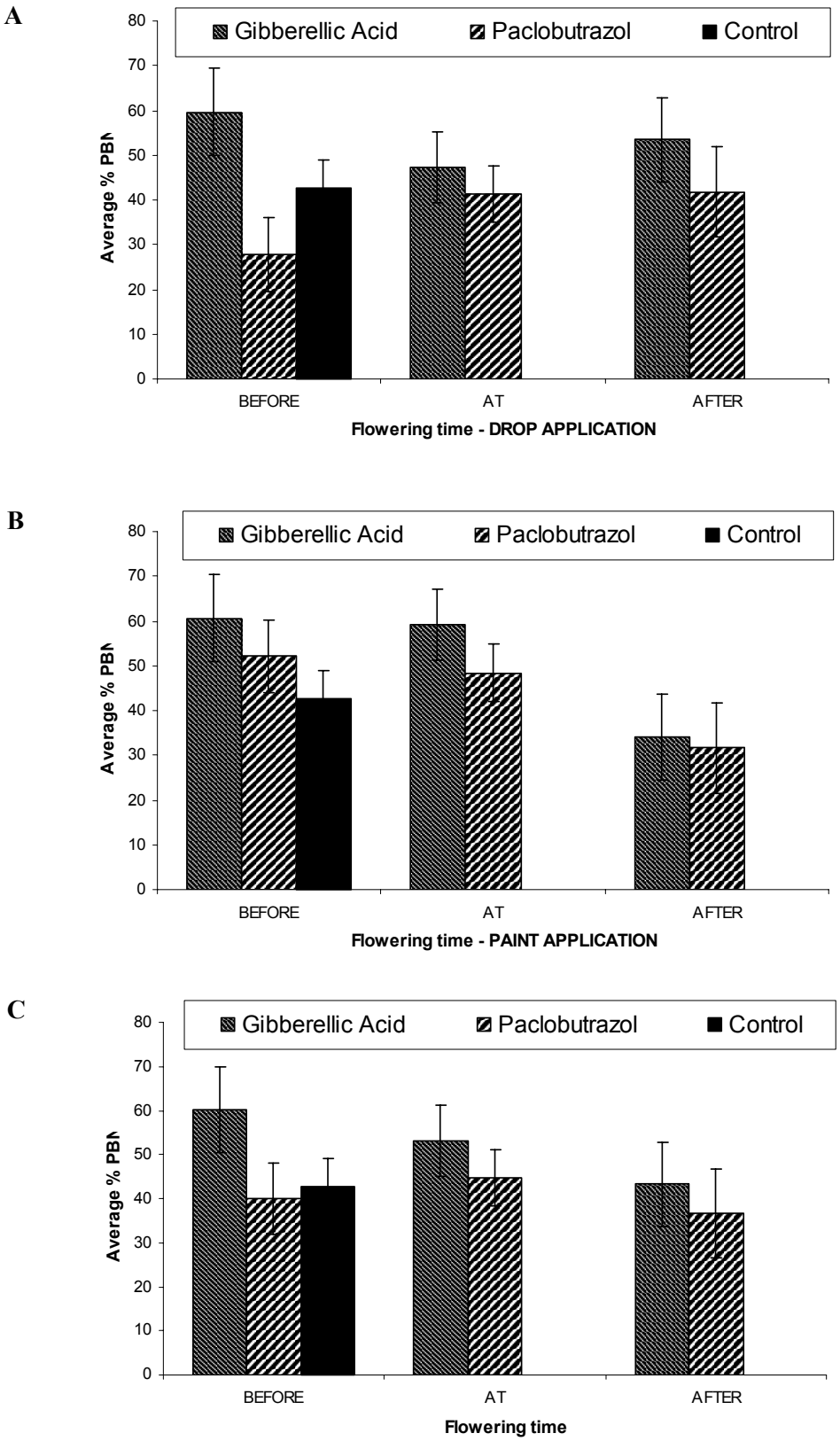


Figure 6.3. Incidence of PBN following application with Gibberellic Acid or paclobutrazol by (A) drop or (B) paint application and (C) combined application methods one week before flowering, at flowering and one week after flowering at Nuriootpa, South Australia.

DISCUSSION

The application of GA_3 increased PBN, with its effect most significant when applied before flowering. Stage of development is crucial in the response of buds to application of GA_3 and paclobutrazol. Flowering is controlled by naturally produced gibberellins (Stephan, 1999). At the time of bud differentiation, GA_3 is at its highest level and is transferred to the new buds. In vigorously growing grapevine cultivars (eg. Shiraz) high levels of GA_3 move to the buds resulting in excessive cell elongation. The imbalance of hormones eventually kills the primary bud. The role of GA_3 in flower initiation may explain why PBN occurs on the onset of flowering.

There is genetic evidence that GA_3 inhibits flowering in grapevine (Boss and Thomas, 2002). GA_3 produced in seeds (such as those in grape berries) can influence the development of uncommitted primordia into tendrils and subsequently inhibit floral development. The inhibition of flowering by GA_3 is normally associated with stimulation of vegetative growth. Natural levels of gibberellins have been shown to suppress flowering in seed plants, such as apple (Unrath and Whitworthm, 1991) and avocado (Salazar-Garcia and Lovatt, 1995). Gibberellins applied exogenously to table grapes can also significantly reduce bud fruitfulness in the following season (Biscay and Badr, 2001).

Different methods of gibberellin application have been used with varying results. In the present study, drop application caused the most significant response. Ziv *et al*., (1981) found that application of gibberellic acid to the leaves of grapevines caused more bud necrosis than when applied directly to the buds through smearing or petiole feeding. However at lower concentrations of GA_3, petiole feeding produced the highest levels of PBN. The direction of translocation of GA_3 will affect the position of necrotic buds along the shoot. GA_3 was applied to every bud on treated shoots and PBN increased progressively along the shoot until bud five. Bud one showed the lowest incidence of PBN compared to other buds and possibly GA_3 from the lower buds moved along the shoot. It is possible that PBN was not high as lower buds did not create a metabolic sink for GA_3 or were already differentiated at the time of treatment.

Paclobutrazol reduced the incidence of PBN in Shiraz. Paclobutrazol inhibits gibberellin biosynthesis. Studies have shown that application of paclobutrazol inhibits vegetative growth leading to reduced shoot extension, internode length and smaller leaf area than untreated plants (Christov *et al.,* 1995; Wolf and Warren, 1995). It is also possible that paclobutrazol causes changes in photosynthetic activity of chloroplasts, resulting in thicker leaves and increased photosynthetic capacity (Christov *et al.,* 1995). In treated trees, morphological modification of leaves may have greater tolerance to environmental stresses and resistance to fungal disease infections (Chaney, 2003). With further research, use of growth retardants in vigorously growing cultivars may be an effective means of controlling PBN.

7. Anatomical development of Primary Bud Necrosis

INTRODUCTION

Primary bud necrosis is characterised by an abortion and subsequent drying of the primary bud within a developing compound bud. The extent and location of necrosis in the primary bud is dependent on stage of bud development. Sections taken of Riesling buds under a light microscope revealed zones of distorted misshapen cells immediately beneath the primary bud axis within 60 days after budbreak. Ninety days after budbreak, cell compression and cell lysis occurred (Vasudevan *et al*., 1998a). Morrison and Iodi (1990) found that necrosis occurred at the base of the primary axis and in other buds, only apical nodes of the primary axis died. In young undifferentiated buds, necrosis developed below the apex causing death of the primary bud (Ziv *et al*., (1981). Although there are conflicting reports regarding the locality of tissue death within the primary bud, scanning electron microscopy has shown that cell destruction was not a result of tissue preparation or microtomy (Vasudevan *et al*., 1998a).

This study aimed to investigate different severity of PBN leading to further insight on the progression of this physiological disorder.

MATERIALS AND METHODS

Grapevine buds from 8-year-old Shiraz vines in a vineyard located at Charleston, South Australia were collected during March 2004. Healthy and necrotic primary buds were removed from shoots in the laboratory using a dissecting microscope. Thirty buds were examined prior to embedding and scored using a severity rating of PBN (Table 7.1)

Table 7.1 Severity ratings for degree of Primary Bud Necrosis (PBN)

Severity Rating	Level of Primary Bud Necrosis (%)
0	0 (healthy primary bud)
1	1-25
2	25-49
3	50-75
4	76-99
5	100 (completely necrotic)

Light Microscopy (LM)

Bud samples were fixed overnight in 3 % glutaraldehyde in 0.025M phosphate buffer, pH 7.2, for a minimum of 48h at 0-4ºC. After fixation samples were put through an alcohol dehydrations series: methoxy-ethanol, ethanol, propanol and butanol. Samples were left for a minimum of 2 h in each alcohol, then infiltrated overnight in a 1:1 mixture of butanol: glycol methacrylate (GMA). Over 4 days samples were then infiltrated with two changes of 100% GMA. Bud sections were then embedded in GMA in gelatine capsules and polymerised at 60ºC.

Embedded buds were trimmed and filed to expose the longitudinal sections (LS) for different PBN severity ratings. Sections 3-4 µm thick were made with an ultra-microtome (Reichert-Jung 2050 supercut) and stained with periodic acid-Schiff's reagent (PAS) and 0.5 % Toluidine blue O (TBO) in 50 mM sodium acetate, pH 4.5 (O'Brien and McCully, 1981). Sections were mounted using microscope slide media (Surgipath, Sub-X mounting medium) and examined using an Olympus BH2 light microscope and micrographs taken using a Nikon TE300 inverted Microscope at magnifications from 4X to 40X. Anatomical components were identified from Esau, (1953) and J. Conran (pers. comm.).

RESULTS

Due to visual similarities in the grapevine buds assessed and scored as severity 1 and 2 and also for severity 4 and 5 these ratings have been grouped together in the results and three levels of PBN recorded: early, medial and late development.

Healthy primary bud cellular structure

Healthy primary and secondary buds show little cell breakdown (Figure 7.1). Microscopic examination of healthy primary buds revealed dividing parenchyma cells which form the shoot apex (Figure 7.2). This area of the bud is known as the apical meristem meaning an apical zone of undifferentiated cells that divide in an organised manner. The apical meristem produces leaf, tendril and inflorescence primordia (Figure 7.2 A (lp, tp, ip)). The leaf primordia contain storage cells with starch granules. During leaf development, these starch granules are replaced with chloroplasts when photosynthesis commences. Individual cells within the leaf meristem produce raphides that are long, slender, needle like crystals of calcium oxalate (Figure 7.3 B,C (r)). The raphides are regularly arranged in bundles along the leaf meristem and act as a feeding deterrent by predators. To protect the bud overlapping scales and hairs are formed (Figure 7.2 A (h)).

Healthy primary buds displayed slight cell degeneration on the outer most leaves. Some degeneration of the cells at the tips was also observed at the second and third leaf layer (Figure 7.2 A (ol)). These cells collapsed as shown by cell walls buckling and folding leading to breakdown of the cellular contents (Figure 7.2 C (de,ep)). Damage was confined to the outer epidermal cell layers

(Figure 7.2C (e)). The outer leaf cell damage was typical of a physiological reaction to stress caused by water loss and dehydration and did not appear to be related to PBN.

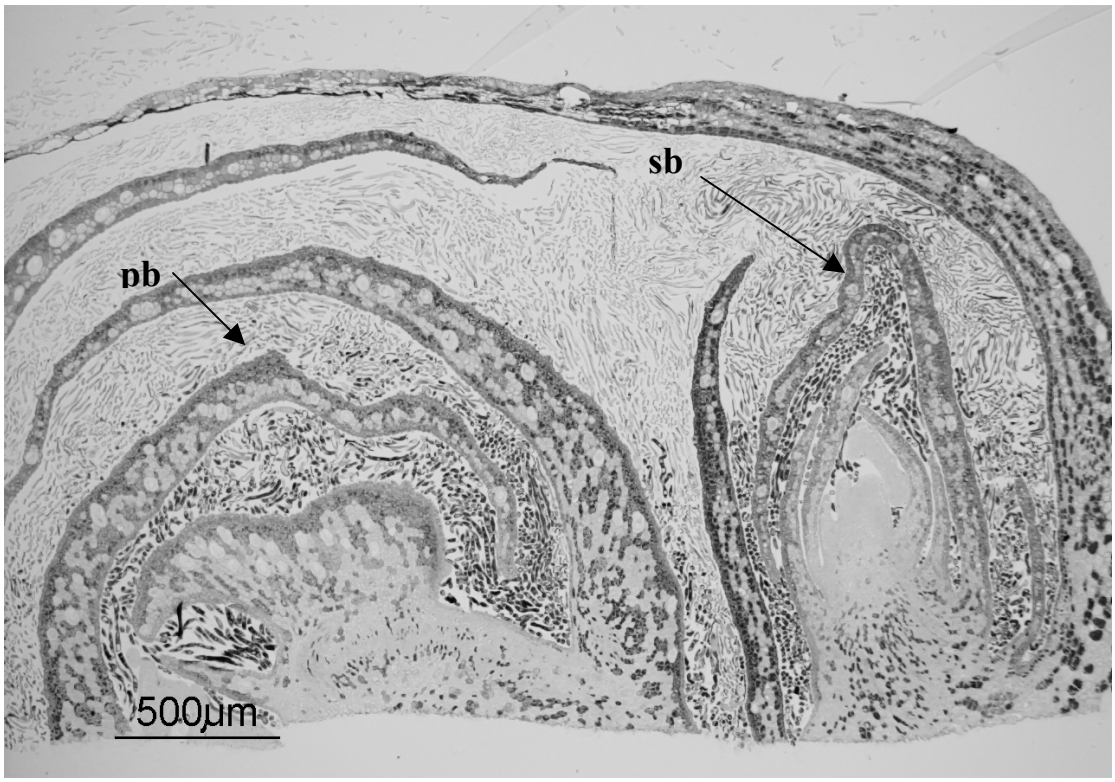


Figure 7.1. Longitudinal section of a 'healthy' primary (pb) and secondary grapevine buds (sb).

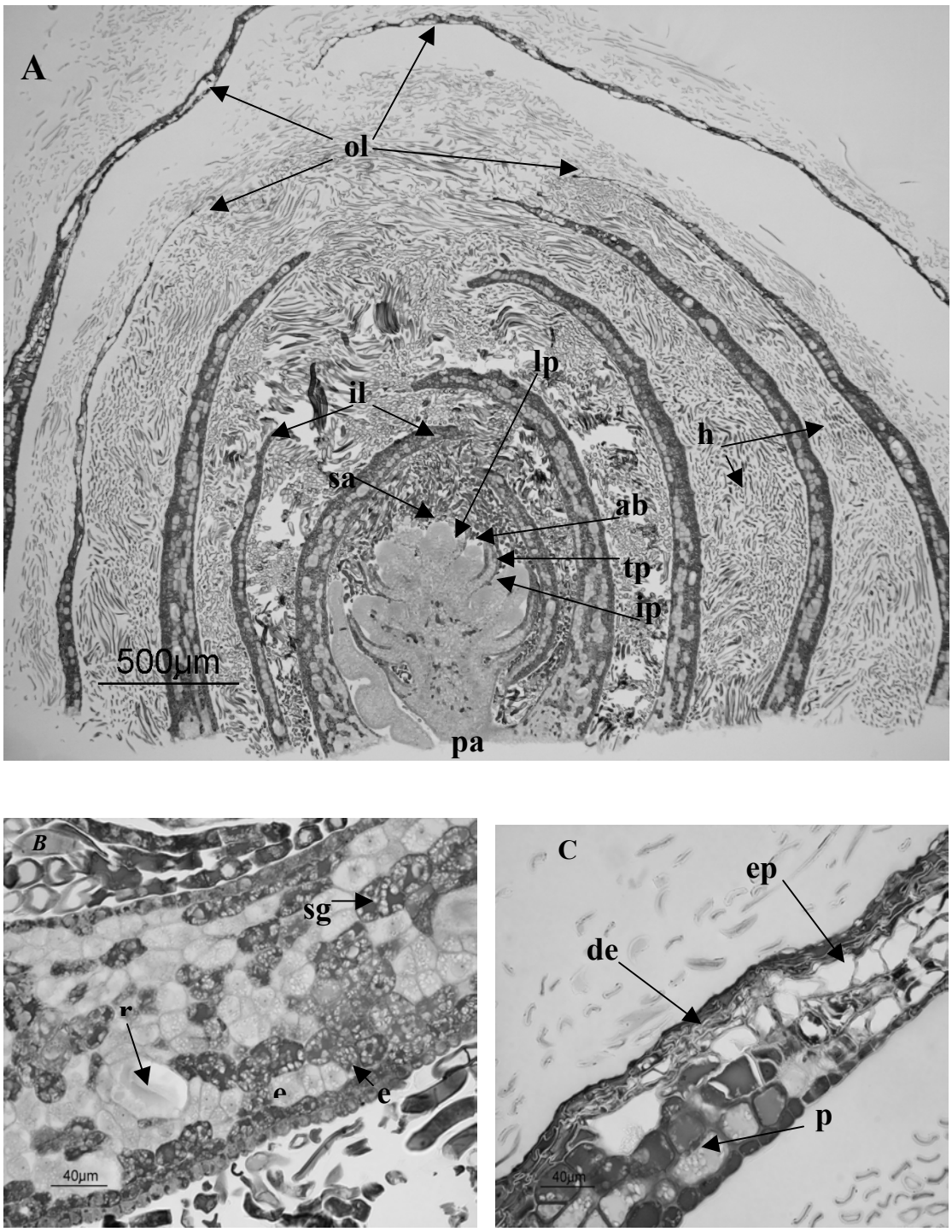


Figure 7.2. (A). Longitudinal section of a 'healthy' primary grapevine bud, Shiraz (*Vitis vinifera*), shoot apex (sa), apical meristem (ap), axillary bud (ab), leaf primordia (lp), inflorescence primordia (ip), outer leaves with some cell degeneration (ol), bud hairs (h). (B). Inner leaf with storage cells containing starch granules (sg), single cell containing a raphide (r), epidermal cell layer (e). (C). Outer leaf near apex with cellular damage caused by dehydration, distorted epithelial cells (de), empty parenchyma cells with thickened walls (ep), parenchyma cell with starch granules (p).

Early development of PBN

There were small differences between healthy buds and those with PBN in severity ratings 0-2. were observed with 0-49% PBN severity compared to healthy buds. Cellular breakdown and loss of cell contents occurred towards the apex of the first, second and third leaf primordia. Breakdown of the epidermis was also observed near the leaf apex in the second and third primordial cellular layers (Figure 7.3 A). This resulted in the production of empty spaces, termed lacunae (Figure 7.3 A (ol, il, l)). These early stages pf PBN appear to be random in their point of origin within the bud.

Medial development of PBN

Damage occurred in the primordia cells however the meristem was still intact with only little cellular damage. Below the meristem, in an area where vascular tissue is developing, multiple layers of collapsed parenchymatous cells occurred. Associated with this region were several layers of cells with thickened walls. Collenchyma cells have non-staining walls and dense staining cytoplasm (Figure 7.4). The nearby parenchymatous cells also have cellular abnormalities with irregular and distorted cell walls, many being smaller than the surrounding cells. They are also associated with a poorly developed epidermal cells. The meristem cells that should be differentiating into the vascular system also show cellular compaction and fewer organisations than those of healthy primary bud sections. Normally this cellular region would differentiate into healthy xylem and phloem parenchyma cells. Instead large luculentus cells formed without cytoplasm in the places where parenchyma cells would have occurred.

Advanced development of PBN

Extensive damage to leaf primordia cells and other cellular disintegration occurred in the leaf mesophyll. Due to cell wall breakdown, rhaphides were deposited between two or more cells instead of one (Figure 7.5). In severely damaged buds (severity rating 5), total breakdown of the leaf structure occurred with breakdown in the leaf mesophyll region and the formation of oil droplets in the epidermal layer. Damage was also recorded in the shoot apex, however the severity varied between rating 5 and rating 4 with some necrotic buds in both severity levels containing healthy cells. This indicated that although the primary bud was visually dead, complete cell breakdown had not fully occurred.

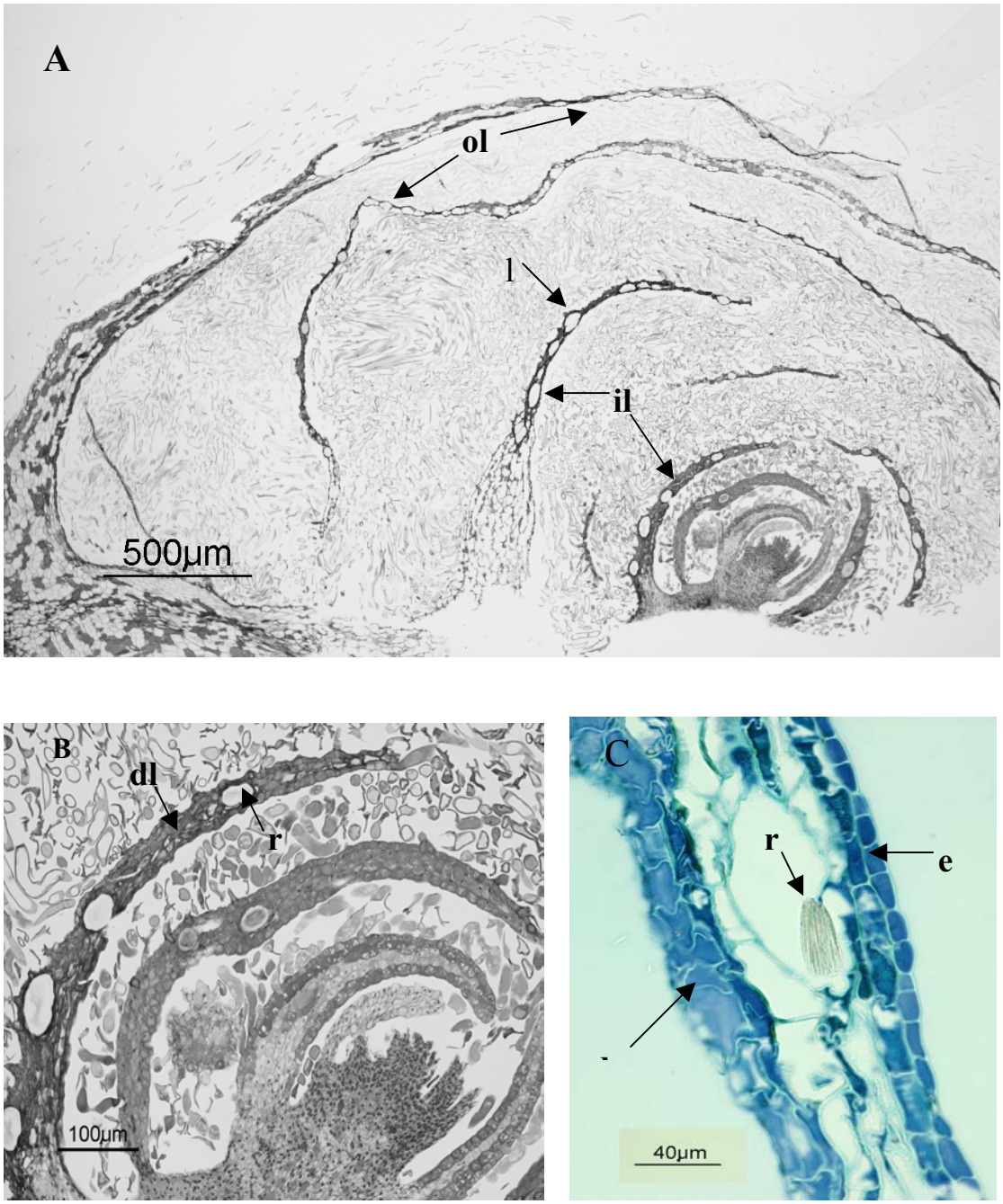


Figure 7.3. (A). Early development of PBN in Shiraz (*Vitis vinifera*). Outer leaves with apical mesophyll damage (ol) and formation of lacunae (l), inner leaf with apical mesophyll damage and formation of lacunae (il). (B). Early stages of leaf primordia raphide cells (r) in damaged leaf mesophyll (dl). (C). Leaf primordia epidermal cells (e), damaged epidermal cells (de), raphide between two cells (r).

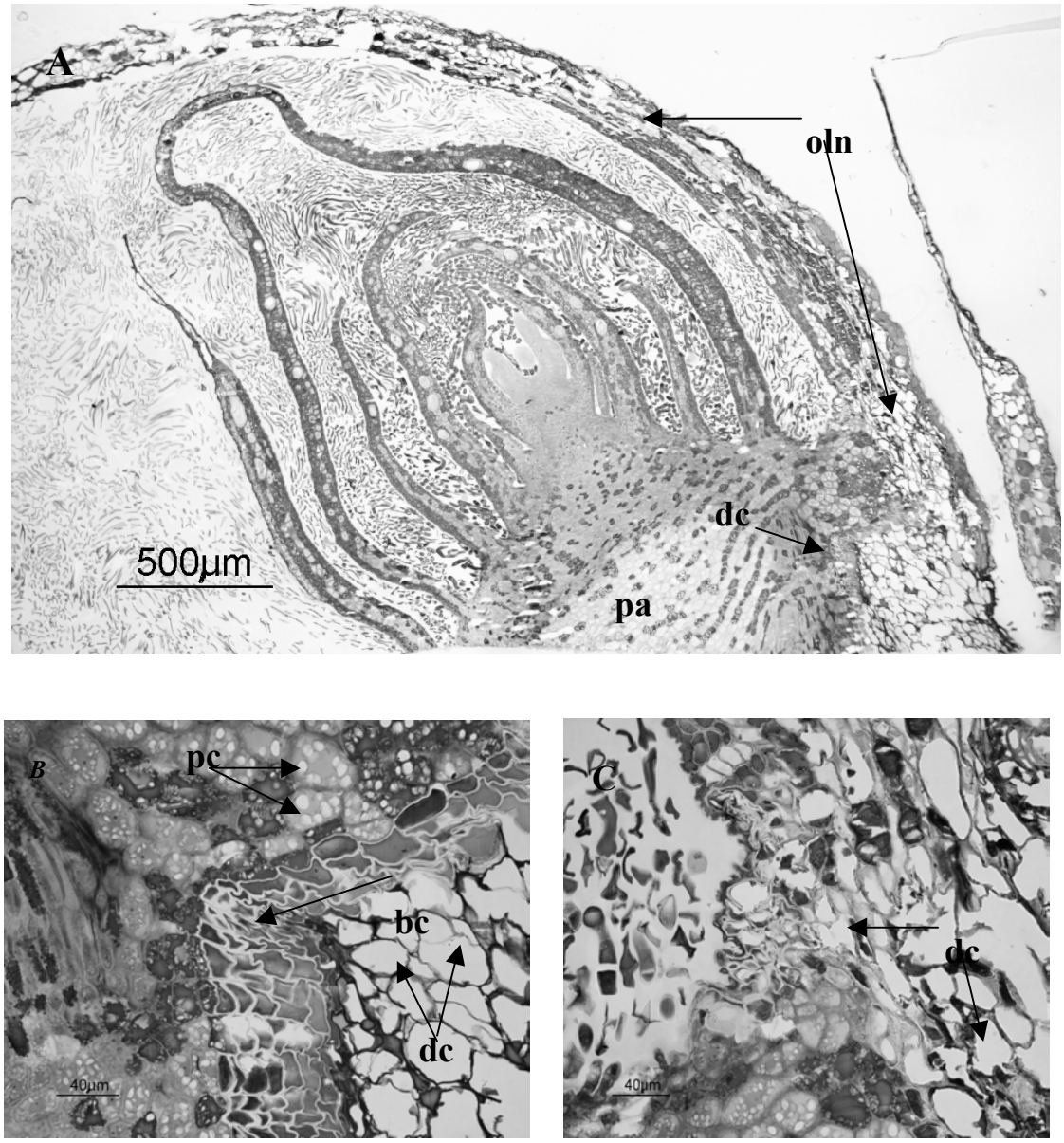


Figure 7.4. (A) Longitudinal section of a primary bud with outer leaf necrosis (oln) and formation of a distorted cellular zone (dc) to the right of the primary bud axis (pa). (B). Healthy parenchyma cells with starch granules (pc), three to five buckled cell layer (bc) and deformed cells (dc). (C). Deformed cells (dc) to the right of the shoot apex.

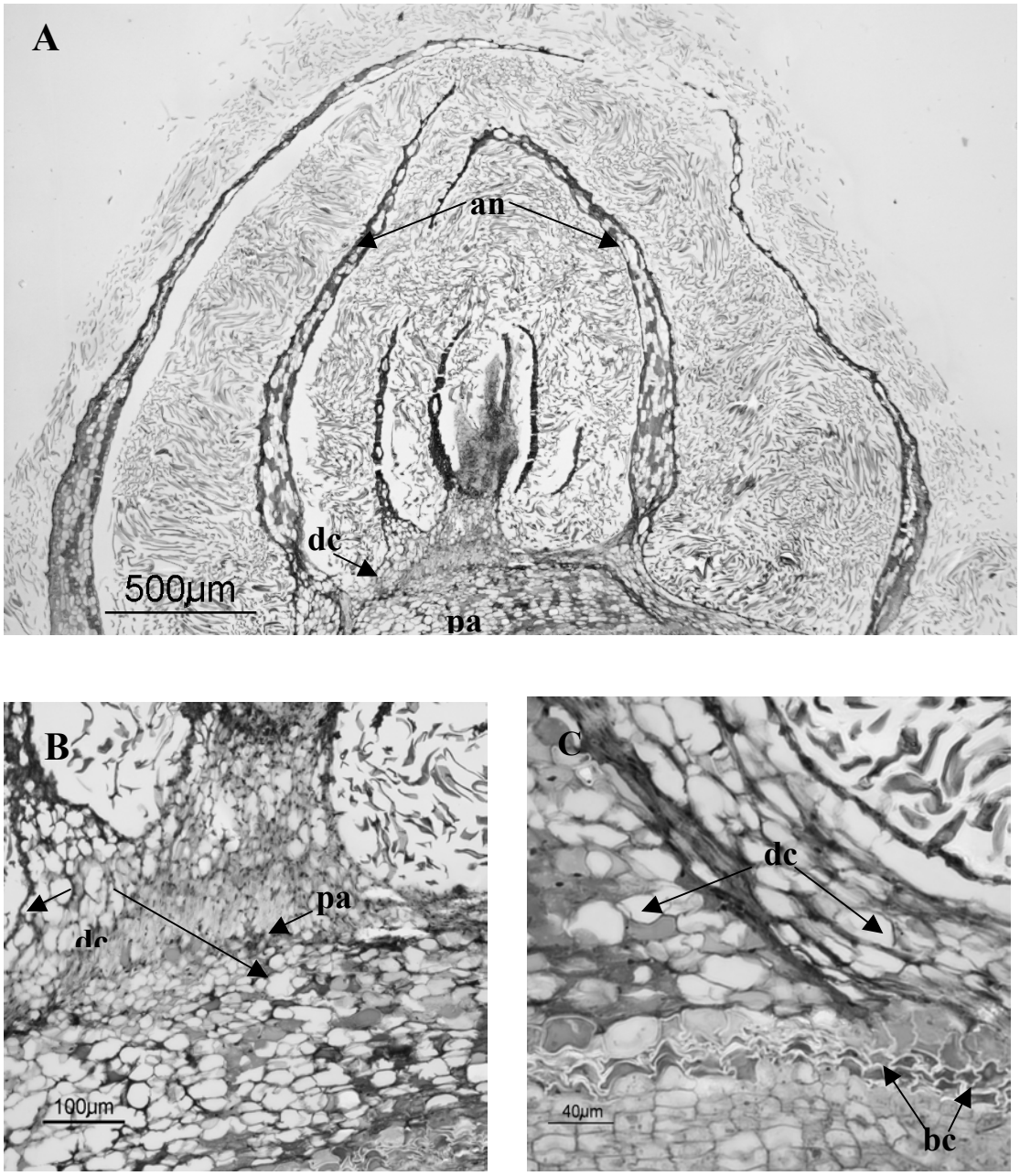


Figure 7.5. (A) Longitudinal section of primary bud, with advanced necrosis of inner leaves (an), and a damaged cell zone (dc) near the primary axis (pa). (B). Damaged cell zone near the primary axis (dc), (pa). (C). Distorted cell layers (dc) and buckled cells below (bc).

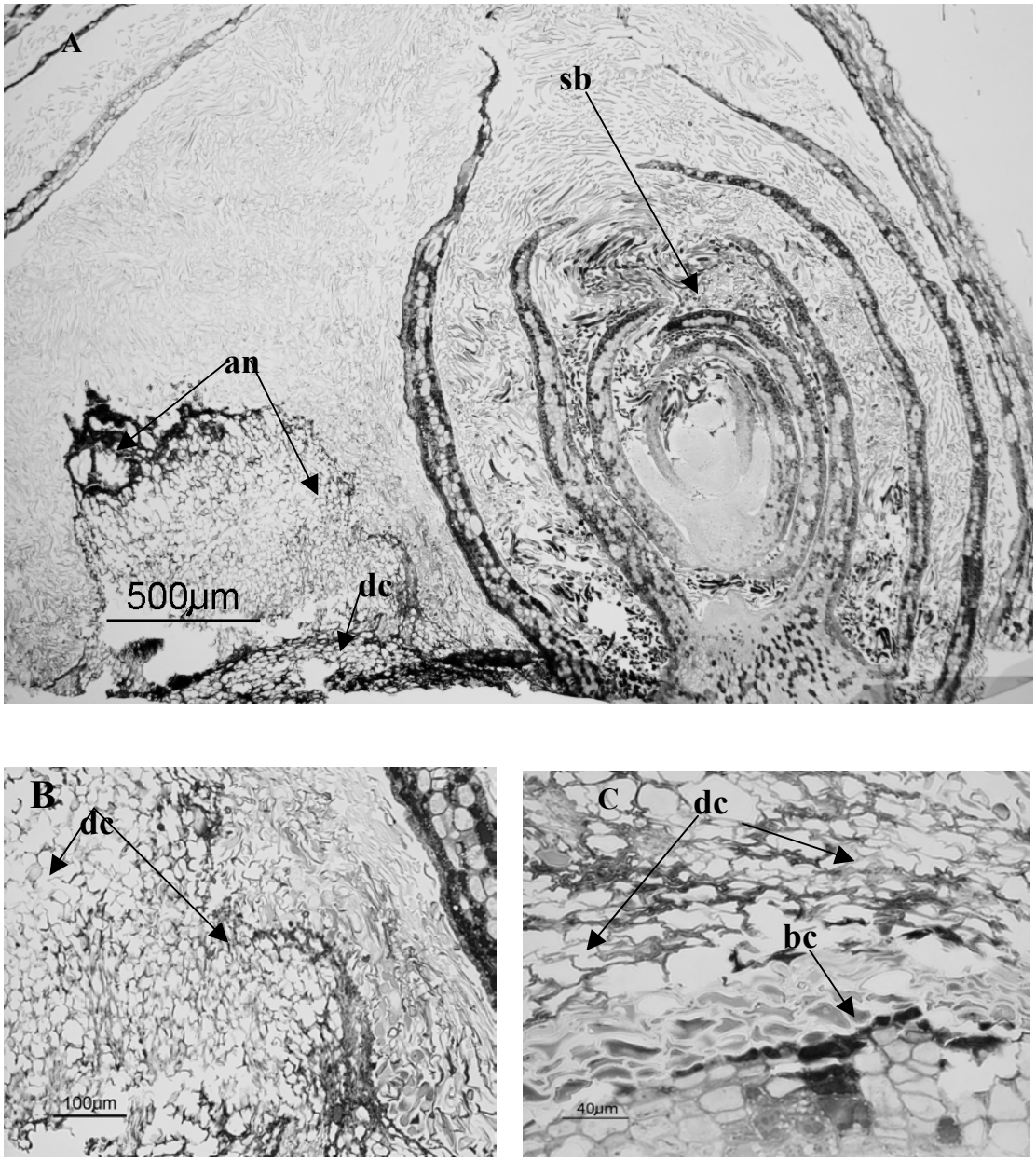


Figure 7.6. (A). Advanced cell necrosis of the primary bud on left (an) with distorted cellular layer below (dc) and healthy secondary bud on right (sb). (B) and (C). Distorted cells remaining in the primary bud (dc) and buckled cell layer below (bc).

DISCUSSION

Similar to the observation by Vasudevan *et al*. (1998a), the first visible symptom of PBN was indicated by the presence of distorted and compressed cells with irregular cell walls. Compressed cells lacked structural integrity and were subject to external pressures, such as the expansion of adjacent cells. There were however some differences in the location of PBN. Vasudevan *et al*. (1998a) found that the zone of compressed cells began at the base of the primary bud and advanced to the leaf primordia. Our observations indicate that PBN can start in the zone of leaf primordia in some primary buds, not just at the base. Morrison and Iodi (1990) also observed the random distribution of PBN in the early stages of development. PBN commenced similarly to other reports (Morrison and Iodi, 1990; Vasudevan *et al*., 1998a) as groups of cells with distorted cell walls, followed by compression of cell walls that subsequently lead to cell breakage.

In Thompson Seedless, PBN is characterised by the formation of a distinct necrotic zone most commonly located at the fourth level of the fourth leaf primordia (Perez and Kliewer, 1990). The formation of necrotic cells in the primary bud caused a rupture or separation between the basal part of the bud and the apex, resulting in death of the primary bud. In this study, similar observations were made, with PBN in severity rating 4 observed at the same time cells in the primordia differentiating into xylem or phloem parenchyma (J. Conran, pers. comm.). PBN stopped further primordial growth, so that cells matured more rapidly without forming whole leaves. This entire cell region of the primary bud then breaks down and, if severe, necrosis extended into the secondary buds. Determination of severity of PBN with a dissection microscope was not as accurate as light microscopy. Buds initially categorised in severity rating 5 appeared completely dead, however upon further examination, it was evident that cells in the lower primary shoot apex were intact. So externally (leaf primordia and surrounding tissue) cells appeared necrotic, but internally (apical meristem), cells were intact.

The shoot apical meristem goes through “maximal” and “minimal” phases between the initiation of one leaf and the initiation of the next. Axillary buds are under the control of the shoot apex, and their further development is usually suppressed by hormonal control. If the shoot apex is damaged a secondary bud may form due to the lack of hormonal suppression.

Further knowledge on the role of hormone levels during this period of development would be valuable in determining in better understanding how and why this disorder occurs.

8. Pruning and Irrigation Management

INTRODUCTION

Bud dissections can provide information on bud fruitfulness and the incidence of necrotic buds. In vineyards with high levels of PBN, it is generally recommended that more buds be retained at pruning to compensate for predicted crop loss. The pruning level (nodes per vine) is determined predominantly by the desired yield target and fruitfulness, but PBN can have a significant influence on yield potential and therefore pruning levels must be adjusted accordingly. In addition, modifying pruning levels can assist with control of vigour, canopy density and regulate crop load. A general recommendation is to retain 30–40 nodes per kg pruning weight for Australian conditions (Tassie and Freeman, 2001) but more buds may need to be retained if PBN is high. Little research has been undertaken to validate the manipulation of pruning levels to minimise PBN in the following season.

The development of inflorescence primordia is sensitive to water stress. In this study we have shown that PBN commences at the onset of flowering, therefore environmental constraints at this time may influence the incidence of PBN. Irrigation maintains an adequate water supply to the vine. Various irrigation techniques are employed in Australia with greater than 71% of vineyards irrigated by drip or micro-spray (ABS 1329.0, 2004). Although standard drip irrigation is most common, other methods have been designed and implemented to minimise water usage. Partial Rootzone Drying (PRD) is a drip irrigation technique used to increase water use efficiency. One side of the vine is wetted while the other remains dry, causing the stimulation of the abscisic acid (ABA). ABA signals leaf stomata to partially close, resulting in decreased water loss from the vine. PRD can reduce vigour, but the affect on bud differentiation and PBN is relatively unknown.

This aim of this trial was to assess the differences between various pruning levels for managing PBN at a number of sites and to determine if irrigation influences the development of PBN.

MATERIALS AND METHODS

To assess the influence of pruning level on the incidence of PBN in Shiraz, three sites were established in three viticultural regions in 2003. Vineyards were located at McLaren Vale, Southern Fleurieu and Nuriootpa, Barossa Valley.

Pruning trial design

McLaren Vale and Southern Fleurieu vineyard pruning trials were designed as randomised complete block designs. Three pruning treatments were applied to McLaren Vale (30, 60 and 90 nodes per vine), while four treatments were applied to Southern Fleurieu (30, 60, 90 and 120 nodes per vine). The site design at Nuriootpa was a split-plot design with two irrigation treatments:

A

B

C

Figure 8.1. Pruning levels applied to vines at Nuriootpa (cv. Shiraz). (A) 30 nodes per vine, (B) 60 nodes per vine and (C) 120 nodes per vine.

Partial Rootzone Drying (PRD) and standard drip randomised to whole plots within each five blocks. Irrigation blocks were split into three sub-plots to which a pruning treatment (30, 60 and 120 nodes per vine) was then randomly assigned. The initial trial was established in 2001. To provide standardised pruning levels between the sites, the pruning levels were obtained by retaining:

15 x 2-node spurs (30 nodes/vine),

30 x 2-node spurs (60 nodes/vine) and

30 x 2-node spurs + 3 x 10 node canes (90 nodes/vine) where applicable.

At Nuriootpa, 120 nodes/vine were pruned according to: 30 x 2-node spurs + 15 x 4 node spurs (Figure 8.1). At Southern Fleurieu, 120 nodes/vine were pruned according to: 30 x 2-node spurs + 6 x 10 node canes.

The measurements recorded during the growing season included: bunches/vine, bunch weight/vine, shoots/vine (budburst percentage), shoot length per treatment and shoot diameter. Forty shoots per treatment were selected for analysis of PBN and fruitfulness up to bud position 10 along the shoot. PBN and fruitfulness were measured within a bud along a shoot on a vine, whereas bunch number was an aggregate measure for a vine. Pruning weights (kg per vine) were recorded for each pruning level at the time of pruning (June) in the following season.

The data was analysed using a standard generalised linear model (GLM) with the logit link function. Fruitfulness consisted of the number of inflorescence primordia in the primary bud and where PBN was observed, from the secondary buds. Both PBN and fruitfulness tested the interaction between pruning treatments, bud position and irrigation (at Nuriootpa only) using deviance tests. Bunch number and weight was analysed by ANOVA assuming a normal distribution with constant variance.

RESULTS

McLaren Vale vineyard

Budburst percentage was 144%, 105% and 101% for 30, 60 and 90 nodes per vine respectively. This indicated that every bud retained produced a shoot and following severe pruning, multiple and water shoots were common. Shoots were also more vigorous and significantly longer on vines pruned to 30 nodes per vine than other treatments (Figure 8.2).

The overall mean fruitfulness was 1.4 inflorescence primordia per bud. The most significant influence on fruitfulness was bud position along the shoot. There was no significant interaction between pruning rate and fruitfulness. When comparing fruitfulness between the bud positions, it was shown that buds 6, 7 and 8 were significantly less fruitful than buds 2, 3 and 4 (Figure 8.3).

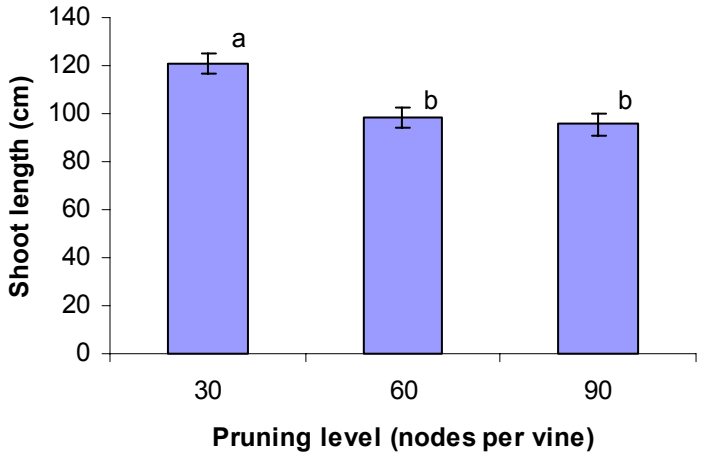


Figure 8.2. Effect of pruning level on shoot length at McLaren Vale, South Australia. Bars represent standard error.

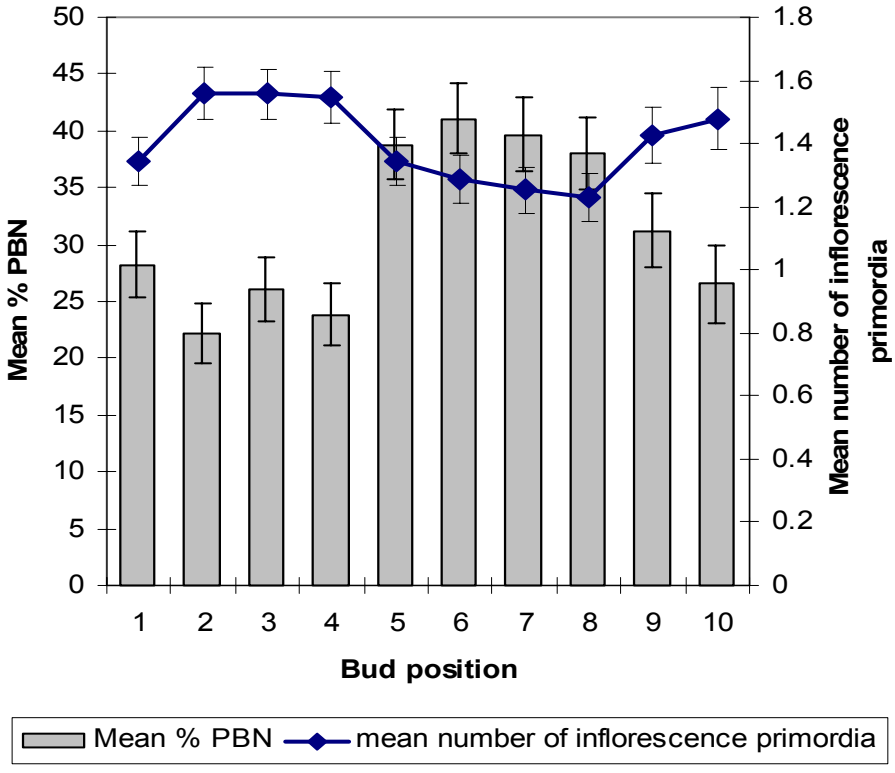


Figure 8.3. Overall mean percentage of primary bud necrosis (PBN) and bud fruitfulness at different bud positions assessed June 2004 at McLaren Vale, South Australia. Bars represent standard error.

Bud 8 was also significantly less fruitful than bud 10. Fruitfulness at buds 1, 5 and 9 did not differ from other buds.

Bud position had a significant effect on the incidence of PBN. It appeared the number of necrotic buds correlated with fruitfulness, whereby high PBN resulted in lower inflorescence primordia in the bud. The incidence of PBN varied greatly between bud positions. Buds 5, 6, 7 and 8 had significantly higher PBN than buds at position 1, 2, 3, 4 and 10 along the shoot (Figure 8.3). Bud 9 (31%) was only different from bud 2 (22%) and bud 6 (41%). Overall, 31% of all buds assessed were necrotic.

The incidence of PBN varied significantly between pruning levels. There was no interaction between bud position and number of nodes retained per vine. Pruning levels were significantly different than one another, with 30 nodes per vine causing the highest incidence of PBN at 44% (Figure 8.4). Retaining more buds per vine reduced the level of PBN in the vineyard.

Pruning level also significantly influenced bunch number ($P<0.001$), hence yield potential of the vine. Comparisons of pruning levels showed that less bunches were harvested from 30 nodes per vine (Figure 8.5). As the number of nodes retained on the vine increased, the mean bunch number significantly increased. Pruning levels of 30 nodes per vine resulted in higher PBN and less bunches.

Bunch weight (kg per vine) also significantly varied between treatments, whereby lower bunch weight was recorded for 30 nodes per vine. The highest yield per vine was recorded for 90 nodes per vine, where more bunches were produced. Individual bunches weighed an average 81g, 69g and 58g for the pruning levels 30, 60, 90 nodes per vine, respectively. So although heavily pruned vines resulted in a yield reduction, individual bunches weighed more than other pruning levels.

Pruning weights were not significantly different between the treatments. The average pruning weight per vine was 1 kg for the three pruning levels. Yield (kg/vine)/pruning weight ratio was 4.5, 6.7 and 7.8 for 30, 60 and 90 nodes per vine, respectively. A value between 5-10 indicates a balance between fruit production and vegetative growth.

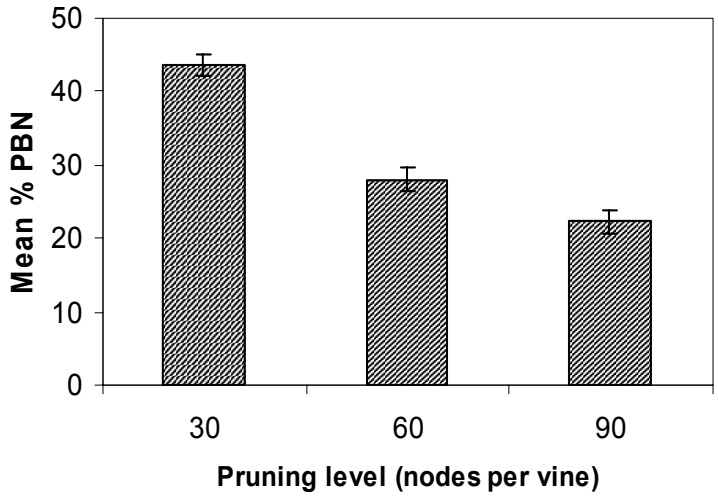


Figure 8.4. The effect of pruning level on the incidence of primary bud necrosis (PBN) assessed June 2004 at McLaren Vale, South Australia. Bars represent standard error.

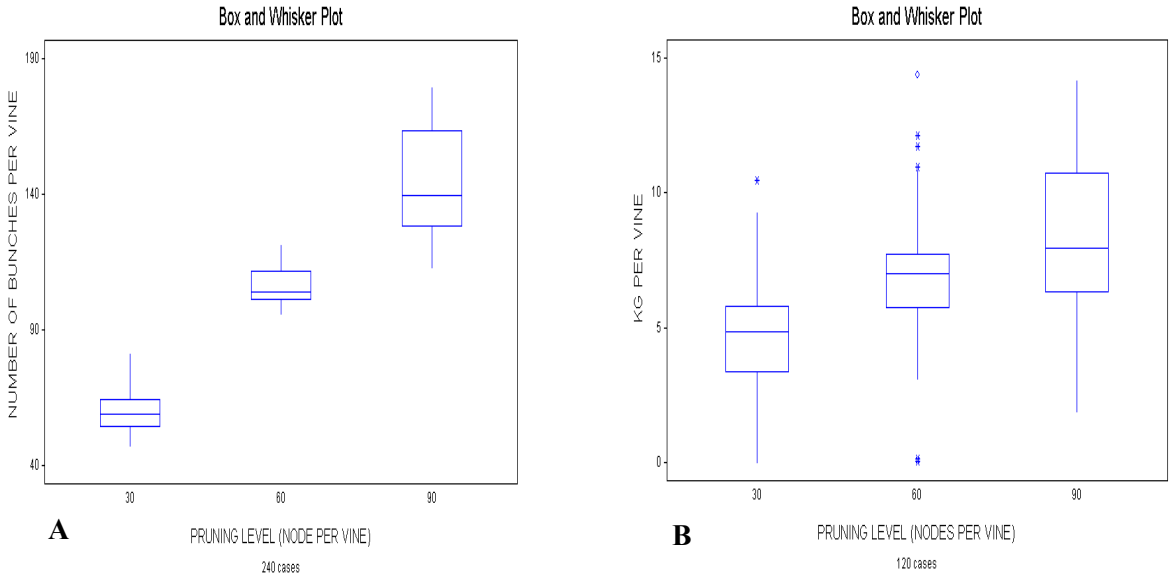


Figure 8.5. The effect of pruning level on (A) bunch number per vine and (B) bunch weight per vine. Assessed June 2004 at McLaren Vale, South Australia.

Southern Fleurieu vineyard

Vines pruned to 30, 60 and 90 nodes per vine had greater or equal to all count nodes producing shoots with 135%, 106% and 100% budburst, respectively. High budburst (97%) was also obtained for 120 nodes per vine. ANOVA showed there was no significant difference between lengths of shoots that developed on the four pruning levels.

Although it appeared buds were more fruitful from vines with more nodes per vine, there was no significant association between fruitfulness and pruning level. Unlike fruitfulness at the McLaren Vale vineyard, there was no significant difference in number of inflorescence primordia along the shoot (Figure 8.6). The coverall mean fruitfulness at Southern Fleurieu was 1.58 inflorescence primordia per bud, which is perceived as high.

However, there was a significant difference between PBN and bud position (P<0.001). Buds 9 and 10 had less necrotic buds than all other buds (Figure 8.6). Buds 2, 7 and 8 had lower levels of PBN than buds 3,4,5. The highest incidence of PBN was observed at bud 3.

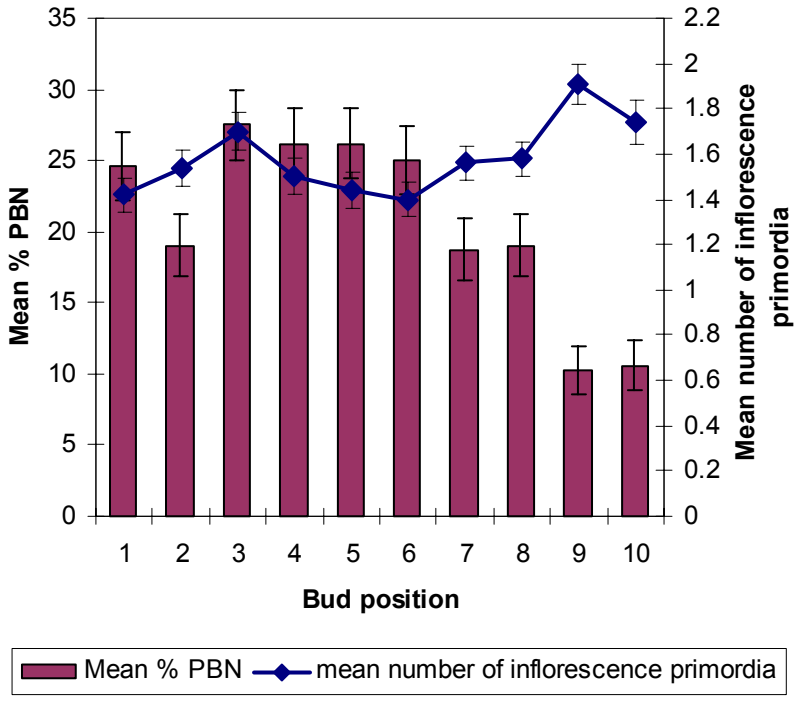


Figure 8.6. Overall mean percentage of primary bud necrosis (PBN) and bud fruitfulness at different bud positions assessed June 2004 at Southern Fleurieu, South Australia. Bars represent standard error.

Pruning level influenced the incidence of PBN, whereby 30 nodes per vine had significantly higher levels of PBN than any other pruning level (Figure 8.7) Additionally, 60 nodes per vine caused higher levels of PBN than 90 and 120 nodes per vine. Bunch number was also significantly affected by pruning level, with 120 nodes per vine having the highest number of bunches. Bunch weight (kg/vine) was significantly less on vines pruned to 30 nodes.

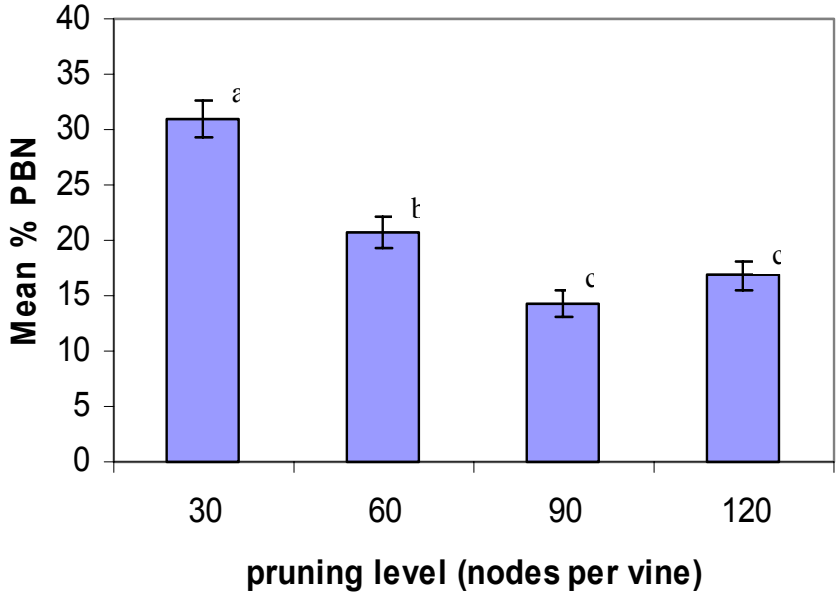


Figure 8.7. Mean primary bud necrosis (PBN) for different pruning levels at Southern Fleurieu, SA. Bars represent standard error. Means with same letter are not significantly different.

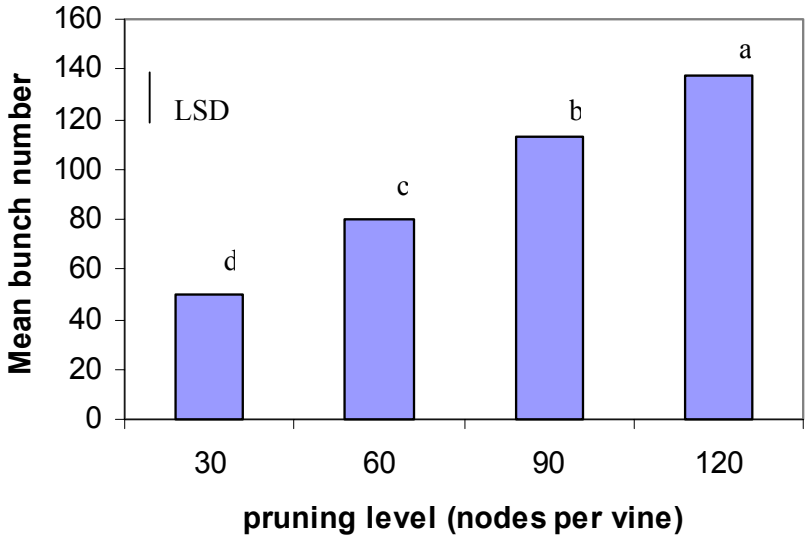


Figure 8.8. Mean bunch number for different pruning levels at Southern Fleurieu, SA. Means with same letter are not significantly different.

Nuriootpa, Barossa Valley vineyard

In all treatments, budburst percentage was exceptionally high. Shoot numbers were significantly greater than number of buds retained. For example, pruning to 30 nodes per vine resulted in an average 98 shoots per vine (325% budburst). Budburst for pruning levels 60 nodes and 120 nodes per vine was 250% and 165%, respectively. There was no significant difference in budburst nor in shoot length between irrigation treatments, however shoot length varied significantly between pruning levels. Shoot length was greater at 30 nodes per vine than other pruning level and shoots were longer on 60 nodes per vine than 120 nodes per vine. There was no interaction between pruning level and irrigation.

Fruitfulness was assessed according to interactions between bud position, pruning level (nodes per vine) and irrigation strategy. The only significant effects on fruitfulness were bud position and pruning level. Irrigation did not have an effect. There was a non-linear incline in fruitfulness along the shoots on average. Bud 1 showed the poorest bud fruitfulness. Buds 9 and 10 were more fruitful than buds 1-6 (Figure 8.9). The overall mean fruitfulness was inflorescence primordia 1.32 bunches per bud. Pruning vines to 30 nodes per vine significantly decreased fruitfulness compared to 60 and 120 nodes per vine, but there was no difference between the latter pruning levels (Figure 8.10). Although pruning to 30 nodes per vine resulted in excessive vigour, number of inflorescence primordia in buds was reduced. There was no interaction between bud position and pruning level.

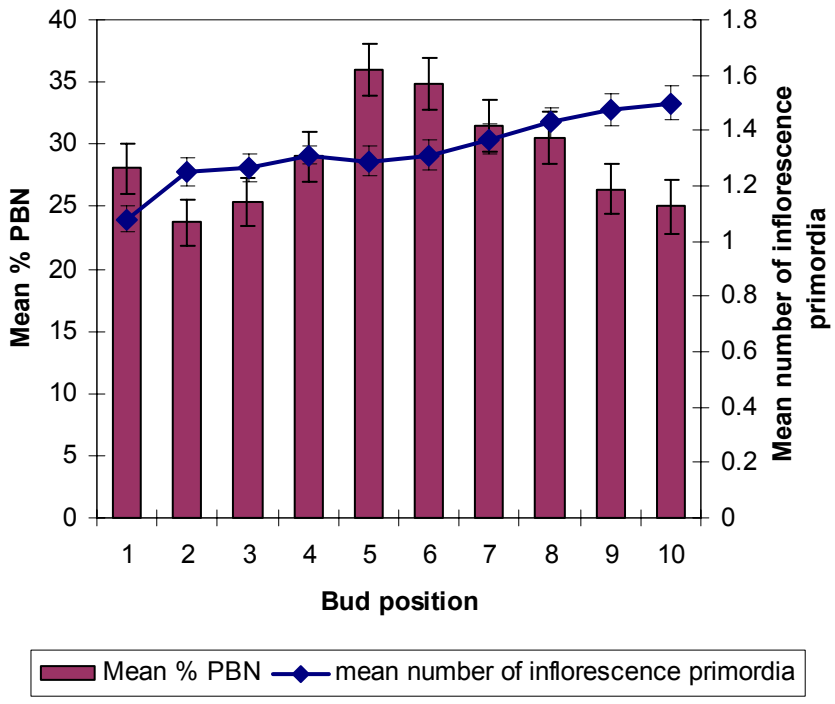


Figure 8.9. Overall mean percentage of primary bud necrosis (PBN) and bud fruitfulness at different bud positions assessed June 2004 at Nuriootpa, South Australia. Bars represent standard error.

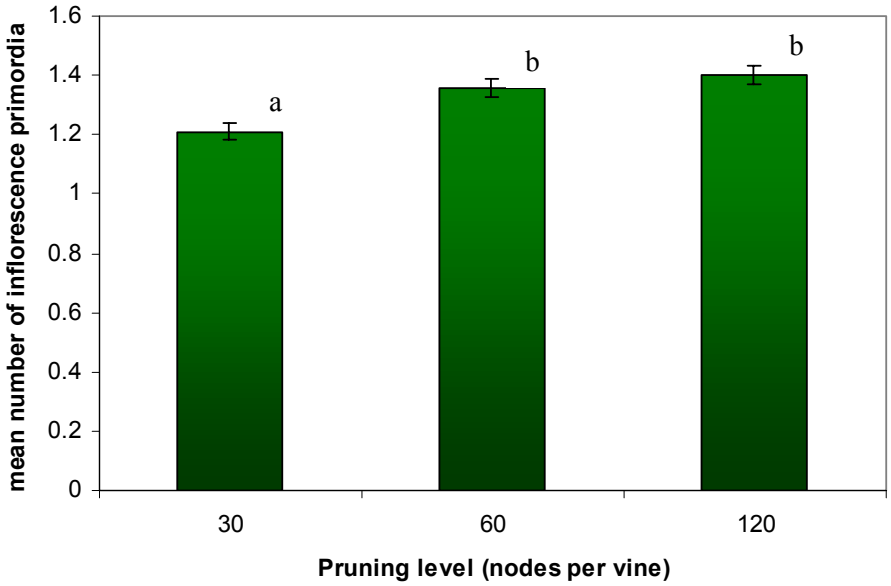
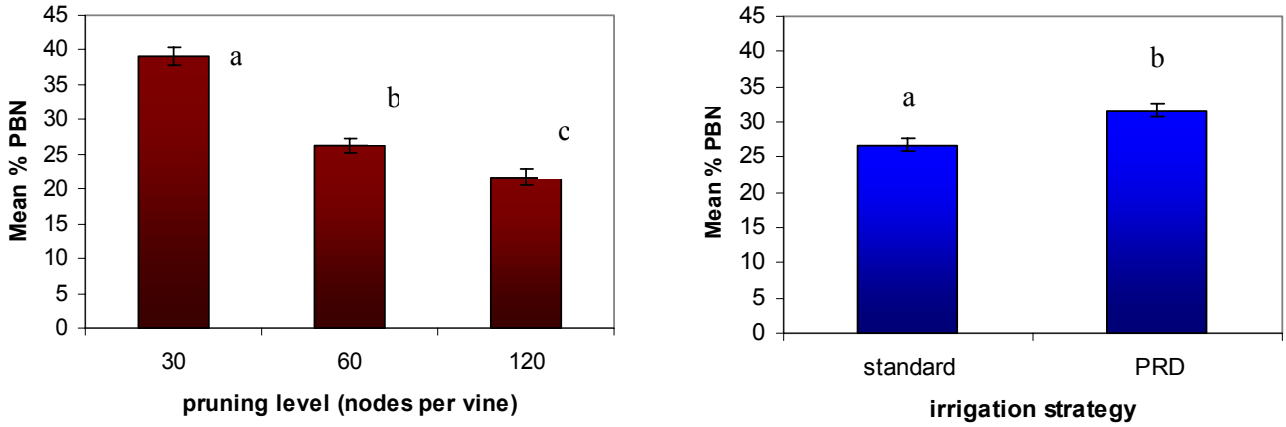


Figure 8.10. Mean bud fruitfulness (number of inflorescence primordia per bud) for different pruning levels at Nuriootpa, SA. Bars represent standard error. Means with same letter are not significantly different.



A B

Figure 8.11. Mean percentage of primary bud necrosis for (A) different pruning levels and (B) different irrigation strategy of standard drip or partial root-zone drying (PRD) at Nuriootpa, SA. Bars represent standard error. Means with same letter are not significantly different.

The incidence of PBN was affected by bud position along the shoot, pruning level and type of irrigation. The two-way and three-way interactions between buds, nodes and irrigation were not significant. PBN was greatest at buds 5 and 6 (Figure 8.9). The pattern of necrosis was similar to vines at Southern Fleurieu where PBN levels declined at buds 9 and 10. PBN at buds 9 and 10 were not significantly different to PNN levels at buds 1-4. As the pruning level increased nodes per vine, the mean proportion of PBN decreased, such that PBN was highest at 30 nodes per vine and lowest at 120 nodes per vine. All pruning levels had significantly different levels of PBN (Figure 8.11A). Irrigation strategy also influenced PBN. Partial rootzone drying (PRD) had a significantly higher proportion of PBN than standard drip irrigated vines (Figure 8.11B). Although there was no significant interaction between pruning level and irrigation, it was observed that 30 nodes per vine under PRD had notable more PBN than other pruning levels of the same irrigation treatment.

Bunch number was significantly different between pruning levels. Similarly to the other two vineyard sites, vines pruned to 30 nodes per vine had less bunches than other pruning levels (Figure 8.12). The highest mean bunch number was obtained for 120 nodes per vine. Less bunches resulted in less weight per vine. Severe pruning (30 node per vine) significantly reduced bunch weight per vine (Figure 8.13A). Although there was no interaction between pruning level and irrigation, there were significant differences between standard drip and PRD. Standard irrigated vines yielded more grapes than PRD (Figure 8.13B). PRD caused yield loss.

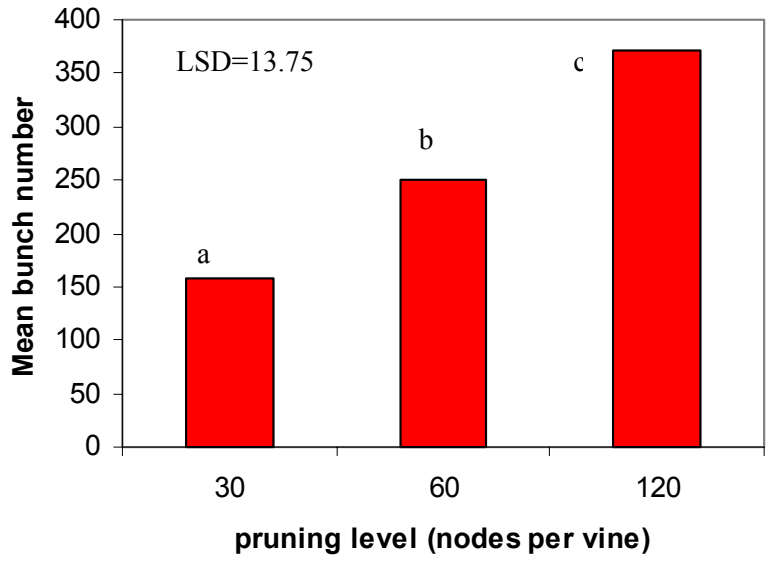
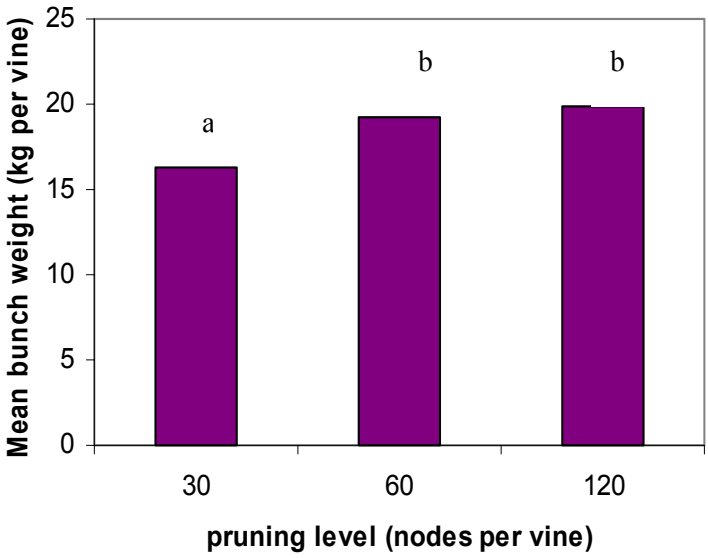


Figure 8.12. Mean bunch number for vines pruned to different nodes per vine at Nuriootpa, SA. Means with same letter are not significantly different.

A



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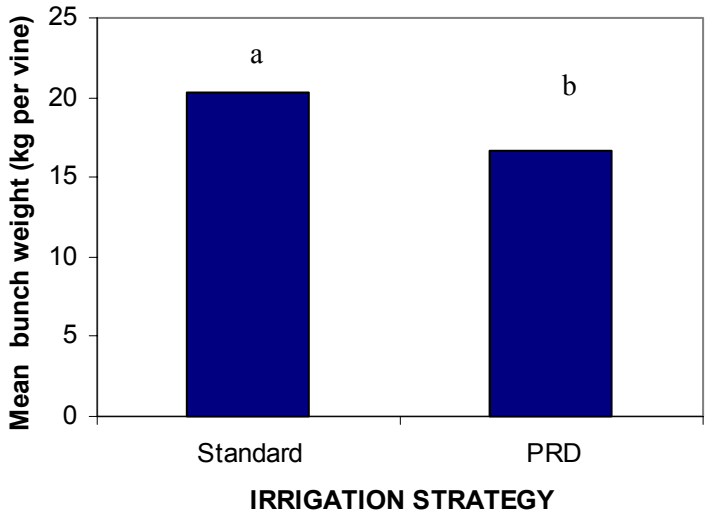

Figure 8.13. Mean bunch weight (kg per vine) for (A) different pruning levels and (B) standard drip and Partial rotozone drying (PRD) irrigation at Nuriootpa, SA. Means with same letter are not significantly different.

Pruning weights varied between pruning level and not irrigation type. Pruning level at 30 nodes per vine produced significantly greater pruning weight (mean 2.54 kg per vine) than 60 and 120 nodes per vine. There was no difference between the other pruning levels. Additionally, individual shoot weights were taken at the time of bud dissection. In this instance, all pruning levels were significantly different, with higher shoot weights on 30 nodes per vine and less on 120 nodes per vine. This supported that vines retaining fewer nodes resulted in greater vigour.

DISCUSSION

This study has highlighted the importance of pruning as a management strategy to combat the incidence of PBN and achieve potential yield. At all vineyards, retaining less nodes per vine resulted in more vigorous growth, less bunches and higher incidence of PBN. Fruitfulness was not affected by pruning level at McLaren Vale and Southern Fleurieu, however more severe pruning at Nuriootpa caused reduced bud fruitfulness. Pruning to 30 nodes per vine caused an imbalance in favour of vegetative growth at the expense of fruit production. Increasing the number of nodes per vine (light pruning) resulted in less shoots, hence reduced shoot vigour and low levels of PBN.

PBN was significantly affected by bud position, pruning level and method of irrigation. Although many reports indicate that basal buds show greater incidence of PBN than at more distal nodes (Dry and Coombe, 1994; Morrison and Iodi, 1990), this was not observed. Conversely, PBN was highest around nodes 5-6 and decreased further along the shoot. Basal nodes however were less fruitful. Poor fruitfulness at basal nodes could be attributed to decreased light interception, especially in denser canopies. Interestingly, internode length was significantly greater at nodes 5-7, further supporting assumptions that more vigorous shoots have greater incidence of PBN.

Pruning level was the main influence on the incidence of PBN. In particular, 30 nodes per vine displayed the highest incidence of PBN despite no detrimental effects on budburst. As a consequence of PBN, the number of bunches declined even though shoot number was high. It is likely that excess shoots were attributed to non-count nodes, water shoots and multiple shoots derived from secondary buds. It is documented that shoots derived from secondary buds produce fewer bunches and, if at all, are typically smaller. Excessive vigour contributed to higher levels of PBN.

Partial rootzone drying (PRD) caused more necrotic buds than standard drip irrigation. As vines receive less water under PRD than standard drip, water stress may be critical in influencing the development of PBN, especially during the period of bud differentiation. Water stress limits cell division processes (McCarthy *et al.*, 2001) and the development of inflorescence primordia. Fruitfulness has been shown to decrease with increase in water stress (Buttrose, 1974). Under PRD, less bunches and reduced bunch weight was recorded. Although there was no true interaction

between pruning level and irrigation method, vines severely pruned under PRD showed reduced yield compared to other treatments. A combination of water stress and pruning management may contribute to a higher incidence of PBN.

There are a number of methods to determine balanced pruning. Yield to pruning weight ratio gives a good indication of the balance between fruit production and vegetative growth (Tassie and Freeman, 2001) and mean shoot weight is a good index of vigour. Vines pruned to 30 nodes per vine at all vineyards were more vigorous, and fruit weight to pruning weight ratio was higher compared to other pruning levels. Pruning level was the major influence on obtained balanced pruning, irrigation had no effect.

This trial demonstrated that balanced pruning is required to (1) reduce the incidence of PBN, (2) reduce excessive vigour and (3) reach a desired yield target with satisfactory quality. Manipulation of pruning levels is necessary in vineyards with highly vigorous cultivars, such as Shiraz, particularly where high incidence of PBN is known.

9. Storage of bud dissection canes

INTRODUCTION

Bud dissection involves collection of lignified shoots (canes) and cutting open buds to determine the number of inflorescence (bunch) primordia at each node position. The service provider may provide advice on the number of cane samples required to obtain reliable estimates of bud fertility. The number of nodes assessed is dependent on pruning strategy and possibly known history of PBN. It was suggested by bud dissection service providers that long-term storage, between sample and dissection time, might enhance the probability of PBN. Length of time and incorrect storage of canes could promote drying of buds, resulting in an inaccurate assessment of PBN. Current practice by some bud dissection service providers includes wrapping of canes in a plastic or paper bag to minimise water loss and storage of canes at 4 °C or lower with no exposure to air. This trial was devised to investigate the occurrence of PBN in storage and to identify the maximum storage time and temperature for accurate bud dissection analysis.

MATERIALS AND METHODS

In June 2003 canes (cv. Shiraz) were sourced from a vineyard in Southern Fleurieu, South Australia. Canes were randomly collected for each treatment and cut to a maximum of 10 nodes. Canes were stored immediately in plastic bags and transferred to the laboratory. The five treatments were:

- Immediate bud dissection on day of collection
- No moisture at room temperature (23°C).
- Moisture at room temperature (23°C).
- No moisture at 4°C in a cool store.
- Moisture at 4°C in a cool store.

Each treatment consisted of 20 canes and was replicated over four storage time intervals: two, four, eight, and 16 weeks after the day of collection. Moisture was added by spraying water into all bags with a spray bottle. At each time, the incidence and severity of PBN was recorded on up to 10 buds per cane for all treatments. For each bud assessed during the trial a severity rating of PBN was given based on the percentage of necrotic tissue visible in the primary bud. Severity ratings were:

0. Healthy (0%)
1. < 25% PBN
2. 26 – 50% PBN
3. 51 – 75% PBN
4. > 76% PBN
5. Dead

RESULTS

The assessment of PBN on the day of collection revealed 29.7% of buds were necrotic (Figure 9.1). The highest incidence of PBN was observed at node 1, with a decline in PBN along the cane. Although more buds were necrotic at basal nodes, there was no significant difference in the severity of PBN across the buds (Figure 9.2). Overall the average PBN severity rating was 3.5 (ie. greater than 50% of the bud showed necrosis). There was no correlation between incidence of PBN and severity.

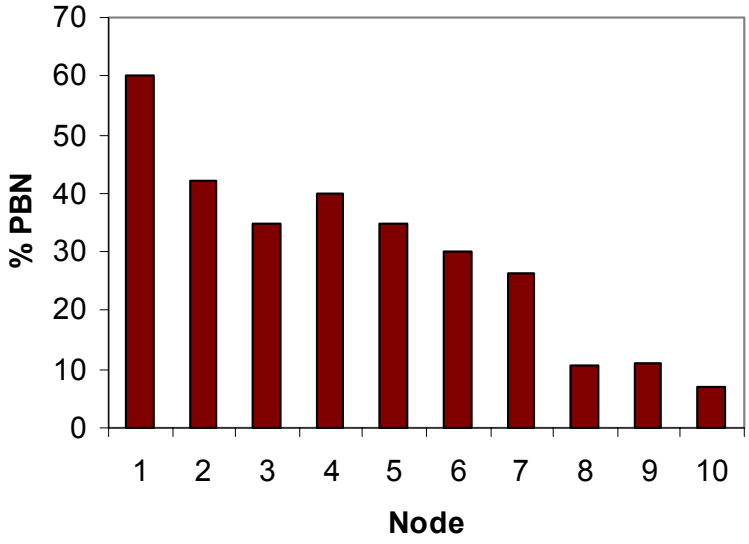

Figure 9.1. Percent primary bud necrosis prior to storage (cv. Shiraz) at Southern Fleurieu, SA, June 2003

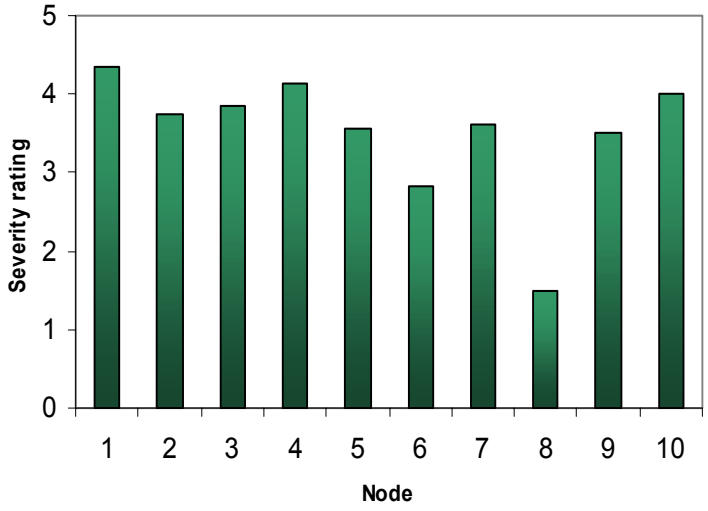

Figure 9.2. Average severity of primary bud necrosis (PBN) across nodes 1-10 prior to storage. Southern Fleurieu, SA, June 2003, whereby severity rating 0= healthy, no necrosis present, 1= <25% PBN, 2=26-50% PBN, 3=51-75% PBN, 4=>76% PBN and 5=dead bud.

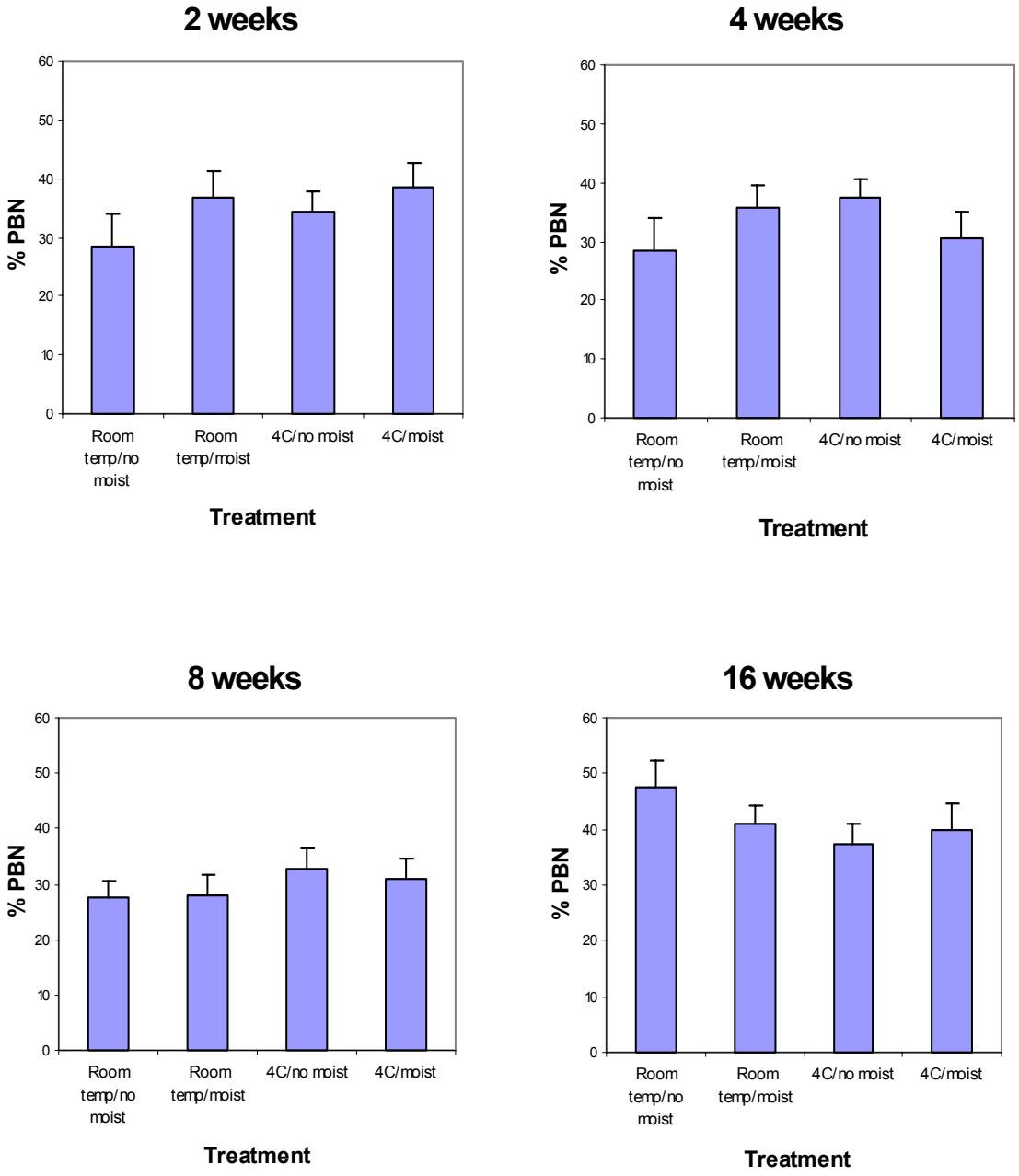

Figure 9.3. Percent primary bud necrosis after (a) two, (b) four, (c) eight and (d) sixteen weeks storage under four different treatments (cv. Shiraz, Southern Fleurieu, SA).

When assessed over time, the percentage of PBN showed slight variation between treatments (Figure 9.3). There was little variance in the percentage of PBN over the two to eight week storage periods. However, after 16 weeks storage the incidence of PBN increased in all treatments from the initial assessment and earlier storage periods. To test the statistical significance of these findings, an analysis of variance (ANOVA) was performed on each of the different treatments and storage periods. Storage of canes using any of the four treatments over two to eight weeks was not significantly different from actual PBN levels in the vineyard. This result was further supported, as the severity of PBN was not significantly different between treatments for these three storage times (P=0.143).

There was however a difference observed in the incidence of PBN from two to 16 weeks (P=0.024). For example, after two weeks, 28% of buds dissected from canes stored at room temperature with no moisture showed signs of necrosis, this increasing to 45% after 16 weeks storage. This result suggests that storage of canes after eight weeks using the tested treatments may alter the incidence of PBN observed.

No significant differences were found between buds dissected from canes stored at room temperature and 4ºC. Additionally, storage with or without moisture showed no significant difference. These results indicated that both temperature and the addition of moisture to a sealed bag are not crucial for the storage of canes for bud dissection analysis.

DISCUSSION

To accurately assess buds for the level of PBN, length of time and storage requirements prior to bud dissection is important. Without this knowledge, the final assessment of PBN may influence final bud fertility and recommendations for vineyard management. Severity of PBN did not vary significantly along the cane, indicating bud dissection analysis results would adequately represent PBN at the various bud positions. However, the results did support the assumption that the incidence of PBN differed along the cane. This has implications for the desired pruning method. The average PBN percentage would be much greater for buds 1-2 from spur-pruned canes than for vines that were cane pruned. Variation in PBN estimates between the nodes would influence how many buds to retain per vine to achieve desired yield.

These experiments have revealed that storing canes up to 8 weeks in a number of different conditions does not significantly increase PBN levels. There was no evidence that temperature and moisture within the sealed plastic bag had a significant effect on PBN. From these findings the following recommendations can be made:

- Store canes in a sealed bag
- Store canes no longer than 8 weeks

10. Conclusion and Recommendations

Primary bud necrosis (PBN) is a problem in most Australian viticultural regions. The increasing use of bud dissection analysis has raised awareness of PBN and the effect on bud fruitfulness. With escalating pressure by wineries to achieve target yields, bud dissections will become an essential tool in estimation of bud fertility. PBN has the potential to dramatically reduce yields but without bud dissection, PBN can go unnoticed.

Shiraz is most susceptible to PBN. Shiraz accounts for the highest production of all grapes grown in Australia, with 436,700 tonnes produced in 2004 (Source: ABS, 1329.0.55.002). A number of other wine cultivars affected by PBN include Petit Verdot, Pinot Gris, Gewürztraminer, Riesling, Sauvignon Blanc, Semillon, Chardonnay and Cabernet Sauvignon. Previous research (Lavee, 1981; Dry, 1986) and findings from this study suggest excessive vigour may be responsible for the high incidence of PBN in Shiraz. Vigour is related to the naturally produced growth hormone, gibberellic acid (GA_3). The endogenous application of GA_3 caused an increase in PBN, whereby the most significant difference was observed before flowering. Flowering is controlled by naturally produced gibberellins (Stephan, 1999). At the time of bud differentiation, GA_3 is at its highest level and is transferred to the new buds. In vigorously growing grapevine cultivars (eg. Shiraz) high levels of GA_3 move to the buds resulting in excessive cell elongation. The imbalance of hormones eventually kills the primary bud. The role of GA_3 in flower initiation may explain why PBN occurs on the onset of flowering. Unlike other cultivars, PBN levels in Shiraz fluctuated greatly throughout the season. However the incidence of PBN was always higher than other cultivars and increased during the season until the commencement of winter. Theoretically, the number of inflorescence formed in the bud is complete by the onset of dormancy and buds can be dissected for yield estimation after this time. However, our research showed that because levels of PBN in Shiraz continued to increase throughout the season, accurate bud dissections needed to be performed as close to pruning as possible.

This study has highlighted the importance of pruning as a management strategy to combat the incidence of PBN and achieve potential yield. Pruning level was the main influence on the incidence of PBN. Retaining fewer nodes per vine resulted in more vigorous growth, less bunches and higher incidence of PBN than lightly pruned vines. At one vineyard, severe pruning reduced bud fruitfulness. Pruning to 30 nodes per vine caused an imbalance in favour of vegetative growth at the expense of fruit production and subsequently, excessive vigour contributed to higher levels of PBN. Increasing the number of nodes per vine (light pruning) resulted in less shoots, hence low shoot vigour and low levels of PBN. Pruning level also influenced yield components such as bunch number and bunch weight. Light pruning resulted in increased bunch number and bunch weight (kg per vine) but conversely, bunches were smaller. Bunch weight itself is critical for yield estimation.

Additionally, cultural practices such as mechanical harvesting and pruning were associated with high levels of PBN.

Balanced pruning is required to (1) reduce the incidence of PBN, (2) reduce excessive vigour and (3) reach a desired yield target with satisfactory quality. This involves managing vine canopy to regulate vegetative growth and fruit production. Some indices of vine balance include: fruit yield to pruning weight ratio, leaf area to fruit yield ratio and pruning weight per metre of row (Tassie and Freeman, 2001; Dry, 2004). Yield, berry weight and prescriptive pruning levels are not appropriate indices of vine balance. A vigorous vine will have excessive shoot growth, many of which derive from non-count nodes and secondary buds that typically lead to production of fewer bunches. More research is needed to investigate the fruitfulness and role of secondary buds in compensating for PBN.

Irrigation method also influenced the incidence of PBN. Water availability determines the balance between vegetative growth and fruit maturation, similar to the effect of pruning level. Reduced water supply can reduce vigour. Partial rootzone drying (PRD) is an irrigation technique that aims to increase water use efficiency. Although there was no difference in shoot length between PRD and standard drip irrigation, vines under PRD produced less bunches and lower yields. PRD caused higher incidence of PBN than standard drip irrigation. In comparison, results from the survey indicated vines irrigated by restricted deficit irrigation (RDI) showed less PBN than standard drip. RDI applies water stress during certain stages of vine development without necessarily reducing overall water supply. Hence water stress may be the critical factor in influencing the development of PBN, especially during the period of bud differentiation. A combination of water stress and pruning management may contribute to a higher incidence of PBN.

One of the difficulties with investigating PBN was block and seasonal variability. High variability was found between vineyards therefore each site would need to be managed on a case-by-case basis. Factors such as seasonal changes, individual block characteristics and cultural practices would need to be considered to accurately estimate PBN levels. One block may show low levels of PBN, and the neighbouring block in the same district of the same cultivar may show high PBN. We do not fully understand why this is so, but high variability may be attributed to vine balance, climatic condition (macro and microclimate), stages of vine development, time of bud differentiation and stress during these critical periods.

It is recommended that:

- growers undertake bud dissection analysis each year to gain a greater understanding of bud fertility and PBN. In general, >20% PBN will be detrimental to yield potential.

- Existing pruning strategy, nodes per vine, vine vigour, labour costs, and target yield need to be carefully considered prior to modifying pruning levels to compensate for PBN.
- Retaining more buds per vine will increase fruitfulness in response to a high number of necrotic buds, but this may solve the immediate problem only.
- Ideally, balanced pruning needs to be implemented every year to eliminate the likelihood of seasonal variability of PBN, particularly in Shiraz. Shiraz is a vigorously growing cultivar and tailored pruning is required to control excessive vine vigour.

To achieve vine balance a number of strategies can be employed.

- Basically, prune to an appropriate node number to achieve desired yield target.
- Prune to a minimum of 15 nodes per kg pruning weight (Dry, 2004), optimum 30-40 nodes/kg pruning weight (Smart and Robinson, 1991) while maintaining 15-20 shoots per metre.
- If fruitfulness is particularly low in one season and PBN high, retain more buds in the short-term, but minimise the pressure of high shoot density by keeping below 20 shoots per metre. Shoot removal may be required to achieve this.
- By identifying characteristics of the vineyard, block-by-block variability and vine capacity, long term vine balance is ultimately the key to management of PBN.

Appendix 1: Communication

Results of this study were presented (Figure I) in 16 workshops and industry meetings as shown below.

Industry presentations and workshops

1.	26 August 2002	Phylloxera and Grape Industry Board of South Australia, grower meeting. McLaren Bocce Club, McLaren Vale, SA
2.	28 August 2002	Phylloxera and Grape Industry Board of South Australia, grower meeting. The Vines, Barossa Valley, SA
3.	29 January 2003	Bud dissection post-mortem. Adelaide Hills Growers Association, grower meeting. Lenswood Research Centre, Lenswood, SA
4.	29 April 2003	Bud dissection workshop. DPI, Tatura, VIC.
5.	22 May 2003	GWRDC Fruitset and flowering workshop. DPI, Tatura, VIC.
6.	28 May 2003	Elders Grapegrowers Day. Waite Research Precinct, Urrbrae, SA
7.	26 June 2003	Bud dissection workshop. IHD Sunraysia Horticulture Centre, Mildura, Vic.
8.	11 August 2003	Wesfarmers Conference. Meridien Conference Centre, Adelaide, SA.
9.	22 August 2003	Barossa Valley Viticulture Technical Group, grower meeting. St. Hallet's, Tanunda, SA
10.	2 February 2004	McLaren Vale Growers Association, grower meeting. McLaren Vale, SA
11.	3 February 2004	Clare Valley Growers Association, grower meeting. Clare Valley, SA
12.	4 February 2004	Adelaide Hills Grapegrowers Association, Lenswood Research Centre, Lenswood, SA
13.	5 February 2004	Coonawarra Growers Association, Chardonnay Lodge, Coonawarra, SA
14.	15 June 2004	Bud dissection workshop. McLaren Vale Visitor Centre, McLaren Vale, SA
15.	24 &26 July 2004	Workshop W17: 'Pest and Disease Monitoring and Identification". 12th Australian Wine Industry Technical Conference, Melbourne, VIC.
16.	3 November 2004	"Sustaining success…the growers challenge", Barossa Viticulture Technical Group Conference, Barossa Arts and Convention Centre, Tanunda, SA

A

B

C

D

Figure I. (A) and (B) Grape growers participate in a bud dissection workshop at McLaren Vale, South Australia, (C) Dr Belinda Rawnsley presents findings of the project at the Adelaide Hills Grower Association symposium, Lenswood, South Australia and (D) Dr Cassandra Collins presents research to the Clare Grapegrowers Association, Clare, South Australia.

Key publications

Rawnsley, B. (2003). What is primary bud necrosis? *The Australian and New Zealand Grapegrower and Winemaker*. Annual Technical Issue. 473a. pp 21-24.

Rawnsley, B. (2003). Inside a bud: healthy or PBN? *Australian Viticulture.* 7(5) pp13-15.

Rawnsley, B. and Collins, C. (2003). Birds eat shoots not just fruit. *The Australian and New Zealand Grapegrower and Winemaker*. 478. pp 25-27.

Collins, C. and Rawnsley, B. (2004). National Survey reveals Primary Bud Necrosis ins widespread in Australian Vineyards. *The Australian and New Zealand Grapegrower and Winemaker*. Annual Technical Issue, 485a, pp. 46-49.

Collins, C., Coles, R. and Rawnsley, B. (in preparation). Anatomical development of primary bud necrosis in Shiraz (*Vitis vinifera*). *Annals of Botany*

Rawnsley, B. and Collins, C. (in preparation). The effect of pruning and irrigation strategies on the incidence of primary bud necrosis in Shiraz. *Australian Journal of Grape and Wine Research.*

Collins, C. and Rawnsley, B. (in preparation). Seasonal development and susceptibility to primary bud necrosis in wine grapes (*Vitis vinifera*). *Vitis*

National and International Conferences

24-29 July 2004	12th Australian Wine Industry Technical Conference, Melbourne, Vic.
20- 25 June 2004	7th International Symposium on Grapevine Physiology and Biotechnology, Davis, California, USA.
28 June-1 July 2004	The Soil Environment and Vine Nutrition Symposium at the American Society for Enology and Viticulture, San Diego, California, USA.

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Appendix 3: Staff

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Appendix 5: Budget Reconciliation