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Anthesis, pollination and fruitset in Pinot Noir

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Summary

Aspects of pollination and resulting fruitset in *Vitis vinifera* cv. Pinot Noir were investigated in a cool climate wine area of Southern Tasmania (Australia). Changes in the appearance of the stigmatic surface and pollen grains were recorded using environmental scanning electron microscopy (ESEM). Flowers with the calyptra intact (before capfall), showed an apparently turgid stigmatic surface and pollen grains present on the surface were slender and elongated (L/D 35 µm/15 µm). Stigmas had a more flaccid appearance after capfall and pollen grains were more spherical and less elongated in shape (L/D 28 µm/20 µm). Pollen was visible on the stigma surface before capfall, indicating that anthesis occurred whilst the calyptra was in place. Pollen viability tests showed that the pollen was already viable at this stage, and it remained viable until after the flower had been open for several days. Fluorescence micrographs showed no evidence of pollen tube growth until after capfall. Flowers covered by waxed paper bags to eliminate external pollen and thus allow only self-pollination, gave a percentage fruitset equal to that of flowers where external pollen was not excluded. Results indicate that Pinot Noir can be self-pollinated, and that while anthesis commences prior to capfall, fertilisation does not proceed until after capfall.

K e y w o r d s : grapevines, flowering, pollination, fertilization, capfall, fruitset.

Introduction

Pinot Noir (*Vitis vinifera* L.), produces highly variable yields in some areas and our own unpublished work in Tasmania has shown that low yields follow cool rainy conditions during fruit-set in some seasons. Poor fruit-set has been associated with inadequate levels of pollen at the style, failure of fertilisation, embryo abortion or abnormal flower development (SRINIVASAN and MULLINS 1981; SEDGLEY 1989; EBADI *et al.* 1995).

Most commercial grape cultivars are functionally hermaphroditic, therefore self-fertilisation is regarded as possible. However the method of pollination is debated, and to date there is little conclusive evidence to indicate how, and when, pollen is transferred from anthers to stigmas. Compared with studies on other factors influencing vine performance, there have been few investigations of pollination and fruit set (MAY 1992). As noted in the review by PRATT (1971), some studies suggest that pollination is primarily by wind, whilst others suggest insects play a role. There is also debate regarding self-pollination. MULLINS (1992) confirmed that the structure of the flower is not suggestive of wind pollination, but conceded that grapevines are primarily wind pollinated. There is a lack of consensus on the mode of pollination but general agreement in supporting *V. vinifera* cultivars may be selfed. GERRATH (1993) confirmed that the issue of self-fertility seemed controversial.

Working with Pinot Noir, STAUDT (1999) showed that, in flowers fixed at opening, anthers had already dehisced. He concluded that anthesis must have taken place before (or during) the opening process. Using measured pollen tube growth rates and observed extension of the pollen tube after capfall, Staudt was able to conclude that 16-18 % of flowers were pollinated before opening and the growth of pollen tubes had already started by capfall.

The objective of the present study was to determine the method of pollination in Pinot Noir as a basis for further studies on managing environmental effects on fruit-set.

Material and Methods

Investigations were carried out in the 2002/2003 flowering season on Pinot Noir clone 2051, in two commercial vineyards in southern Tasmania. Weather data from the nearest Australian Bureau of Meteorology Sstation at Grove Horticultural Research Station was collated for November and December 2002 and January 2003, these corresponding to the flowering and fruitset periods (November and December 2002 and January 2003).

F l o w e r s a m p l i n g : At different stages of flowering, inflorescences were removed from the vines in early morning and kept in a sealed vial in an insulated container for transport to the laboratory, where they were refrigerated at 4 °C for up to 4 h.

S c a n n i n g e l e c t r o n m i c r o s c o p y : Flowers were examined on an ElectroScan ESEM2020 Environmental Scanning Electron Microscope (ESEM) to record apparent changes in stigma surface and to look for the presence of pollen grains. The instrument was operated at an accelerating voltage of 15 kV in environmental mode, using water vapour as the imaging medium. Secondary electron images were acquired using the proprietary ElectroScan Gaseous Secondary Electron Detector (GSED) using water vapour as the imaging gas at a nominal pressure of 5 Torr (666.5 Pa).

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Flowers were sampled at different stages of the flowering cycle, ranging from fully closed with the calyptra intact, through to flowers that had been open for several days (tagged at capfall). To obtain images of the stigma surface prior to natural capfall, the cap was carefully removed using fine forceps, just prior to placing the flower in the ESEM chamber.

Anther dehiscence: The change in appearance of anthers during dehiscence was recorded photographically using a dissecting microscope with a camera adapter. Caps were carefully removed from mature flowers for photographs. Assessment of pollen deposition on the stigmas of flowers was made for both opened and capped flowers. Stigmas were mounted in a drop of basic fuchsin gel and examined under a light microscope.

Pollen viability: Bulk pollen samples were collected from flowers with intact caps, flowers at capfall and flowers which had been open for some time, but showing no evidence of fruit development. Flowers (with caps removed) were shaken over a 1.5 ml Eppendorf tube, and the collected pollen desiccated over freshly dehydrated silica gel in a small sealed container. After dehydration the tubes were sealed and stored at -80 ºC. Samples (150-300 grains) were taken from the stored bulked pollen and re-hydrated by placing open tubes containing pollen over a water bath at 35 ºC for 60 min. Viability was determined using the fluorochromatic reaction test (FCR) of SHIVANNA and RANGASWAMY (1992).

Pollen tube growth: Both capped and naturally open flowers were collected for examination of pollen tube growth on stigmas. Stigmas were excised, placed on a slide, covered with a drop of aniline blue stain, and a cover slip and left at room temperature for 4 h. A fluorescence microscope was used to view the mounts and the number of pollen tubes counted (SHIVANNA and RANGASWAMY 1992).

Selfed or open pollinated: Ten inflorescences developing mid-way along canes on separate cane-pruned vines were selected at random from a commercial vineyard. On 22 December 2002, 5 un-opened infloresences were enclosed in small white waxed paper bags and sealed with cable ties around the base of the rachis to eliminate any transfer of pollen into or out of the bag. Five un-opened inflorescences were also selected, labeled and left uncovered. Sixteen d later, on January 7, 2003, after fruit expansion had commenced, bags were removed and the number of expanding berries present and the total number of flowers in all 10 bunches were recorded. At commercial harvest each bunch was removed and bunch weight and berry number counted. Fruitset was calculated as the ratio of developing fruit to original flower number.

Results

Weather data: Monthly average maximum temperatures were 19.9 °C for November, 21.5 °C for December and 25.0 °C for January. Corresponding minimum temperatures were 7.4 °C, 9.3 °C, and 10.4 °C whilst average relative humidity for the three months was 66.6 %, 61.4 % and 60.5 %. Precipitation was 33 mm for November, 13 mm for December and 23 mm for January.

Scanning electron microscopy: ESEM images showed that pollen was present on the stigmas of capped flowers. These flowers also showed a turgid stigmatic surface and any pollen grains present were slender and elongated, with a length of around 35 µm and a length/diameter ratio of 2.33 (Fig. 1 a). Stigma surfaces of open flowers had a more flaccid appearance and pollen grains were less elongated in shape, as shown in Fig. 1 b and d, with a length of around 28 µm and a length/diameter ratio of 1.4. Pollen grains

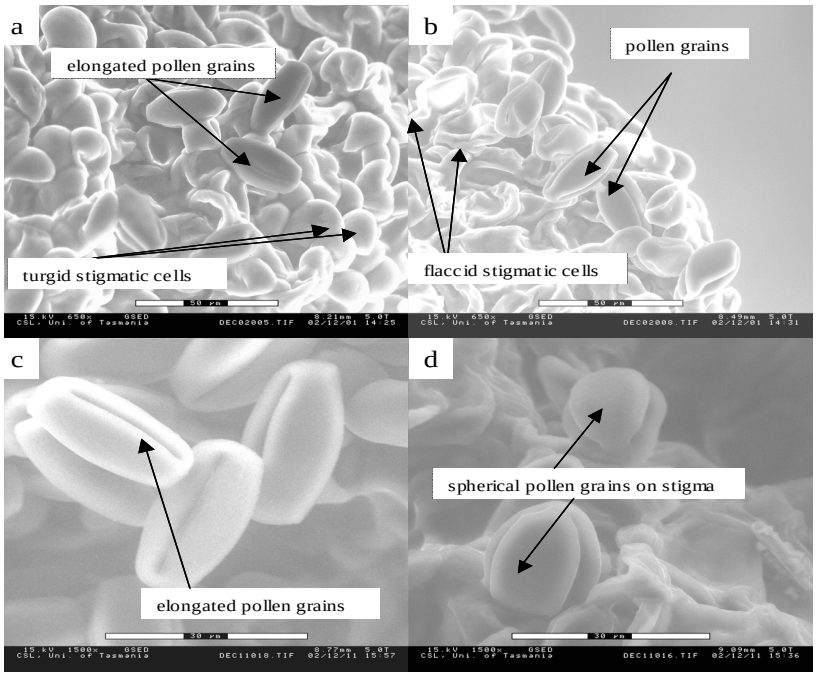

Fig. 1: Electronmicrographs of flowers before and after capfall showing: (**a**) the stigma surface of a capped flower with turgid stigmatic cells. (**b**) the stigma surface of an open flower, with flaccid stigmatic cells. (**c**) elongate pollen grains contained in an open anther of a capped flower. (**d**) pollen grains on the stigma of an open flower, where pollen grains are less elongate.

appeared to be tricolpate and of medium size when contained within the anther (Fig. 1 c). There were no pollen tubes or evidence of pollen tube growth in ESEM images for flowers before or after capfall.

Anther dehiscence: When closed, the anthers contained two lobes, each smooth in texture and plump in appearance. Opening of the lobes commenced with a gradual split up the center. When the lobes were completely open, each consisted of two discs of pollen. The latter stage was evident in flowers where the anthers were exposed by removal of the cap. The fuchsin stain technique confirmed the presence of pollen grains on the stigma of capped flowers. The mean number of pollen grains present on the stigma surface after natural capfall, 423.7 (sd = 39.2), was far greater than the mean number present on flowers examined prior to capfall, 92.3 (sd = 15.3).

Pollen viability: The pollen viability tests showed that viability was similar for capped flowers, flowers at capfall and for open flowers. Viability of pollen removed from the capped flowers was 51.6 % (n = 155), removed from flowers at capfall 55.2 % (n = 324) and from open flowers it was 57.0 % (n = 219).

Pollen tube growth: The staining method gave definitive results without the need for clearing of the stigmatic tissue. In each of the 30 open flowers examined, there was obvious pollen tube growth in the style, as shown in Fig. 2. Mean pollen tube length was 513 µm (sd = 203) with shorter pollen tubes being found in recently opened flowers and longer tubes in more mature flowers. In the thirty flowers with caps intact, there was no evidence of pollen tube growth.

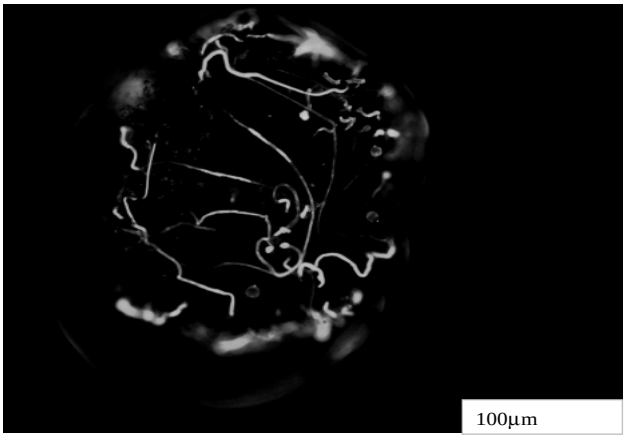

Fig. 2: Fluorescent micrograph showing pollen tubes on the stigma of an open flower.

Self or open pollinated: There was no significant difference in fruit set between the self and open pollinated bunches. Fruitset of the self-pollinated bunches was 79.8 % (sd = 6.7) and in the open pollinated bunches 73.2 % (sd = 7.3).

Discussion

The flowering season of 2002/03 was generally representative of previous seasons. The long-term averages from 1952 to 2003 showed average maximum temperature to be 18.4 °C for November, 20.1 °C for December and 22.3 °C for January, with average minimum temperatures of 7.0 °C, 8.6 °C and 9.4 °C respectively and relative humidity 68 %, 67 % and 66 %. Rainfall was however substantially lower than the long-term averages of 68.7 mm, 63.3 mm and 47 mm for the three months.

There were marked changes in the appearance of pollen grains and the stigma surface after capfall. Before capfall, elongated pollen grains were present and the stigma surface presented as apparently turgid cells. After capfall the surface cells appeared more flaccid and the pollen grains had become more spherical in shape. These observations all agree with MIAJA *et al.* (1999) who, working with the self-fertile cv. Barbera, showed that fresh flowers before anthesis had turgid papillae (provided the sample did not dehydrate within the ESEM) and there was pollen on the stigma before capfall. Collapse of papillae on fresh material was observed when the calyptra began to rise and pollen was already visible on the stigma. The authors also reported that, when the pollen tube elongated and the contents of the grain migrated through the pore, the pollen grain partially collapsed, leading to a more spherical appearance. Pollen tubes were not visible on the ESEM images obtained in the present study, but the observed changes in pollen shape were consistent with the observations by MIAJA *et al.*

The ordered anther opening sequence, evident in flowers with caps in place suggests that accidental damage to the anther and associated scatter of pollen during cap removal was not responsible for the appearance of pollen on the stigma before capfall.

In the field pollination trial, there was no difference in fruit set between bunches formed from inflorescences exposed to external pollen sources, insect activity and ambient wind flow and those inflorescences contained in bags. Whilst it was not possible to show whether non-bagged flowers were exposed to genetically different pollen, the results do confirm that self-pollination must have occurred in the bagged flowers resulting in fruit set equivalent to flowers exposed to normal vineyard conditions. Further, our data also confirm that pollen transfer from anther to stigma to produce normal fruit set was not dependent on wind or insect activity. KIMURA (1998) reported an increase in fertilization and consequent berry set with cross pollination in *V. coignetiae*, but there appears to be a general acceptance that *V. vinifera* is self fertile and the present results support this view.

Pollen viability tests showed that dehisced pollen was already viable underneath the cap but contrary to the results of STAUDT (1999), there was no evidence of germination and pollen tube growth until after capfall. STAUDT (1999) calculated that 1 h prior to capfall, 35 % of the flowers had pollen tubes, but this percentage decreased to less than 5 % at 24 h prior to capfall. It may be that unopened flowers in the present experiment were not sampled close enough to capfall, but none of the 30 flowers sampled with caps in place showed any pollen tube growth. STAUDT's (1999) results were based on extrapolation using pollen tube growth rates, not observation of flowers prior to capfall. Thus, although Pinot Noir is self fertile, and (on present evidence) pollen transfer occurs in the protected environment inside

the capped flower, pollen germination and subsequent pollen tube growth appear to be delayed until after capfall.

The conclusion reached by STAUDT (1999) that pollen tube growth commences before capfall, means that capfall has no significant role in pollination and subsequent fertilization. In contrast, failure of pollen to germinate under the cap, as shown in the present study, suggests that although flowers are at least partly self fertile and pollen transfer occurs under the cap, capfall is an essential step in the processes leading to fertilization. If this is the case, then research on factors influencing cap fall may give a useful guide to environmental influences on fruit set and bunch size in this variety.

To date, there is no indication of whether failure of pollen germination under the intact cap is related to the stage of development of the grain itself, the condition of the stigma surface, or the physical environment of the capped flower. The link between cap fall and germination of the pollen provides a possible explanation for the common view that weather conditions at flowering influence fruit set. The observations suggest that cool wet weather will have no direct influence on pollen shed or transfer, but if such weather delays cap fall then fertilization and subsequent berry development may be restricted by delayed and variable pollen germination.

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