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Continued development of *V. vinifera* inflorescence primordia in winter dormant buds

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Summary

Continued development of inflorescence primordia in winter dormant buds of *Vitis vinifera* 'Pinot Noir' is reported. In buds sampled from a commercial vineyard in the cool climate wine region of Southern Tasmania, mitotic activity was evident throughout the period from harvest to bud swell the following season. Results showed that in spite of buds entering apparent dormancy, cell division and inflorescence development continued throughout the winter months.

K e y w o r d s : *Vitis vinifera*, floral initiation, inflorescence initiation, inflorescence primordium.

Introduction

The formation of inflorescences and flowers in grapevines involves three well-defined stages; formation of anlagen, formation of inflorescence primordia and finally the formation of flowers (Barnard 1932, Barnard and Thomas 1933). Despite the extensive body of published research, uncertainty continues to surround the possible interaction between temperature and continued development of these critical processes.

May (2000) reported that there is general agreement that flower initials are not formed before the onset of bud dormancy, but that the question remains to be answered whether the first order branching continues after dormancy has ended. In a later review, May (2004) concluded that inflorescence primordia branch to form an inner and an outer arm and globular initials of side branches before the onset of bud dormancy and that conversion from inflorescence primordium to inflorescence starts as soon as spring growth commences. The review by Boss *et al.* (2003) reported that the immature inflorescence which forms before the buds enter dormancy survive winter in a quiescent state.

Lavee and May (1997) use Vegis' (1965) definition of dormancy of buds as "partial or growth dormancy which experience temporary cessation of growth, while metabolic processes including respiration continue". These authors consider that the diffuse passing of grapevine buds from one phase of dormancy to the next make it unwise to name each phase (para-, endo- and eco-dormancy), and that predormancy, dormancy and post-dormancy place the correct emphasis on the dynamic processes of dormancy development and release.

As a result there appears to be general acceptance that inflorescence primordium initiation occurs before bud dormancy but development ceases during bud dormancy. Srinivasan and Mullins (1976, 1981) reported that when dormant latent buds are activated in the following spring the inflorescence primordia undergo a period of rapid development however there is no mention of development during the dormancy phase. This was reinforced by Lavee (1985), in the review by Gerrath (1993), and Dunn and Martin (2000). Studies have concentrated on the periods of pre-dormancy and post-dormancy and the possibility and potential significance of ongoing cell division and expansion during dormancy have been rarely considered.

A study by Carolus (1970, reported in Lavee and May 1997) stated that in Bordeaux in the northern hemisphere, during dormancy the nucleoli are small and stain weakly and mitotic figures are absent, in contrast to when the buds grow, when the meristematic cells have large and deeply staining nucleoli and mitosis is evident.

In cool climate regions, where varieties like 'Pinot Noir' produce widely varying yields from year to year (Heazlewood 2005), interest surrounds inflorescence development, and extent of branching and resulting inflorescence size. The present study was part of an ongoing investigation into yield variability in cane pruned 'Pinot Noir'. In this case the emphasis was on the development of the inflorescence primordia from harvest and leaf fall.

Methods

Buds from node position four on randomly selected canes of eight-year-old 'Pinot Noir' (clone D5V12) grapevines, in a commercial cane pruned vineyard in Southern Tasmania, were sampled weekly from harvest in April 2006, through to bud swell at the beginning of the 2007 season. Excised buds were fixed in 10 % FAA and stored for histological examination. Inflorescences were excised and prepared on a squash mount stained with 2% acetoorcein, and examined for mitotic activity using Nomarski Differential Interference Contrast (Zeiss 4753366) microscopy. Mitotic indices were calculated to describe the sum of cells in prophase, metaphase, anaphase and telophase, expressed as a percentage of the total number of cells observed in a sample from the tip of an inflorescence (Sanchez-Moreiras, Coba de la Pena *et al.* 2001). For microscopic examination, buds were dehydrated, embedded in paraffin wax, sectioned using a microtome, attached

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to slides with Eupharel mountant, stained with 0.1 % safranin in 50 % ethanol, the wax cleared using an ethanol and histoclear series (4 drops of HCl in 50 % ethanol, 50 % ethanol, 70 % ethanol, 80 % ethanol, 90 % ethanol, ethanol / histoclear 50/50, 100 % histoclear), and images were obtained using a Zeiss Axioskop 2. A second sample of buds was taken from the same position and at the same time, and the apex and intact inflorescence primordia were removed by dissection under a light microscope (Nikon SM2-1B) and fixed in 2.5 % gluteraldehyde. These were then dehydrated using a water-acetone-carbon dioxide series, critical point dried (Polaron CPD E3000), mounted on aluminium stubs with carbon tabs and sputter coated (Edwards 5150B) for examination under an environmental scanning electron microscope (Philips XL30 FEG).

Results and Discussion

The presence of mitotic figures in inflorescence tip cells was used as an indicator of continued development. In all of the 'Pinot Noir' buds sampled (n = 54), mitotic activity was evident throughout the sampling period, confirming that cell division was occurring within the inflorescence throughout the winter months, and that the inflorescence primordia were not entering complete winter dormancy, as shown in Figs 4 and 5. The mean mitotic index across the period from leaf fall to bud swell was 3.11 (SD = 1.69) and there was no significant change with time. The mitotic index is comparable with results from floral tissue of *Lolium temulentum* 'Ceres' (Jacqmard, Bomans *et al.* 1993). As the mitotic index did not change with time, it can be assumed that the rate of cell division did not change significantly, and that the rate of inflorescence development did not change over the sample period. This is in contrast to the findings of Carolus (1970, reported in Lavee and May 1997), who found that mitotic figures were absent during dormancy. Based on long term average temperature data, the coldest winter month in Bordeaux has a mean minimum temperature of 3.3 °C and mean maximum temperature of 10 °C, whereas Southern Tasmania has a mean minimum temperature of 4.1 °C and mean maximum temperature of 12.4°C. It is possible that this small difference in temperature may account for the continued development of inflorescence primordia in this study, but not in that of Carolus (1970, reported in Lavee and May 1997)

In the series of scanning electron micrographs, inflorescences were distinctly larger at the end of dormancy. There was a gradual increase in inflorescence primordia size over the sampling period, towards the end of vine dormancy the inflorescence had almost doubled in size, due to increased branching (Figs 1-3). At the beginning of dormancy the number of branch primordia did not exceed 4 (Fig. 1) and at the end of the dormancy period branch primordia were in excess of 10 (Fig. 3).

These results confirm that the inflorescence within the buds on field grown vines of 'Pinot Noir' in this region do not enter complete dormancy during winter. Consequently the timing of the phases of inflorescence initiation and floral development in *Vitis vinifera* L. may not be as distinctly separated by winter dormancy as suggested by May (2004) and earlier publications. Further investigation is suggested as continued development of inflorescence primordia during the winter period may indicate the potential for winter conditions to influence inflorescence size or time to complete an intermediate phase of development during bud dormancy and hence time of bud burst. Thus, further research into the effects of winter temperature on cell divi-

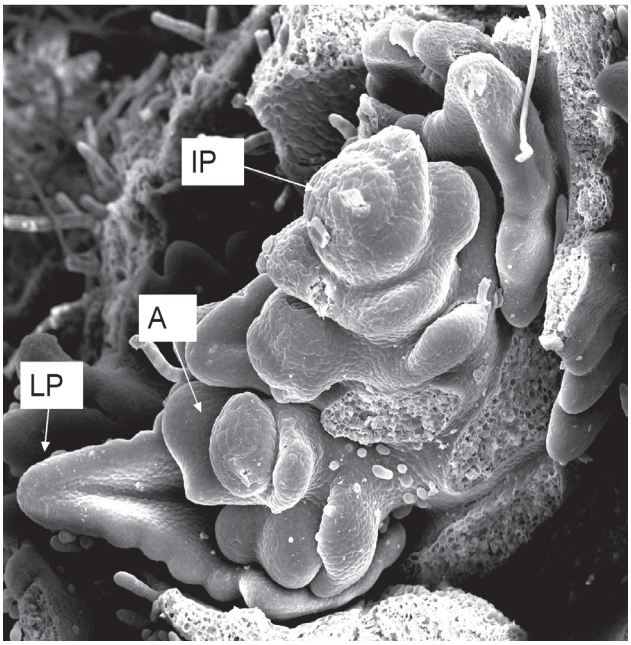

Fig. 1: Inflorescence primordium present in a bud, April 2006, showing shoot apex (**A**), inflorescence primordium (**IP**) and leaf primordium (**LP**). Image collected 28/11/2006, HV = 20.0 kV, Mag = 325 x, HFW = 0.83 mm, WD = 9.5 mm, Det = ETD, Sig = SE, Scale 3.5 cm = 200 µm.

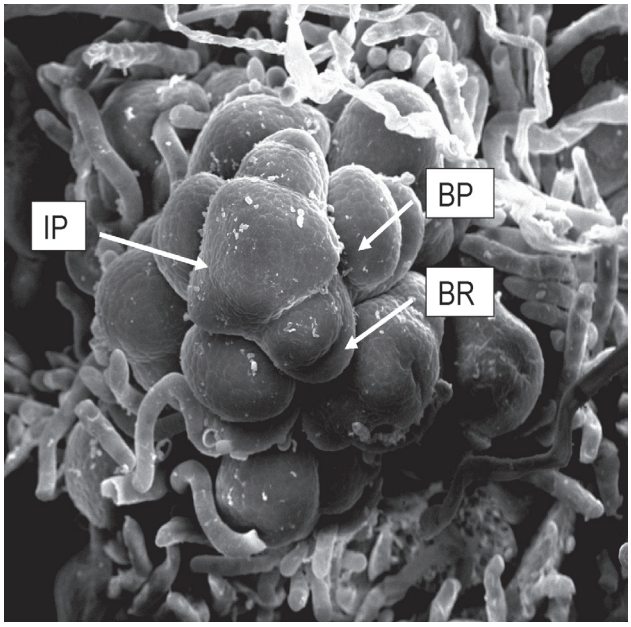

Fig. 2: Inflorescence primordium present in a bud, July 2006, showing inflorescence primordium (**IP**), branch primordia (**BP**) and bract primordia (**BR**). Figure collected 28/11/2006, HV = 20.0 kV, Mag = 541 x, HFW = 0.50 mm, WD = 15.2mm, Det = ETD, Sig = SE, Scale 3 cm = 100 µm.

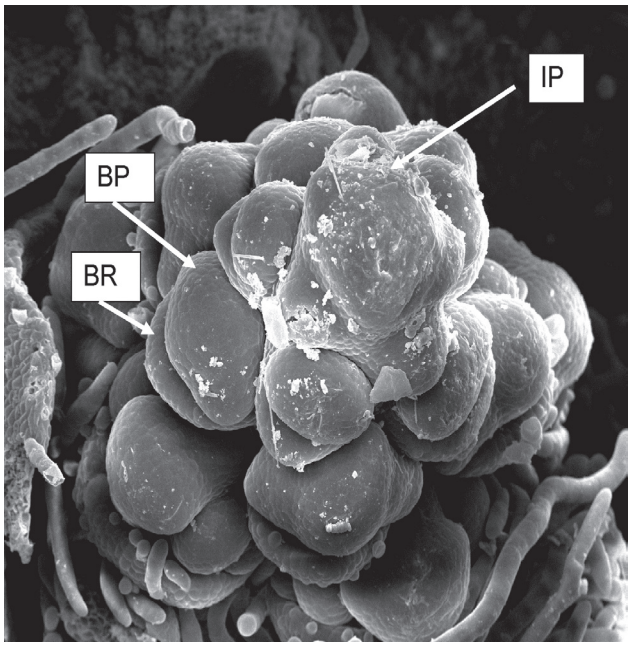

Fig. 3: Inflorescence primordium present in a bud, September 2006, showing inflorescence primordium (**IP**), branch primordia (**BP**) and bract primordia (**BR**). Figure collected 28/11/2006, HV = 20.0kV, Mag = 500 x, HFW = 0.54 mm, WD = 14.5 mm, Det = ETD, Sig = SE, Scale 3 cm = 100 µm.

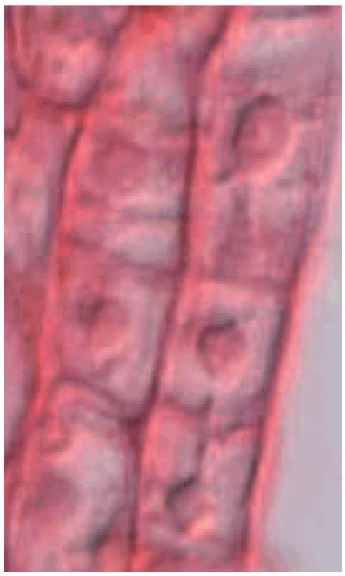

Fig. 4: Cells from inflorescence primordium tip showing nuclei (mag 40 x). Image recorded July 18th 2007.

sion and inflorescence development may provide greater insight into yield potential.

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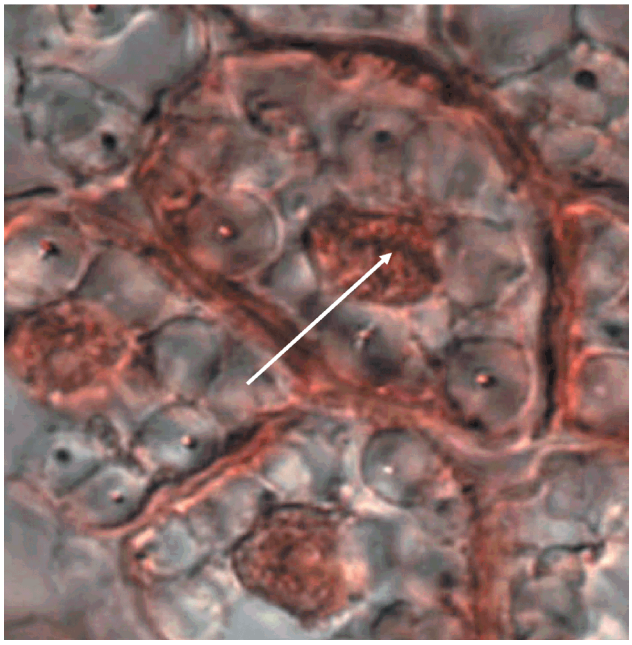

Fig. 5: Metaphase, chromosomes align at the equatorial plate within the nucleus (mag 100 x oil immersion). Image recorded July 18th 2007.

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